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# RACIAL AND INDIVIDUAL VARIATION IN ANIMALS, ESPECIALLY FISHES

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## I

JUST before the explosive evolution of genetics during the opening years of this century, the study of variation was growing with a promise of becoming a major subfield of zoology. This development of variation studies then became obscured, in part only apparently because of an overshadowing shift in biological attention, but largely in fact. The promise of a new field (an allurement in itself), the greater satisfaction in the newer experimental as contrasted with the older circumstantial evidence which had been gained in biometry, the greater definiteness of individual over population analysis—such factors attracted biologists from biometry into genetics. And with the natural pride and satisfaction in their epochal progress, the geneticists developed a feeling which often approached scorn toward the variation studies of the preceding generation.

The field of variation, following the principle of the phylogeny of animals, has continued to exist on, as an evolutionary strand during the flourishing rise of the derived field of genetics. Perhaps a new progressive evolutionary movement may sometime arise from the generalized relict.

In certain fields and for special purposes, the population analysis of biometry was not, or could not be displaced entirely by the individual experimentation of genetics. In human heredity, for example, genetic experiments have been inhibited by the slow breeding if not the unwillingness of the possible subjects, and recourse must still be had to the cruder, less efficient tool of observational statistics.

In the new systematics, likewise, genetics can not replace variation investigations. This is not because genetics does not broaden the general biological view of the systematist, nor because genetics may not offer him detailed data of actual value in his systematic interpretations—but because systematic groups are populations, and must be investigated by population analysis.

It has been in large part the increased use of statistical methods and the development of a statistical sense, in species and subspecies and local race discrimination, which has caused systematic zoology—I speak particularly for systematic ichthyology—to make distinct progress during the last half-century. To be sure many biologists (unacquainted with the systematic field, certain physiologists and geneticists for instance, still display their ignorance by insisting that no advance in systematics has taken place since the days of Linnaeus. I hold the view, perhaps in extreme contrast, that much the same order of difference exists between the fish systematics of so excellent a last-century ichthyologist, as for example Günther, and that of the coming if not of the present school, as contrasts the heredity of Galton with the genetics of Morgan. Not only is it becoming increasingly the habit of the systematist to search the exterior and even the interior of his animals for possible distinctive features, but he is also coming to consider distribution, habitat, habits and life history, and the characters of the young and even of the germ cells. But experience is clearly teaching the systematist that even such refined systematic analysis may only formulate his problems: to determine whether the observed differences are consistent he is often forced to obtain a much larger series of specimens than his predecessors would have considered accumulating, and to analyze statistically the variation in the characters of his many specimens, whether they be internal or external, of bone or of flesh, of anatomy or of color.

In the analysis of the local races of fishes, the variation

# THE IMPORTANCE OF DEVIATION AMPLIFYING CIRCUITS FOR THE UNDERSTANDING OF THE COURSE OF EVOLUTION

by

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SUMMARY

The importance of deviation-amplifying processes for the emergence of major evolutionary novelties is discussed by exemplifying the evolution of birds and the term 'chain evolution' is proposed.

It is suggested that the importance of deviation-amplifying networks for the evolution of major systematic groups indicates that the changes leading to the origin of these groups progressed within a single genetic pool. The probability of polyphyletic origin of such taxonomic units as Tetrapoda, or Mammalia is regarded as extremely low.

The diversity of the structure of the central nervous system in different vertebrate groups is explained as resulting from multiple connections between it and the biology of the group. These connections form networks which may act either in a stabilizing or a deviation-amplifying way. Some examples of the networks are briefly discussed.

## INTRODUCTION

Cybernetics introduced the concept of feedback mechanisms as obligatory elements of every self-regulating and equilibrating system. In such systems the feedback is negative and therefore deviation-counteracting. But the feedback can be also deviation-amplifying, when the links between the elements within a system are positive. MARUYAMA (1963) demonstrated the importance and the ubiquity of deviation amplifying systems, BIELICKI (1965, 1969) has recently used this concept in a discussion of events which led to the origin of man, BARTHOLOMEW (1970) developed with its help a model explaining the unusual features of reproduction of Pinnipedia. It is the aim of the present article to demonstrate the importance of this concept for the evolutionary theory.

## STABILIZING VERSUS DEVIATION AMPLIFYING SELECTION

The importance of natural selection for the stability of the species was most extensively demonstrated in the publications of SCHMALHAUSEN (*e.g.* 1960), who was the founder of the "theory of stabilizing selection".

Still, every change in the environment may initiate a directional selection, driving some of the characters of the species towards a new optimum. There are examples of selectional forces changing direction even within a year. Thus SEMEONOFF & ROBERTSON (1967) have demonstrated that in *Microtus agrestis* the natural selection increases the amount of gene for blood esterase in summer and decreases it in winter. MERREL & RODELL (1968) have found that the "burnsi" genotype of *Rana pipiens* decreases in number during summer and increases during winter. BISHOP (1969) described the doubling in number of "yellow" variants of the crustacean *Sphaeroma rugicauda* during winter and the return to the previous value during summer. As the environment of every species is constantly changing, many opportunities arise for a shift in the direction of evolution. Usually the directional selection either influences one feature only or a limited numbers of features. However, as the elements of the whole organism are interconnected by numerous relations influencing each other in a very complicated pattern (compare *e.g.* DULLEMEIJER, 1968), a new value assumed by any one of parameters tends to create forces aiming at a change in the other elements. Thus *e.g.* the change in the dimensions of the animal may influence its metabolism, its food choice, its thermal preferendum etc. The correlated shifts in various features may be disadvantageous and, therefore, the selection changing one feature may be counteracted by a negative force. In an arid climate, for instance, it may be advantageous for toads to shorten the period of larval life as this will decrease the risk for tadpoles of being killed in drying up pools. But early metamorphosis produces small toadlets which are more vulnerable to desiccation. The extreme acceleration of the larval life is therefore deleterious, the selection acts as a stabilizing agent.

It may happen that adaptations in a feature promoted by selection create a secondary selectional force working toward a change in another feature, and the changed feature increases the original pressure for the change in the first characteristic. So *e.g.* when the ancestor of *Rana cancrivora* attained some ability to withstand the brackish water it simultaneously acquired the possibility to extend its ecological niche. By entering the diluted sea water the animal increased the selection forces acting for a further improvement in the tolerance to increased salinity and every progress in this direction caused a further extension of the ecological niche of the species until the ability to live in undiluted sea-water was reached (GORDON, SCHMIDT-NIELSEN & KELLY, 1961). In this way a positive feedback loop between the physiological and the ecological factors was formed and the initial process of change was

accelerated. If several positive feedback circuits of that kind are simultaneously initiated the species enters a period of rapid and profound reorganisation, possibly giving origin to a major evolutionary radiation. It has often been stressed that a taxon evolving inside an adaptive zone is changing slowly (SIMPSON, 1947), whereas the entrance into a new zone is accompanied by an acceleration of all evolutionary processes (*e.g.* HEYER, 1969). THOMSON (1969) says that "the mode of origin of the major groups of organisms is one of the most interesting and least completely understood aspects of evolutionary biology". The realization that this process depends on the formation of a complicated network of numerous deviation-amplifying processes explains the speed with which the "second order evolutionary changes" (THOMSON, 1969) are known to have occurred. I have used the term "chain evolution" to emphasise the similarity of such evolutionary processes with a nuclear chain reaction (SZARSKI, 1967).

To exemplify the evolutionary process probably directed by a network of deviation amplifying circuits, let us consider the development of birds from arboreal reptiles. As the frequency of jumps from tree branches, probably to avert a dangerous situation increased, the natural selection forcefully promoted every enlargement of scales, the improvements in the ability to judge distance, and the ability of steering in the air. The attainment of progress in any of these features increased the pressures for more frequent jumps thus closing the first deviation-amplifying loop. The existence of the loop increased the pressures for progress in the features and thus hastened the appearance of further loops. The changes in the biology of the animal caused further shifts in a direction of natural selection.

The speed of movement caused the rise in body temperature and also induced the appearance of mechanisms capable of controlling it. Owing to the formation of closed circuits the discussion whether the enlargement of scales results from the tendency to make jumps, or the ability of making jumps is the result of the enlargement of scales is irrelevant. Every element of a closed system influences the remaining parts and is in turn influenced by them. Consider an example of a negative feedback: it can be said as well that the level of thyroxine in the blood of a vertebrate is a result of the level of the thyreotropic hormone of the pituitary, or that the thyroxine level determines the amount of circulating thyreotropic hormone. Both statements are true.

When the active movements increased the body temperature of birds ancestors it simultaneously improved the performance of the nervous

system and the strength of muscles. A constant supply of food is needed to maintain a high rate of metabolism and the necessity of constant active search for food puts a high premium on every improvement in the functioning of the brain and on the refinement of sense organs. The bipedal locomotion on the ground necessitates a highly sensitive control of equilibrium. The development of the parental care is also linked with the flight ability. It is only due to the parents feeding their offspring that the vulnerable early stages of life are shortened. Consequently, parental care asks for a highly complicated behaviour which can exist only when the brain has already reached a certain grade of development. Flight would be dangerous without a perfect visual memory and orientation in space which are conspicuously demonstrated by the homing ability of many bird species. In this way a highly complicated network of deviation amplifying processes came into existence. In this network every improvement in one factor constituted a stimulus for further progress in the remaining ones.

It can thus be stated that the most important factor for the development of radical evolutionary novelty lies not in the small initial change in the structure or in the behaviour which started the process, but in the formation of many interdependent deviation amplifying circuits which may rapidly induce a profound reconstruction of the whole organisation of the species and its ecology. It is *e.g.* obvious that the important difference in the mode of finger reduction of the Perissodactyla and of the Artiodactyla is a consequence of the very small difference in the structure of the feet of their ancestors. The differences between the "inverted" and the "everted" type of telencephalon development in vertebrates are probably the result of some minimal differences in the structure of the brain of their ancestors. The mutual interdependence of evolutionary changes tends to amplify any trend once that trend has started rolling.

The explosive process of chain evolution does not proceed for a long period of time at an accelerating rate. A phase of accelerated change is followed by a stage of a stable progress, and as the possibilities for improvement are successively exhausted a slowing down ensues. Finally the whole process peters out and comes to a halt. This may result from different causes. The process of changing of a certain feature into a specific direction may reach a limit beyond which the selection pressure is reversed. Thus the tendency for the feet bones to grow more slender and lighter in fast running Ungulates cannot proceed indefinitely. Another possibility is the full realisation of a trend. BIELICKI (1965) has pointed out that the evolution toward bipedalism in human ancestors was an

element in deviation-amplifying loops but it ceased to influence the evolutionary processes when the bipedalism was complete. In a similar way the gradual shortening of the tail of the ancestors of Salientia ceased to influence other evolutionary tendencies when the tail disappeared completely.

Finally the process of directional evolution may be halted when all the potentialities of the architectural plan are exhausted. It seems that at present there are no possibilities for an overall improvement in the bird's structure. In the more immediate past the evolution of the behaviour of birds and of their anatomy was restricted to diversification. Many different modes of flight arose, each adjusted to the specific ecological niche.

In contrast to the speedy change observed in the evolution of birds from reptiles some conservative vertebrates have persisted over long periods of time. Some biologists imagined that this could result from a lowered variability of these forms. But recently SELANDER, YANG, LEWONTIN & JOHNSON (1970) demonstated that in *Limulus polyphemus*, a representative of Xiphosura, a highly conservative systematic unit, the genetic variability is approximately similar to that of the rodent *Peromyscus polionotus*, and is higher than in some birds. It must therefore be assumed that the conservatism of Xiphosura reflects the fact that this group is under the pressure of a stabilizing selection which counteracts every deviation from the ancestral structure and mode of life.

## THE DEVIATION AMPLIFYING CIRCUITS AND THE PROBLEM OF MONOPHYLETISM

The possibility that some taxonomic units may have a polyphyletic origin has often been debated. Thus for instance JARVIK is a strong advocate of amphibian polyphyletism (1968), whereas SZARSKI (1962), PARSONS & WILLIAMS (1963), THOMSON (1968) and many others favour a monophyletic origin of all terrestrial vertebrates. The assumption that the process of chain evolution was operational in the origin of every major taxonomic group before the stage of adaptive radiation throws additional light on such questions.

In the complicated network of mutual relationships of deviation-amplifying circuits every element forms an important part and influences the whole course of events. Therefore to obtain a similar result in two parallel cases the networks ought to be formed around organisms very similar in structure and living in a similar environment. Such two processes cannot, however, proceed simultaneously in the same geographic

area, as the competition between the two groups would tend to amplify every difference between them. The probability that in two distant areas two similar groups will simultaneously enter into an identical process of chain evolution seems very remote. Every known example of parallel evolution among vertebrates shows features speaking against a common ancestry. Thus, although Tachyglossidae, Pholidota, Myrmecophagidae and Tubulidentata are modified in a very similar way for a resembling mode of life, namely eating of ants and termites, their common ancestor was undoubtedly unspecialized, and hence all these groups retain in their structure the particular characters which speak against their inclusion in one single taxon. An identical situation is found in different orders of ungulate mammals. The numerous specialization trends toward similar niches in Metatheria and Eutheria are never really parallell. It may be added that recent evidence speaks clearly toward the monophyletic origin of mammals (*e.g.* HOPSON & CROMPTON, 1969; KIELAN-JAWOROWSKA, 1970; JENKINS, 1970).

The importance of self-accelerating, deviation-amplifying circuits in the origin of new evolutionary levels suggests that every major change arose within a single genetic pool. The well known definition of SIMPSON (1961) of monophyly, according to which a taxon is to be regarded as monophyletic if it is derived "through one or more lineages from one immediately ancestral taxon of the same or lower rank" is therefore ill suited for the description of the actual history of animals. I am not suggesting that the intermediate systematic units, say between reptiles and birds, consisted of a single species. Several branches probably evolved simultaneously, but as one of them attained a definite improvement in one of the features *e.g.* in the care of young, it began to expand and invaded various ecological niches, whereas the descendants of other lineages were eliminated by competition.

## THE SPEED OF EVOLUTIONARY CHANGE AND SOME TAXONOMIC PROBLEMS

Recently KIRSCH (1969) discussed the problem of rates of change in evolution. He lists other researchers inclined to assume that the amount of evolutionary change is strictly time-dependent. The tendency for such generalization is also noted by BEHNKE (1970) in biochemical publications. KIRSCH demonstrates, however, that data pertaining to biochemical and serological affinities of Marsupialia disagree with this proposition. He attracts the attention to the fact that all classification systems accept the existence of the progressive and of the conservative taxons. The

paleontological data demonstrate that some groups have greatly changed during their known history, whereas others remained nearly untouched by the passage of time. This is incompatible with the assumption that the evolutionary change is proportional to the time elapsed.

The fundamental importance of deviation-amplifying processes for the evolutionary change explains the causes of these differences in the speed of reorganisation. It ought to be assumed that the groups displaying a high degree of conservatism remained for a very long period under the prevalent influence of stabilizing selection, while the other groups which underwent radical reconstruction were forced to change by the pressure of mutually reinforcing deviation-amplifying loops. The average lifespan of a species, or of any other taxonomic unit gives therefore little information about the nature of the evolutionary process. The speed of change may be either very low, or very high, and it is just the mere intermediate values which are most probably rarely found. MAYR (1963) wrote "the only thing we can say is that there is no standard rate of speciation". The distance in time separating present living forms from a common ancestor is not correlated with the amount of difference between organisms.

Some authors are influenced by the well known ideas of HENNIG (1966). In a recent publication NELSON (1969) proposes a new classification of vertebrates in which *e.g.* Aves form a series of superorder Archosauria. The impracticability of such an arrangement is obvious. MAYR (1965) and BOCK (1968) have listed many convincing arguments against HENNIG's ideas. It may be added here that such systematic schemes as presented by NELSON, disregard the fact that some groups remain conservative and therefore the forms belonging to them are similar notwithstanding that their common ancestor is very remote in time, whereas other forms are simultaneously changed profoundly. Two recent dipnoans, *Protopterus* and *Neoceratodus* are in fact much more similar to each other than two birds *e.g. Passer* and *Grus*, although the lineages leading to the two fish genera were probably separated when the archosaurs had not even begun to diversify.

## THE EVOLUTION OF THE VERTEBRATE BRAIN

The large differences between the structure of the brain in different vertebrate animals are usually discussed in order to find the causes of the evolutionary course which is responsible for the mammalian and especially the human brain, whereas the conservative structure of the brain of many animals was rarely commented upon. Many authors are convinced that a

more complicated brain and a more plastic behaviour are invariably promoted by natural selection. So *e.g.* SIMPSON wrote (1958): "whether a leg is better than a fin depends on where they are, but there is no doubt that an eye is in some valid and more universal sense better than a pigment spot and a central nervous system better than a nerve net". According to RENSCH (1967) "probably all improvements of the brain had a great selection value because they allowed more plastic action due to an increase or an improvement of reflexes, instincts and actions based on experience".

But even the presence of eyes may constitute a handicap rather than an asset. In burrowing species the eyes are easily injured and thus may open the way for infection, while their utility is none. They have accordingly disappeared. A network of complex relationships connects the structure of the brain with the environment and the mode of life of the species. If the elements of a system, say transistors or nervous cells share some possibility of failure then the number of possible mistakes of the whole system depends on the number of elements forming it, unless every complication in the system is provided with a regulating and devation-counteracting device.

A simple brain and a simple rigid behaviour are therefore undoubtedly superior if the species persists for a long period of time in an uniform and stable environment. The ability to individual modification of the behaviour in accord with the changing conditions will be promoted by natural selection only if the conditions of life of the species are really diversified. NISSEN (1958) was undoubtedly right in saying that "the rigidly fixed responses to innately determined stimulus patterns may be highly adaptive in a stable environment".

The links connecting the evolution of the nervous system with the mode of life of the species may form a deviation amplifying or a stabilizing network. The selection will promote complications in the brain structure and encourage versatility of behaviour in animals having high metabolism, high mobility, which can live in many different environments, or which have exploited in the past many different modes of life. A completely different course of evolution of the nervous system is characteristic of species that live in stable, conservative environments, which have a low rate of metabolism and a limited mobility. The Tunicata may be cited as an example of animals having a very primitive nervous system due to a very stable environment.

Among fishes the most primitive brain structure is characteristic of Dipnoi and Latimeria. These animals live in environments of their

distant ancestors. They are poor swimmers and they live near the bottom. Both Elasmobranchii and Actinopterygii abandoned the primitive pattern of brain structure and developed new complications which are linked with the ability of these groups to invade pelagic waters (WAHLERT, 1963, 1968). The different structure of the cerebellum in Elasmobranchii and Actinopterygii agrees with the actual views as to how the pelagic waters were independently invaded by these animals. In Elasmobranchii the deviation amplifying network connecting the evolution of the brain with the swimming ability included the reduction of weight due to the loss of dermal armour, the accumulation of fat in the liver and the change in the body shape. In Actinopterygii the principal elements of the network were the progress in the hydrostatic function of the air bladder, the ability to swim in schools and to spawn in open water, and the evolution of the shape and of the position of fins.

The fish brain never attained the size comparable to that found in some land vertebrates. The following circumstances are probably responsible. Water forms a much more stable and uniform environment than air. In water the sense organs, and especially the eyes, are able to obtain detailed information only from the vicinity. The high conductivity of water prevents the development of homeothermy and therefore tends to limit the metabolism. Even in those forms among sharks and actinopterygians which have the ability to rise the temperature of trunk muscles above that of the surrounding water, the temperature of the brain remains as low as the blood that feeds it and which was cooled in the branchial vessels (CAREY & TEAL, 1969).

It is only in two fish groups that the brain is large, in Gymnarchidae and Mormyridae, which possess the ability to emit electric pulses and to obtain the information about the surroundings by evaluating the reflection of these pulses (LISSMAN, 1958; LISSMAN & MACHIN, 1958). Their brain is modified by the enormous increase in the size of cerebellum. A detailed study of their behaviour and learning ability would be very welcomed.

The amphibian brain is very conservative (STARCK, 1962; SCHOBER, 1966). The lack of pressures for an increase in brain size is probably connected with the following amphibian characters. The naked humid skin prevents the rise of body temperature. The lung ventilation mechanism is inefficient. Land movement of amphibians is usually very slow, except in escape activities when some forms are capable of a rapid dash for cover by a few jumps, or by a short run (SZARSKI, 1964). Amphibia are able to withstand a complete lack of hemoglobin which may be advantageous in some circumstances but is probably incompatible with the activity of

higher nervous centra. The retical filter of stimuli (MATURANA, LETTVIN, MCCULLOCH & PITTS, 1960; HIMSTED, 1969) probably restricts the possibilities for a sophistication in brain performance.

The invasion of land was a major environmental shift, its correspondence with the evolution of the brain is apparent only in reptiles and their descendants. The network connecting the evolution of the brain structure of birds with their biology has already been described. Similar processes have influenced the development of the brain of mammals. Some birds have brains that are proportionally larger than that of some mammals (STRELNIKOV, 1970), but mammalian behaviour is more plastic when compared with the behaviour of birds. The evolution of birds was for a long time centered on the perfection of flying. Early mammals, by contrast, evolved in a more general way, their mode of locomotion being far less specialized. The ability to fly is so important for the safety of birds and so complicated that it cannot be learned individually, but appears as a fully formed, or nearly fully formed hereditary ability. On the other hand young mammals acquire many of their reactions in their early youth by individual experience collected during play. The instinct of play together with the correlated changes in the brain structure formed a closed circuit that tended to diminish the importance of hereditary behaviour patterns in mammals.

It will be the task of future research to determine the different networks of deviation-amplifying relations which have shaped the nervous systems of different orders of reptiles, birds and mammals. BIELICKI (1965, 1969) recently described the factors which were probably forming the network of deviation-amplifying circuits responsible for the evolution of the human brain. I would like to add that owing to the diversity of brain function selfaccelerating circuits may arise by the mutual interdependence of selection for different functions. An increase in the capacity of the memory may act *e.g.* as a factor promoting the selection for a more sophisticated mechanism for the analysis of stimuli, and the improvement in analysis will in turn favour the selection for a more prolonged duration of recollections. Once the memory is improved then natural selection may preserve individuals more prone to behaviour modified by experience, and when the behaviour of the species is appropriately changed the selection for a good memory will be increased. The effects of mutual influences of different deviations in structure of the nervous system and in the behaviour was probably most pronounced in the evolution of the human brain as it is undoubtedly concerned with the most complex and versatile system.

I am at present unable to devise an experimental procedure which would imitate the work of deviation amplifying mechanisms in the course of evolution. I hope that it will be possible to plan such experiments *e.g.* with *Drosophila* cultures. To attain this aim it would be necessary to culture the flies in two different media, between which the animals could move freely, and find such a method of selection which would induce the surviving individuals to select this environment which would reinforce the tendency for the change initiated by the original selection. But even before experimental demonstration of the role of deviation-amplifying circuits for the course of evolution is obtained, the theoretical analysis of the probable course of major evolutionary changes is made easier by the application of this concept as prove the papers of BIELICKI (1966) and of BARTHOLOMEW (1970).

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[1964]

# Organisms and Molecules in Evolution*

GEORGE GAYLORD SIMPSON

(*Editor's Note*) Among zoologists the counterpart of the physical anthropologist is the ecologist or the population biologist rather than the biochemist or molecular biologist. In part this is because even the most "biological" human biologist is not immune to the eventual necessity of considering relevant phenomena not characterized as natural science. It is also because physical anthropology has traditionally been concerned with phenotypic, rather than genetic aspects of man. This organismal and, more recently, populational approach to an understanding of human evolution can now, in most respects, be carried on as "scientifically" as the research of white-clad investigators working in laboratories. But is there any point at which the organismal and molecular approaches meet and complement each other? This is the concern of Simpson's article, and it's a very important one because mistaking points of emphasis for different and opposed basic viewpoints about nature can lead to artificial distrusts and misunderstandings which may impede scientific investigation. Physical anthropologists might pay particular attention because the traditional emphasis on gross anatomy, biometry, and descriptive morphology has led them up many a blind alley. On the other hand, the realization of this is no cause to abandon all the original questions, or to assume they will be answered in one fell swoop by jumping on the biomolecular bandwagon. Simpson suggests (and his own work is proof that it can be done) that it is possible to make the best of both the Cartesian and compositionist worlds by working within the framework of the synthetic theory of evolution, which requires the coordination of both kinds of approaches.

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It is universally recognized that molecules of biological importance may evolve—that is, they may change in the course of time as have the organisms in which they occur. Some molecules, like adenosine triphosphate, are so nearly universal and invariable as to suggest no evolutionary sequence, but many others surely have evolved, notably groups of proteins and, obviously, DNA. Before the importance of DNA was known, Florkin (*1*) had already discussed the systematics and evolution of various families of molecules. In such instances evolutionary interpretation of the biochemists' findings requires information from paleontologists and systematists, information especially on the time scale involved and the phylogeny and relationships of the species in which varying molecules are to be compared. An example is the hypothesis that serum proteins (*2*) or cytochromes (*3*) have changed in a regular if not linear manner with respect to time—that they have evolved by some sort of internal constant-rate mutational process and not in an irregular or a specifically adaptive way. In fact, when the data are replotted with what seem to be the most probable time coordinates they indicate that the hypothesis is incorrect or, at least, that these data do not support it. Williams now tells me that the hypothesis has been modified, but it exemplifies the clarifying confrontation of molecular and organismal data.

Other interesting examples of such confrontation arise from further studies of serum proteins, such as that by Goodman (*4*). Phylogenetic relationships of the animals concerned, primates in this case, are inferred from the apparent degrees of homology in their various serum proteins. The lineages thus inferred then permit conclusions as to the evolution of the proteins themselves. Similar inferential methods have been applied to the evolution of hemoglobins, also in primates, by Hill and the Buettner-Janusches (*5*). When phylogeny is inferred from the molecular data and molecular evolution is inferred in turn from that phylogeny, there is an element of circularity, which does not wholly invalidate the method but does warrant some reservations. A necessary cross-check is to arrange the molecular data in the framework of a phylogeny based entirely on nonmolecular evidence. It should be mentioned in passing that this, too, has sometimes led to semi-circular reasoning when molecule-based phylogeny has been compared with phylogeny with other bases: agreement between the two has been taken as the requisite validation of the molecular approach to phylogeny, but nonagreement has been taken as evidence of the greater reliability of the molecular method.

However, the most important reason for relating organismal and molecular evolution to each other is not simply the testing of hypotheses or the validation of methods. It is the balancing of points of view and the achievement of more complete explanations. Wald (*6*) has said that "living organisms are the greatly magnified expressions of the molecules that compose them." Anfinsen (*7*) believes that "we may almost define the life sciences as those concerned with the elucidation of the mechanisms by which molecules exert their specific actions in living cells." In fact there are many respectable and even eminent students of the life sciences who have no concern whatever with molecules or their actions. Concentration on one level of organization to the practical exclusion of others is often a necessity of specialized research, but nowadays almost everyone agrees that eventual understanding of relationships between levels is also necessary. Sonneborn (*8*) has emphasized the fact that molecular genetics could only have arisen through, and would now have little meaning apart from, "classical" or Mendelian organismal genetics. Weiss (*9*) has pointed out that there is a "cellular control of molecular activities" as well as a molecular control of cellular activities. There is also an organismal control of cellular activities, and, for that matter, a populational control of organismal activities. Indeed both Wald and Anfinsen, in the works from which one-sided aphorisms have been quoted, were concerned with relationships of molecules to higher organizational levels in evolution.

The sort of problem that can arise from a limited approach is exemplified in a recent article by Mora (*10*). He points out that living organisms have a teleological or purposive aspect which he proposes to label "urge." He finds that this aspect is inexplicable at the molecular level as hitherto studied. He proposes, but does not describe, a new approach, to be frankly permeated by teleology. Although he seems to think or hope that this may still be naturalistic, he does not clearly state what a naturalistic teleology might be. Now, this is precisely the problem with which organismal biologists have been coping for generations. Unknown, it would seem, to some biochemists, they have achieved a naturalistic (or, in a sense, materialistic) explanation of what is now often called [after Pittendrigh (*11*)] the teleonomic aspect of organisms. The teleonomic, or *apparently* teleological or purposive, characteristics of organisms are adaptations. They include "urge" itself in Mora's sense, its manifestations, and its results in the activities of individuals and the evolution of populations. Teleonomic adaptations arise in the course of evolution, and the factor governing their origin and maintenance



is natural selection. That is surely as true at the molecular level as at any other. However, the ramifications of natural selection at various levels are far from simple.

## Natural Selection

The process of natural selection, as now understood, is complex rather in its concrete working and its interactions than in its basis. That basis is simply differential reproduction correlated with genotypic constitution. If some individuals in a population have more surviving and breeding offspring than others, and if there is a consistent average difference, however small, in the genotypes of those who have more and those who have fewer, that is natural selection at work. The actual selection—that is, the determination of which individuals have more or fewer offspring that survive to breed in their turn—is an interaction between environment, in the broadest sense, and the population, in all its individuals throughout their complete ontogenies. Aspects of this process are discussed at length in recent works (see, for example, *12–14*) which supply many details not given here.

Natural selection requires, first, reproduction and, second, hereditary variation of such a kind as to influence the success of reproduction under existing circumstances. When those factors are present, natural selection *necessarily* occurs. In precellular evolution [a principal concern for Mora (*10*)] it necessarily began when there were replicating molecules that differed in the rate or efficiency of replication (see *15*). However, the pertinent unit is not the replicating molecule but the reproducing system. This was presumably a molecule at first but became a cell at the protistan level, and is a dynamic unicellular-to-multicellular ontogenetic individual at metaphytic and metazoan levels. Selection acts on the whole phenotype and can single out genes only to the extent that they have phenotypic effects separable both phenotypically and genetically from those of other genes. Although selection apparently does act in an analytically separable way on some particular molecules, it evidently does not do so as a rule. It usually acts on supramolecular phenotypic characters, on whole complexes of them, or indeed on all of them at once. Since most genes are pleiotropic and most characters are polygenic, it follows that selection usually is not concentrated on single genes, as might appear from the necessarily oversimplified models first formulated by population geneticists. Although the connection is not yet well understood, this presumably means also that it is unusual

(it may even be impossible) for intermediary molecules such as enzymes and other proteins to be selected for or selected against independently of other molecules.

### Effect of Selection on Particular Features

In considering the effect of selection on particular features of an organism, it is important to judge how far these are in one direction from the genes and in the other direction from the phenotypic characters directly subject to selection. Behavior is subject to particularly strong selection, and it is probably farthest removed from the genes and also most elaborately polygenic as a rule. Some single-gene determinants of behavior are known, but they are exceptional (see *16*). Proteins or, at least, intracellular enzymes are believed to be almost directly and uniquely determined by one or a few particular genes. The effect of selection will surely be influenced by the length of the functional chain from the genes to the character selected for or against. As a rule, with exceptions, the effect becomes more, not less, diffuse and less, not more, direct as the level of the gene is approached.

Zuckerkandl (*17*) has argued that a molecule like hemoglobin is preferable to most "structural," or more remotely phenotypic, characters for the determination of affinities because it is so near the genes, so nearly a direct reflection of part of the DNA code. It may be added that hemoglobin is so literally vital that natural selection may here act at a level near the gene. Those are advantages in certain respects, but they are accompanied by disadvantages, and the more distantly phenotypic approach also has advantages, as Zuckerkandl notes but possibly understresses. Zuckerkandl has shown that, "from the point of view of hemoglobin structure, it appears that gorilla is just an abnormal human, or man an abnormal gorilla, and the two species form actually one continuous population." From any point of view other than that properly specified, that is of course nonsense. What the comparison seems really to indicate is that in this case, at least, hemoglobin is a bad choice and has nothing to tell us about affinities, or indeed tells us a lie. (It does show that men and gorillas are rather closely related, but that has long and more accurately been known from traditional morphological comparisons.) Of course, as Zuckerkandl points out, we should use not just one kind of molecule but many, preferably proteins. However, if one can be misleading, so can many! (Let me add that Zuckerkandl's discussion of the phylogenetic interpretation of molecular data is invaluable and, unfortunately, almost unique.)



In some respects it is a drawback that hemoglobin, various enzymes, and some other proteins are so near to the genes in the functional chain. It means that each sample is genetically determined by, and therefore provides a sample of, only an extremely minute part of the whole genetic system—apparently only two genes in the case of hemoglobin and probably only one for many enzymes. The farther a character is from the genes, the more likely it is to sample a number of genes or a really significant part of the whole genetic system. The complexity of the genetic determination of a characteristic is a positive advantage, not a disadvantage, when the purpose is to determine affinities of whole organisms. Moreover, such characters are in almost all cases those which were in fact subject to selection. On an average, the farther we are from genes the nearer we are to the action of selection, and thus the better able we are to interpret the adaptive processes involved.

When, as is usual, selection is on the phenotype and well removed from the genotype, all that matters is that the genotype should in fact result in the selectively favored phenotype under the existing conditions of development. In this sense, or beyond that point, it really can be said that the genotype does not matter in adaptive evolution. There is ample evidence (much of it summed up in *14*, with references) that genotype-phenotype determination is not unique in either direction. Phenotypes that are apparently identical and that seem to be equal in the face of selection can have markedly different genotypes. There are also many systems—genetic, ontogenetic, and selectional—that tend to channel phenotypic development in the face of considerable change or variation in genes and hence, presumably, also in many families of macromolecules (*18*). I am arguing not that any one kind of evidence on evolution—genetic, molecular, phenotypic, or other—is superior but, on the contrary, that no one kind suffices in itself.

### Special Problems

The evolutionary study of molecules has raised a number of special problems, not always seen in the same way by molecular and organismal biologists. The phenomenon that has caused most trouble in attempts to determine evolutionary affinities is convergence: the development of similar characteristics by organisms of different ancestry. Any addition of evidence would be most welcome, especially

if it involved characters unlikely to converge. Here the molecular biologists do not agree; Wald (*6*), for example, says that convergence is much more likely at the molecular level, while Zuckerkandl (*17*) independently maintains that it is less likely. To me, as an organismal biologist, it seems that Wald is probably right. Convergence to the point of identity or of seriously confusing similarity would appear to be more likely in a single kind of molecule, even one as complicated as a protein, than in such phenotypic characters as are end results of the interactions of a very large number of such molecules. Anfinsen (*7*) cited an example (from the work of Sanger *et al.*) indicating from insulin composition that sperm whales are identical with pigs and quite different from sei whales! (*19*). To be sure, a sequence of only three amino acids is involved, and both differences and resemblances could be incidental without even true convergence, but the lesson is there. Fortunately, the fact that protein and morphological convergence may be independent of each other gives a double check if the evidence of both is available.

Another problem, discussed at some length by Anfinsen (*7*), arises from the evidence that proteins have parts that can vary greatly or even be removed altogether without seeming to affect function. There is also the concept of "dormant genes" [discussed by Zuckerkandl (*17*), among others, and in studies which he cites; see also Zuckerkandl and Pauling (*20*)]. This concept is, again, related to the hypothesis of regular, secular change in molecules, mentioned in the opening paragraph of this article. Essentially the same question has long been discussed by evolutionary biologists, in this form: Can a gene (or allele) be neutral with respect to selection? (Much of the discussion is summarized, with citations, in *14*.) It is impossible to establish complete absence of exceptions, but so far every supposedly neutral gene that has been adequately investigated has turned out not to be neutral. There is a strong consensus that completely neutral genes or alleles must be very rare if they exist at all. To an evolutionary biologist it therefore seems highly improbable that proteins, supposedly fully determined by genes, should have nonfunctional parts, that dormant genes should exist over periods of generations, or that molecules should change in a regular but nonadaptive way.

This unsettled question could have far-reaching significance, for instance through the hypothesis [suggested but not fully supported by Anfinsen (*7*)] that the invariable or fully homologous parts of proteins in different animals are the functional, or at least the most significantly functional, parts. It would then seem to follow that the



actual specific differences in proteins may be little or not at all adaptive, and this again seems unlikely to an organismal biologist. However, Anfinsen also points out (and the examples could be largely multiplied from other sources) that, for instance, serum proteins with no immunochemical similarity at all may be fully and identically functional. It is certainly not true as a generalization that molecular differences among species are commonly nonfunctional or nonadaptive, and indeed I think no molecular biologist would go to that extreme.

It is undoubtedly on questions related to adaptation that an evolutionary synthesis of molecular and organismal viewpoints and data will be most useful. I shall here give briefly two further examples from work by Wald (*6*, and earlier papers cited therein), not because I happen to disagree with his interpretations but because his brilliant studies provide such ideal data on the molecular basis of organismal adaptation. He shows that freshwater vertebrates generally have retinal pigments containing vitamin $A_2$, while marine and land vertebrates generally have $A_1$. He interprets this as a phylogenetic phenomenon, with $A_2$ in ancestral (true) fishes, supposedly freshwater forms, and $A_1$ developed in progressive phylogeny by marine and land descendants. He finds it inexplicable and almost an unnecessary complication that, for instance, reptiles, primitively having $A_1$, "revert" to $A_2$ when they adapt to fresh water. To an organismal biologist, the picture, including the apparent anomalies and supposed reversions, suggests interpretation in terms of adaptation, primarily, and phylogeny only secondarily. Many, but perhaps not quite all, of the observations would be explained if we assumed that $A_2$ is adaptive in freshwater forms and $A_1$, in land and saltwater forms—so much so that selection usually produced these adaptations rapidly and tended to erase purely phylogenetic effects. I have no idea what the difference in adaptation might be, but suggest that study from this point of view might clarify the molecular function involved.

A second example from Wald is his demonstration that tadpoles resemble fishes in a number of biochemical characteristics, whereas adult frogs have a biochemistry more like other land vertebrates. Amphibians were of course derived from fishes, and Wald interprets these changes as "the most striking instances we know of recapitulation." In my opinion there is no reason to invoke recapitulation and definite reason not to. As regards the species in question, it would appear that tadpoles are adapted to live in the water and adult frogs to live on land. In spite of some complications, this is the plausible

explanation for nitrogen excretion: ammonia in water, urea out of it. Other changes may be less clearly adaptive but are likely, at least, to be adaptive. Some of the evidence, also given in part by Wald, is that when amphibians go from land to water, as some do, the changes tend to go in the opposite direction; they antirecapitulate!

### The Adaptive System

Finally, let us turn (or return) to the structure of the whole adaptive system, its causations, and the place of molecules in it. The most basic of all molecules, in this context at least, is DNA. Its influence is exerted, in part if not altogether, through RNA. Recognizing the RNA as an agent of DNA in this sequence, we conclude that RNA is not the cause of the eventual action: synthesis of a protein. (One could raise some delicate semantic problems here, but I think the statement can stand as written for present purposes.) Then is the DNA the causative agent in a really explanatory sense? It carries, as we say, a message (another semantic problem!) and is indeed a messenger and an agent just as much as messenger RNA is. In following the chain back we reach a really significant point of causation not when we locate the message, which is in the DNA, but when we learn where the message came from to begin with, what composed it. Any message composed, so to speak, by the DNA itself would be in the language of mutation. But mutations are predominantly inadaptive, and the message, beyond doubt, is almost entirely adaptive. Mutations form what may be called letters or words, to continue the now somewhat shopworn metaphor, and in that way they supply materials that permit something new to be said and that limit what can be said. However, they certainly do not compose the message in any meaningful sense.

The message, or at very least the greater part of it, relates to interaction of organism and environment. The interaction involves the whole organism, and hence arises and expands from the molecular level. There must be some sort of feedback from the organism-environment interaction into DNA, and hence into the other molecules. There are, as is well known, innumerable feedback mechanisms at the molecular level itself, and many or most of these are responsive to interactions with the environment. The Neo-Lamarckians, before much was known about feedback or anything at all was known about molecular genetics, supposed that evolutionary feedback was of the same kind, within individuals and into the genetic system, whatever that might prove to be. Now, however, we do know about DNA and



other essentials of the genetic system, and we know beyond serious doubt, even though it seems rather odd, that DNA is not subject to feedback within individuals. That is, as Pontecorvo (*21*) has put it, "the *structure* of the genetic material is not subject to regulatory change . . . although the *expression* of the genetic material . . . is subject to regulation—qualitative and quantitative—at all levels of organization. . . ."

Changes in individual expression—to put it figuratively, the way the message is read—do not affect the message itself. The necessary message-constructing feedback is not here but in a system of higher order: in the population and not the individual. It operates through natural selection, which operates in populations, just as populations are what really evolve. Thus, through a different approach we come again to natural selection and now see it as the most truly causative (although not the only) element in the adaptive system. Viewed in this way, it is the composer of the genetic message, and DNA, RNA, enzymes, and the other molecules in the system are successively its messengers.

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# The Role of Systematics in Biology

The study of all aspects of the diversity of life is one of the most important concerns in biology.


Ernst Mayr


There are many ways of dealing with the topic that was assigned to me. One might give a history of the role which taxonomy has played in the development of biology; or one might concentrate on the present status of systematics in biology; or finally one might attempt, in a timeless and somewhat philosophical way, to delineate the niche which systematics occupies within the total conceptual framework of biology. Further thought makes it evident that the three approaches are interdependent to such a degree that one has to give due consideration to all three of them.

Let me start with the question, what do we mean by "systematics," the role of which I am to describe? To be able to answer this question meaningfully requires an excursion into the history as well as philosophy of biology. The ancient Greeks saw a natural order in the world which, they thought, could be demonstrated and classified by certain logical procedures. They tried to discover the true nature of things (their essences) and approached classification with the methods of logic. Indeed, Aristotle, the first great classifier, was also the father of logic. The underlying philosophy, now usually referred to as essentialism (from essence), dominated the thinking of taxonomists up to and including the time of Linnaeus. Taxonomic nomenclature and the so-called typological thinking of taxonomists right up to our day have been permanently affected by the Aristotelian heritage (*1*).


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## History of Taxonomy

During the early history of biology this was no great handicap. Botany and zoology, to state it in a highly oversimplified manner, arose from the 16th century on as applied sciences, attached to medicine. Botany started as a broadened study of medicinal herbs and early botanical gardens were herb gardens. With but one or two exceptions all the great botanists and herbalists from the 16th to the 18th century (Linnaeus included) were professors of medicine or practicing physicians. Zoology arose in connection with human anatomy and physiology. When botany and zoology became independent sciences, the first concern of the two fields was to bring order into the diversity of nature. Taxonomy was therefore their dominant concern, and indeed in the 18th and early 19th century botany and zoology were virtually coextensive with taxonomy. Moreover, by sheer necessity, taxonomy was essentially the technique of identification.

The middle third of the 19th century was a period of decisive change to which many separate streams of development contributed. Increasing professionalism was one, and increasing specialization was another, to mention just two. Taxonomy itself helped in accelerating the change by introducing several new concepts into biology. The greatest unifying theory in biology, the theory of evolution, was largely a contribution made by the students of diversity, as we might call the taxonomists. It is no coincidence that Darwin wrote his *Origin of Species* after encountering taxonomic problems dur-

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- Are sibling sp. genetically more similar to each other than either to a 3rd morphologically distinct sp. in genus? - Mayr - Dobzhansky hold sibling sp. although high premium on phenotype morphologically distinct (due to genetic homeostasis). are just as genetically diverged as good morph. sp.
- Is it necessary for major reorganization of genome - for speciation to sp. level

Genus Drosophila - Subgenus (Drosoph).
species group - (repleta)
sib. A ——
sib B ——
- relative in sp. group ——

- 3 subgenera w/ 6 sp. groups used in 3 way test for sib - relationships. - 27 sp.
- Electroph. tech. & enzyme & protein assay described N. (63) H & C. (66) - compared elect. mobilities of 6 enzymes + larval hemolymph proteins
- esterase = 5 bands. hemolymph 9 to 19 dif. proteins compared
- Results sibling sp. share about 40% protein similarity only w/ each other - only 8% only w/ near relative all 3 about 9% - - About 50% overall similarity between sibs.
- Assume each site of enz. activity or protein staining on gel (elect. mobil.) - results from single loci -

Library

- my ms.

Zeiner, 7. E. Pleistocene ?

- may not be - 2 or more structural genes may contribute polypeptide subunits to a migrating entity.

Sarcophaga

- Coverage sib. pr. 49.7% of loci identical
- non sib. 17.5%

rate of genetic differentiation varies w/ proteins

- study essentially typological & species polymorphism not understood

- D. pseudoobscura a polymorphic sp. 11 of 18 loci monomorphic 7 polymorphic - some highly so.

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# Non-Darwinian Evolution

## Most evolutionary change in proteins may be due to neutral mutations and genetic drift.

Jack Lester King and Thomas H. Jukes

Darwinism is so well established that it is difficult to think of evolution except in terms of selection for desirable characteristics and advantageous genes. New technical developments and new knowledge, such as the sequential analysis of proteins and the deciphering of the genetic code, have made a much closer examination of evolutionary processes possible, and therefore necessary. Patterns of evolutionary change that have been observed at the phenotypic level do not necessarily apply at the genotypic and molecular levels. We need new rules in order to understand the patterns and dynamics of molecular evolution.

Evolutionary change at the morphological, functional, and behavioral levels results from the process of natural selection, operating through adaptive changes in DNA. It does not necessarily follow that all, or most, evolutionary change in DNA is due to the action of Darwinian natural selection. There appears to be considerable latitude at the molecular level for random genetic changes that have no effect upon the fitness of the organism. Selectively neutral mutations, if they occur, become passively fixed as evolutionary changes through the action of random genetic drift.

The idea of selectively neutral change at the molecular level has not been readily accepted by many classical evolutionists, perhaps because of the pervasiveness of Darwinian thought. Change in DNA and protein, when it is thought of at all, is thought to be limited to a response to activities at a higher level. For example, Simpson (*1*) quotes Weiss (*2*) as stating that there is a cellular control of molecular activities, and Simpson adds that there is also an organismal control of cellular activities and a populational control of organismal activities, and concludes (*1*):

> The consensus is that completely neutral genes or alleles must be very rare if they exist at all. To an evolutionary biologist, it therefore seems highly improbable that proteins, supposedly fully determined by genes, should have nonfunctional parts, that dormant genes should exist over periods of generations, or that molecules should change in a regular but nonadaptive way . . . [natural selection] is the composer of the genetic message, and DNA, RNA, enzymes, and other molecules in the system are successively its messengers.

We cannot agree with Simpson that DNA is a passive carrier of the evolutionary message. Evolutionary change is not imposed upon DNA from without; it arises from within. Natural selection is the editor, rather than the composer, of the genetic message. One thing the editor does *not* do is to remove changes which it is unable to perceive.

The view that mutations cannot be selectively neutral is not confined to organismal evolutionists. Smith (*3*) states:

> One of the objectives of protein chemistry is to have a full and comprehensive understanding of all the possible roles that the 20 amino acids can play in function and conformation. Each of these amino acids must have a unique survival value in the phenotype of the organism—the phenotype being manifested in the structures of the proteins. This is as true for a single protein as for the whole organism.

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Carl B. Schreck

1969. Sci. 165(3899):

# Differentiation of Populations

## Gene flow seems to be less important in speciation than the neo-Darwinians thought.

Paul R. Ehrlich and Peter H. Raven

Most contemporary biologists think of species as evolutionary units held together by gene flow. For instance Mayr (*1*) writes "The nonarbitrariness of the biological species is the result of . . . internal cohesion of the gene pool." Merrell (*2*) states "The species is a natural biological unit tied together by bonds of mating and sharing a common gene pool." This idea is founded in the pioneering work of Dobzhansky, Mayr, Stebbins, and others integrating the theory of population genetics with laboratory and field experiments and observations to produce the neo-Darwinian or synthetic theory of evolution. These workers quite logically concluded that differentiation of populations would be prevented by gene flow, and they focused their discussions of speciation on various means of interrupting that flow. In other words, they emphasized the role of mechanisms isolating populations from one another. Until quite recently there has been little reason to question this view. In the past few years, however, growing evidence from field experiments has led us to reevaluate the processes leading to organic diversity, and to conclude that a revision of this section of evolutionary theory is in order.

In this paper we suggest that many, if not most, species are not evolutionary units, except in the sense that they (like genera, families, and so forth) are products of evolution. We will argue that selection is both the primary cohesive and disruptive force in evolution, and that the selective regime itself determines what influence gene flow (or isolation) will have. Threefold evidence is presented for this. We will show that (i) gene flow in nature is much more restricted than commonly thought; (ii) populations that have been completely isolated for long periods often show little differentiation; and (iii) populations freely exchanging genes but under different selective regimes may show marked differentiation.

We finally reiterate the point (*3*) that a vast diversity of evolutionary situations is subsumed under the rubric "speciation," and that this diversity tends to be concealed by an extension of a taxonomic approach from the products of evolution to the processes leading to the differentiation of populations. *Euphydryas editha* and *Festuca rubra* are both species to the taxonomist, but knowing this does not tell us if they are evolutionary units or how they evolved. Nor does it permit us to guess how similar are their evolutionary pasts, in what way they are similar today, or to predict anything about their evolutionary futures.

### Gene Flow in Nature

To what extent do populations considered to be conspecific ordinarily share a common gene pool? Mayr (*4*) estimated that "genetic exchange per generation . . . due to normal gene flow is at least as high as $10^{-3}$ to $10^{-2}$ for open populations that are normal components of species." He considered that gene flow was the principal source of genetic variation in natural populations, and we would agree that the introduction of genetic novelties into natural populations, even at a low level, may be important in supplying raw material for selection (*5*). The problem of testing Mayr's estimates and the conclusions to be drawn from them is complex. First, we must ascertain how much gene flow ordinarily occurs in nature. Second, we must determine the amount of gene flow at which significant sharing occurs. That is, we must find the amount at which subpopulations of a species affect the evolution of other subpopulations. Both questions are difficult to answer, but at least a general picture of patterns of gene flow in nature has started to emerge recently.

### Movement and Gene Flow in Animals

For many animals there is information on the movement of individuals. For instance, butterflies (except those few species which are migratory) seem to be quite sedentary as compared with what one might expect in view of their powers of movement (*6*). Birds also often seem to show less movement than they are capable of—the young of migratory species often nest near the parental nest site (*7*). There also is some evidence that birds may be stopped by "psychological barriers" (*8*). Similar restriction of movement not associated with insurmountable physical barriers has been observed in many nonaerial organisms, such as the rusty lizard (*9*). Twitty's (*10*) studies demonstrate that California newts show great perseverance and navigating ability in returning precisely to a particular stretch of stream to breed. Individuals displaced several miles in mountainous country have successfully returned to their "home pool." And, of course, the great accuracy with which salmon return to their birthplace to breed is well documented (*11*).

On the other hand, there also is abundant evidence in the literature that individuals may travel very long distances, such as in Bishopp and Laake's (*12*) release-recapture experiments with flies in which individuals were recovered as far as 17 miles (27 km) from the point of release. Small wind-dispersed terrestrial organisms may travel tremendous distances, as may some mammals (*13*). It is also clear (*14*) that extremely careful work covering the entire life history under a variety of weather conditions is necessary before reasonably definitive statements on amounts of individual movement may be made.

Of course, movement of individuals does not necessarily indicate gene flow. Anderson (*15*) has shown that the pres-

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[1969]
J-Stor

# Molecular Taxonomy and Typology

**David I. Rasmussen**

The development of techniques for the separation and identification of molecular components of various body fluids, tissue homogenates, and extracts has placed numerous new phenotypes at the disposal of biologists interested in judgment of affinities between groups of organisms. A voluminous amount of this literature on molecular taxonomy, while paying homage to the molecular revolution of biology, also bears homage to the typology of Plato's *eidos*. If it appears that taxonomic typology has been replaced by the concept of a taxon as a representation of a Mendelian population of variable individuals, a review of the literature related to molecular characterizations of taxa will dispel this illusion.

The potential importance of molecular studies in the understanding of evolutionary processes and in assessing taxonomic affinities is generally obvious. The primary importance of molecular studies appears to be the increase in the number and kinds of measurable phenotypes useful in assessing affinities. Accepting the potential importance of molecular studies, the following criticism regards the use of molecular attributes of individual organisms in evaluating populations. The debate regarding phenetic or phylogenetic inferences of measured differences will not be considered.

## Premises

This criticism is based on three premises, apparently necessary for the evaluation of likenesses and differences between sexual populations. First, taxa consist of populations of heterogeneous individuals. Biological individuality is real; the taxa are generalizations. These generalizations assist biologists in handling the immensity of individual variation which occurs within populations. Second, taxonomic evaluations (whether phenetic or phylogenetic) are based on characters which reflect the action of genotypes present in the population. Certain characters relate to few genotypes, others represent larger samples of the population's genome. Third, the importance of specific phenotypes for the characterization of taxa cannot be judged a priori. An assessment of the extent of individual variation and the patterns of geographical variation must precede taxonomic evaluations of populations.

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## The Problem

Citation of specific authors indicating the extent of the existing typology will not be made in order to avoid the suggestion that the problem results from only a few authors.

Publications regarding biochemical and molecular differences and affinities of taxa often neglect to present data indicating the number of individual organisms comprising the sample and the location from which the sample was collected. Not only are individual organisms often used to characterize a species, but molecular samples from single species are used to characterize higher taxa.

It is not uncommon for authors to omit a detailed description of their methodology used in discerning molecular differences. This oversight often makes it impossible for another investigator to compare data. For example with starch gel electrophoresis, the variables of gel density, buffer molarity, buffer pH, and migration distance affect the separation and resolution of proteins. Such a multivariable system leads to different protein patterns for the same serum samples as variables are altered. Authors utilizing antisera for the measurement of immunological affinities often neglect to state the number of individuals used as the source of antigens for immunization or absorption procedures. The antigenic uniqueness of individuals is especially well documented in vertebrates as is the heterogeneity of the antigen population within an individual. These aspects of antigenic heterogeneity deserve greater respect and clarity when evaluating immunological relationships between populations of individuals. Comparison and repetition of data require specific methodology statements.

Some general statements regarding a recent collection of papers dealing with molecular taxonomy will indicate the reality of this typological philosophy in diverse groups of organisms (Leone, 1964). Several authors in this volume, including two immunologists who began their investigations of molecular relationships in the 1930's, before the so-called biochemical revolution of biology, carefully tabulate the number of individual organisms comprising their samples. They also characterize populations in terms of the frequency of molecular phenotypes and indicate an obvious respect for the reality of individual differences in their discussions. However, within this same volume more than half the authors presenting data directly comparing various populations of organisms neglect to mention the sample size and location of the sample. Over half the authors utilizing some form of immunological precipitin reaction fail to mention the number or sample location of organisms used as the antigen source for direct precipitation or for immunization and absorption procedures. Several authors clarify the organism investigated by such terms as dog, pig, shark, frog, or fish. Which of the approximately 15,000 species of fishes does the designation "teleost" represent? And even if given the specific name, does this fish represent "the teleosts in general"? Similar lack of respect for the uniqueness of individual

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Tryggve Gustafson
Mark I. Toneby

# How Genes Control Morphogenesis

*The role of serotonin and acetylcholine in morphogenesis*

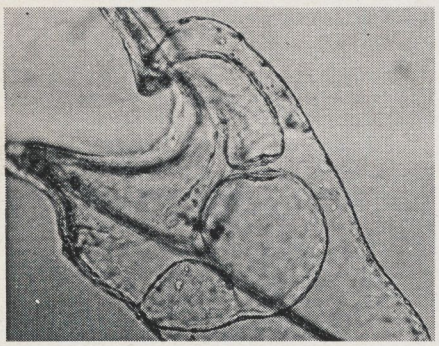
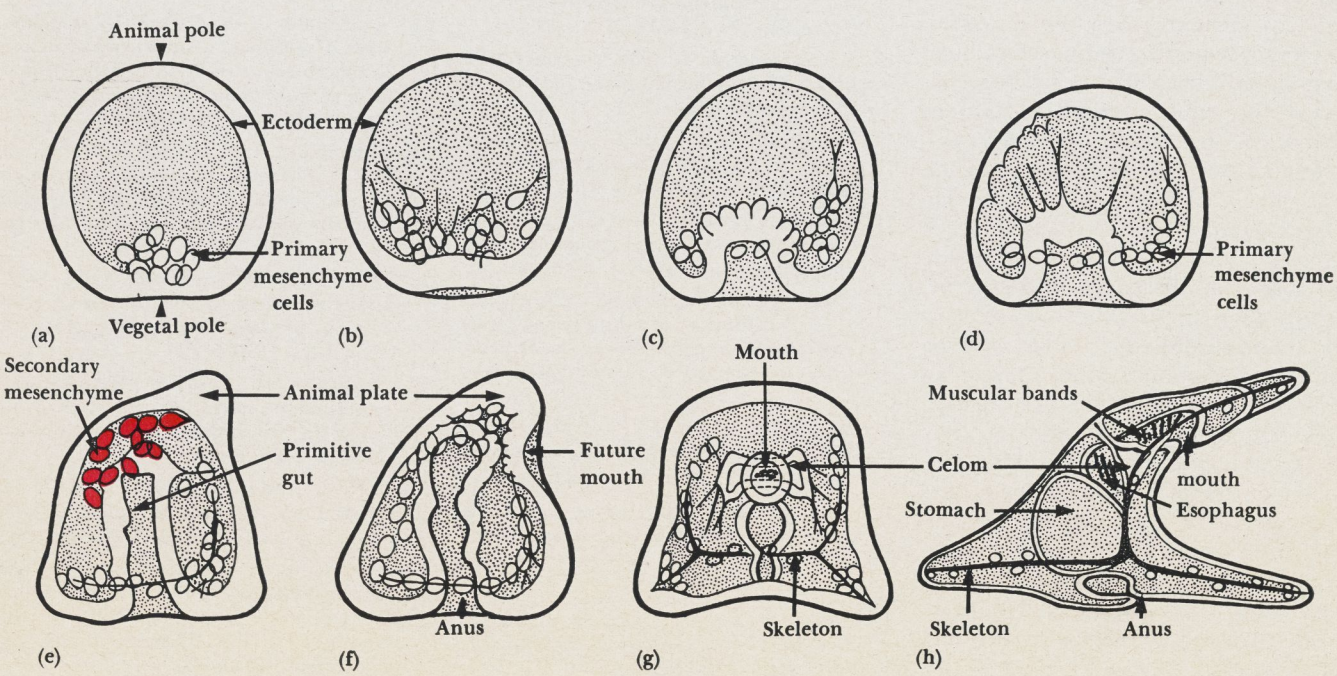


Figure 1. Some stages in early development of the sea urchin larva (*Psammechinus miliaris*). *a* and *b:* the primary mesenchyme cells are released from the blastula wall at the lower (vegetal) pole and enter the blastula cavity. The impocketing of the lower part of the wall (the formation of the rudiment of the primitive gut) occurs in two steps; the end of the first step is illustrated in *c* and the beginning of the second step in *d*. *g* and *h* represent an early pluteus stage seen from the mouth side and in lateral view, whereas the photograph above is of a somewhat more advanced stage.

During the past decade the molecular biologists have been successful in revealing the nature of the genes and the way in which genes direct the formation of messenger-RNA and thereby protein synthesis. We have also begun to learn how inactive cell nuclei and even individual chromosome regions can be switched on to produce messenger-RNA, and we know that the manufacture of different types of messengers and proteins varies as development of an embryo proceeds. We also realize that molecular species in one group of cells may interact with those in adjacent regions, e.g. during embryonic induction. Further work along these lines may gradually reveal the mechanisms of biochemical differentiation.

That molecular events in the long run have morphogenetic consequences is well known. It is quite superfluous to point out that gene mutations and disturbances of the biosynthetic processes in the embryo may result in abnormalities in shape. However, whereas much is known about causes and consequences at the molecular level, and in spite of an enormous accumulation of chemical and morphological data on embryos of various kinds, our understanding of how genes control morphogenesis is still far from complete.

One reason for this situation may be that molecular biologists and morphologists speak different languages. While the former speak about messenger-RNA, transhydrogenases, cyclical AMP, and conformational changes of protein molecules, the latter deal with ectoderms, hypoblasts, celoms, neural crests, and kidney tubules. The situation is hardly improved if the molecular biologist



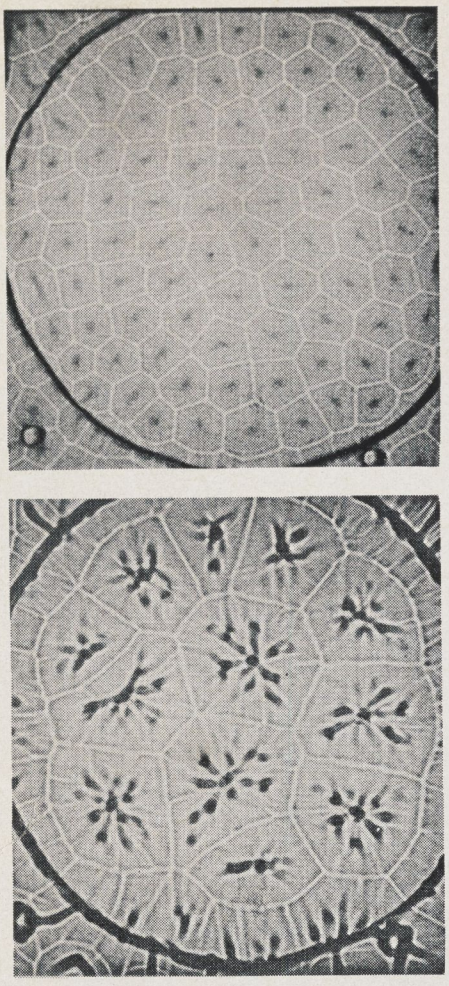


Figure 10. The emergence of biharmonic flow in hexagonal convection. Rayleigh number for the top is 5,000; that of the bottom is 30,000. Depth of the fluid in the larger Rayleigh number experiment is twice that of the smaller.

complicated flows. The years ahead offer excitement as the physics of these higher transitions is uncovered. Particularly intriguing are the powerful new upper bounding techniques, which have already been shown by Busse to possess the same qualitative features of discrete transitions observed experimentally. The true potential of these new techniques has yet to be fully explored.

We note that turbulent flow can be described as non-unique, non-steady, and non-reproducible, and already cellular convection has been found to be both non-unique and non-steady. It is reasonable to expect that the further transitions will contain yet more features of turbulent flows. We close by noting the irony of the fact that this fruitful work was originally stimulated by the beautiful surface tension-driven experiments of Bénard—which involved a different phenomenon!

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jects—objects that are the least likely to be involved accidentally. But physically we know the least about these peculiar objects, and they are the ones for which there is the greatest a priori chance that new and unknown physical mechanisms are at work.

In the end, however, we must all agree that the ultimate criterion for science is experiment and observation. If the observational paradoxes discussed in this article can be demonstrated to be false or accidental, then we can say that the paradoxes are solved on the basis of our present knowledge. If the observations stand, then we must conclude that something new of vast importance is happening and we should get on with the exciting job of finding out more about it.

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41. I thank O. C. Wilson for helpful advice, comments, and encouragement in the preparation of this manuscript.


+ Origins of Taxonomy

# Heterochromatin, Satellite DNA, and Cell Function

Structural DNA of eucaryotes may support and protect genes and aid in speciation.


Jorge J. Yunis and Walid G. Yasmineh


The term heterochromatin was first introduced by Heitz (*1, 2*) to denote chromosomes or chromosome regions that are condensed in interphase and prophase and do not unravel in telophase like the rest of the chromosomes. Although Heitz made his initial observation in primitive plants (liverworts and mosses), his definition of heterochromatin generally holds true for most organisms. In mammals two main types of heterochromatin are recognized: constitutive heterochromatin, or the heterochromatin that is present in homologous chromosomes, and facultative heterochromatin, or the heterochromatin that results from the inactivation of one of the two X chromosomes in females. This inactivation is an effective mechanism to reduce the number of functional X chromosomes to one in both sexes (*3*).

Recent reports indicate that the DNA of constitutive heterochromatin is composed to a large extent of short repeated polynucleotide sequences, termed satellite DNA. This discovery has necessitated a critical review of current ideas concerning the origin and function of this portion of the genome of higher organisms (*4–12*). A careful appraisal of the information that has accumulated about heterochromatin since the time of Heitz (*1, 2*) and on satellite DNA during the last decade suggests that these entities have vital structural functions: they maintain nuclear organization, protect vital regions of the genome, serve as an early pairing mechanism in meiosis, and aid in speciation.


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## Satellite DNA

Satellite DNA was first detected in the early 1960's by the technique of density gradient centrifugation. When DNA of the mouse, guinea pig, calf, and crab was centrifuged in neutral CsCl, a minor component or components differed in buoyant density from the bulk of the DNA (*13–15*), and the DNA of different density was termed satellite DNA. The observation a few years later that the complimentary strands of mouse satellite DNA reassociate rapidly after denaturation by heat (*16, 17*), strongly suggested that satellite DNA is composed of relatively short, repeated polynucleotide sequences.

The relation in many organisms between repetitiveness and rate of strand reassociation was investigated soon thereafter by Britten and Kohne (*17*). They introduced the variable *Cot* (where *Co* equals the initial concentration of DNA in moles of nucleotide per liter and *t* equals the reassociation time in seconds) to estimate

present epoch by condensation in rather quiescent, outer regions of galaxies.

Another, perhaps more fundamental, paradox is: What is the cause of the nonvelocity red shift? It may be possible, by using known physical mechanisms, to explain this type of red shift. The models, however, would probably be very complicated. For example, Sisteró showed that collimated photons overtaking and scattering off relativistic electrons will give red shifts where $(\Delta\lambda/\lambda)$ is constant throughout the spectrum (*7*). Since relativistic electrons are very forward-scattering, this mechanism would also circumvent the usual second objection to variants of the "tired light" theory, namely, that the apparent angular diameter of the source is enlarged appreciably by numbers of scatterings. Some support for Compton scattering models might be forthcoming from the fact that, in order for photons to penetrate the densities of relativistic electrons that are derived on the assumption of cosmological distances, the electrons and photons would indeed have to be moving in closely similar directions. But a difficulty arises in that the nucleus or source of the spectrum must be shielded from direct view by the observer. This restriction suggests that the nucleus is shielded by nondiscrete blue scattering from electrons in other directions. Another possibility is that the nucleus is shielded by dust. This mechanism in turn introduces the possibility that a nucleus seen reflected from a dust cloud moving away from the observer would be shifted by the velocity of the moving reflector. Rees has mentioned the additional possible mechanism of red-shifting within an optically thick, expanding shell around a spectral source (*39*). He has also mentioned the possibility that we see objects ejected with high velocities but that, for some reason, possibly dust obscuration in front of the objects, we do not "see" the approaching velocity.

In fact, it seems that there are a number of possible explanations that can be derived from conventional physics. Although these explanations could give only very complex and intricate solutions, they cannot at the present time be ruled out as solutions. It would be of utmost importance to see if these models could all be demonstrated to be very unlikely explanations (as the gravitational red-shift models were shown to be). The importance of this step would lie in the existence of new physics which would then be implied. It is possible that in the year 1971 we do not know all the physics there is to know. Perhaps in the nucleus of a galaxy or in the process of ejection physical conditions are encountered that are so extreme that local geometry is affected or clocks run slow. If there were, in fact, new physics to be discovered, it would most naturally be in the realms where observations most fundamentally contradict the current theoretical expectations. But, of course, before we consider seriously new physics, we must first exhaust the more conventional alternatives.

Fig. 13. Region aound the large Sb spiral galaxy NGC 7331. Stephan's quintet is shown about 30 arc minutes southwest.

## Whose Move Now?

Most of the evidence that I have reviewed here is in the literature in various places. The reason for bringing it all together here and trying to evaluate it on more than a purely technical level is that there seems to have been some sort of impasse reached in astronomy with regard to this evidence, and it is of interest to examine why this is so and how the deadlock might be broken.

As I have stressed throughout this article, if the observations are correct, some of our fundamental assumptions are wrong. The only escape from this conclusion is to say that each association of discordant red shifts and observed ejection phenomena is accidental. For example, if the string of quasars coming out of the exploding galaxy is not accidental, then quasars are closer than cosmological distances and their red shifts are due to some other cause. I have tried to reduce these questions to crucial sets of associations—either "yes" or "no," with the decision hinging on the facts of the observation. Many astronomers either reject these cases as "selected" accidents or adopt a neutral attitude. But it seems to me that the accumulation of evidence is now very difficult to either reject or ignore.

It could be argued that each case is somewhat different—that sometimes quasars, sometimes compact radio galaxies, sometimes companion galaxies are involved in associations. The answer to this argument is that all these objects are varieties of galaxies and that there is a continuity of attributes involving all these various kinds of extragalactic objects. In these associations it is usually the most extreme and peculiar objects that have the most discordant red shifts. Actually this observation strengthens the conclusion that the associations are significant, because, if there were accidental associations, they should, for the most part, involve normal objects, which are the kinds of objects most frequently observed. Instead they tend to involve peculiar ob-

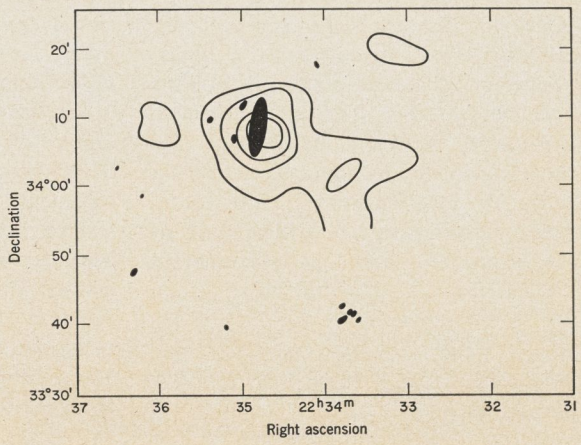


Fig. 14. Radio map of the region around NGC 7331 [adapted from DeJong (*37*)]. The map stops just north of Stephan's quintet. [Courtesy of *Astrophysical Journal*, University of Chicago Press, Chicago]

—Hull-Gardner-Rosen Am. Nat 72

"Chromosome Number, DNA Quantity and Phylogeny"

(Presented at Concurrent Session, First International Congress on Systematic and Evolutionary Biology, University of Colorado, Boulder, Colorado, Thursday, August 9, 1973)

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## INTRODUCTION

Cultivation of monophylogenetic "trees" is once again becoming popular. Actually this is a return to prominence of a pasttime that was considered an integral part of nineteenth century biology. According to many leading biologists, new attention to broad phylogenetic questions that were only shelved 50 years ago rather than answered is required because of the outstanding developments in modern genetics and molecular biology in recent decades.

As I see the situation, one question is, "How do biologists in the 1970's try to establish affinity between animal groups and plant groups as they build monophylogenetic trees?" And a corollary question is apropos: "How do these attempts qualify as being at all scientific according to rigorous application of scientific methodology?" I shall attempt brief answers to these questions, as a continuation of a paper read before a Society for the Study of Evolution session at the 1971 AAAS meetings in Philadelphia. At that time I concluded:

> Based upon a careful, five-fold examination, no empirically demonstrable data can be found which can "fit" the commonly, popularly accepted monophylogenetic explanation of relationship in diversity among animals and among plants. ....
>
> All the empirical data available from breeding records and from over 100 years of research into the fossil record can be used to support the conclusion that "fixity of kinds" exists today and has existed in the past in contradistinction to all the "trees" drawn by proponents of the monophylogenetic viewpoint. (Moore, 1973)

And for that analysis "On Chromosomes, Mutations, and Phylogeny" about two years ago I used the attached two pages of "Identities or Axioms" as a frame of reference. I do likewise for this paper.

Of course, the scientific reviewer Phillip Morrison expresses a dubious mood when he admitted in 1971:

> For 50 years after Darwin biologists cultivated their phylogenetic trees. These were subjective, wishful and barely testable, everything that today's sharp reproducible and tightly argued molecular biology is not. Trees are out of fashion. But who can tell us how point mutations and sundry tape doublings, crossings and writhings made the oak and the squirrel, the gull and the gall by summing up the changes in many a piece of enzyme? (Morrison, 1971) (Emphases added)

Yet, monophylogenetic trees are fashionable again. Whereas nineteenth century biologists based their contentions of affinity upon basic similarities of skeletons, muscles, embryos, geographic distribution, rudimentary (vestigial) organs, and such, this time, monophylogenetic trees are being built upon basic similarities correlated with microscopic and sub-microscopic manifestations, such as, 1. chromosome size and number, 2. DNA quantity, 3. protein complement, and 4. other cellular organelles. Nevertheless, all

efforts at building monophylogenetic trees are only as reliable as the reliability of the basic assumption involved; namely, the degree of relationship depends upon or is a function of the degree of similarity. These bases of modern, present-day tree planting must be examined against criteria of rigorous application of scientific methodology.

## STRUCTURAL AND NUMERICAL CHANGES

Some biologists still persist in searching for support of the monophylogenetic explanation of relationship of living things at the more macroscopic level, that is, at the chromosomal level. Structural and numerical changes of cellular inclusions recognized as chromosomes are still utilized as means to formulate monophylogenetic explanations of relationship. In introducing his chapter on chromosomal changes in evolution, one textbook writer makes this statement:

> Apart from cases of differences in chromosome number which are due to polyploidy, cytotaxonomic differences between karyotypes of related species must have arisen by chromosomal rearrangements, such as inversions, translocations, deletions and duplications, or combinations of these. (White, 1961) (Emphasis added)

Before writing about breakage and joining of chromosome parts, deficiencies, duplications, inversions, translocations, and position effects, another author ended the introduction of his chapter on chromosomal structural modifications:

> Structural modifications of chromosomes are common in nature and have apparently played a significant role in evolution. They occur spontaneously, that is, without any known cause. (Gardner, 1968) (Emphasis added)

Even French geneticists try to use structural changes to "explain" the origin of the genus Homo. For instance onebiologist (Ruffé, 1970) has imagined chromosome translocation in genus Pan karyotype such that chromosome #9 supposedly became added to chromosome #16, thus resulting in formation of chromosome #9 in the Homo sapiens karyotype!!! Such imagined changes are ingenious but they are hardly scientific. In what way is the ingenious scheme of Dr. Ruffé scientific, as being testable? Or, is the idea merely ad hoc and totally untestable, unscientific?

Similar examples could be multiplied many fold. But the significant point is the fact that all reference to different phenomena of ploidy and chromosomal rearrangements constitute nothing more than ad hoc, untestable hypotheses, as far as any attempt to explain any relationship between or among major groups of animals or major groups of plants is concerned. Absolutely no genetic connections are ever established between any major groups of living things by means of any mechanisms involving ploidy and chromosomal rearrangements.

That inviolate genetic barriers exist between major groups of living things may be stated conclusively on the basis of available genetic evidences. Unbridgeable breeding gaps are known; and, no amount of reference to ploidy and/or chromosomal rearrangements will truly erase the undeniable evidence that breeding gaps between major groups of living things do in point of fact exist.

Hence any effort to construct monophylogenetic trees based on structural changes of chromosome parts partakes of ad hoc hypotheses and constitutes a long exercise on the use of circumstantial evidences. It is true that empirical findings can be amassed for changes of chromosomes by means of duplication, deletions, translocations, and inversions, but such data are always associated with studies of organisms within one species or one genus. Never do we find reports of research or structural or numerical changes of chromosomes that may be used to document any true genetic relationship between major groups of animals or major groups of plants.

# IDENTITIES OR AXIOMS

STUDY OF ORIGIN OF FIRST LIFE : BEYOND { LIMITS OF MAN'S APPLICATION OF CAUSE AND EFFECT (I. E., TESTING)

MODERN IDEAS OF ORIGIN OF LIFE $\equiv$ SPONTANEOUS GENERATION (HETEROGENESIS) AT SUB-MICROSCOPIC LEVEL

ANY LABORATORY "PRODUCTION" OF LIVING SUBSTANCE $\equiv$ SYNTHESIS OF LIVING FROM NON-LIVING ACCORDING TO KNOWN FORMULAE AND NOT ACCIDENTAL (NOR ANY CHANCE COMBINATION)

EVOLUTION $\not\equiv$ DEVELOPMENT

MICROEVOLUTION $\equiv$ GENETIC VARIATION WITHIN LIMITS OF KIND



JOHN N. MOORE
December 1971

## IDENTITIES OR AXIOMS

MEGAEVOLUTION
(SOMETIMES MACROEVOLUTION)
OR
TRANSMUTATION OF KINDS

$\equiv$

TREE OF LIFE
OVER
GEOLOGIC TIME

KINDS $\equiv$ TYPES $\equiv$ FORMS $\not\equiv$ SPECIES

THEORY OF
ORGANIC EVOLUTION
[ENDS]

$\not\equiv$

THEORY OF
NATURAL SELECTION
WITHIN LIMITS OF KIND
[MEANS]

ORGANIC EVOLUTION
(TRANSMUTATION
OF KINDS)

$\not\equiv$

ANY
GENETIC
CHANGE

JOHN N. MOORE

December 1971

All this notwithstanding, Babcock (1947) and Stebbins (1950) gave evolutionists early "models of thought" regarding the supposed "evolutionary" importance of chromosome number and polyploidy. Though Stebbins (1966) wrote extensively about correlation of chromosome size (translated to mean DNA content) and so-called evolutionary advancement in his monophylogenetic scheme, his argument is heavily loaded with such terms as, "indicate", "suggests", "probably", "appears", "would", "could", and "in my opinion". He even admitted to nothing but speculation in more than one place in shoring up his "hope" of stimulating further exploration of chromosomal variation and monophylogenetic schemes. Just how truly scientific, as judged by the criterion of testability, are the speculations of Dr. Stebbins? Absolutely no genetic connections between major groups of organisms, living or dead for that matter, have ever been detected. As stated above, Stebbins' speculations about chromosomal changes involving ploidy, deletions, and translocations are purely ad hoc hypotheses, which are totally untestable, totally unscientific.

I have explored the matter of chromosome number as being in correlation with monophylogenetic postulations. Textbook authors often offer different lists of chromosome numbers which supposedly contain the basis for establishing a progressive increase in chromosome number from least complex organisms to most complex organisms. In other words, students studying such lists might well gain the impression that an increase in chromosome number correlates with so-called increase in differentiation and complexity of organisms. Nevertheless my own "exploration", to again borrow the term from Stebbins, resulted in two original diagrams of 2n counts of chromosomes. (See attached diagrams)

The groups of organisms have been arranged according to the commonly accepted monophylogenetic "sequence" of relationship of major groups and the various entries within the major groups of animals and major groups of plants are merely representative. Let me point out explicityly that these two brief diagrams contain a much more detailed range of chromosomes than that usually found in textbooks. I wonder why this is so. (?)

From just "spot inspection" of these diagrams, with major groups and chromosome counts positioned according to the commonly accepted monophylogenetic system for denoting supposed relationship, at least one conclusion is obvious. There is absolutely no pattern of increase of chromosome count that might possibly be construed or correlated with so-called increase in complexity or organization of major groups of animals (and the same generalization can be maintained for ma jor groups of plants). No ad hoc, untestable hypotheses about chromosome duplication or deletion, or even any type of ploidy, seem at all satisfactory or applicable to explain the separate arrays of representative chromosome counts in the two diagrams.

Is the previously mentioned basic assumption, that the degree of similarity of physical characteristics is a basis for degree of relationship, at all applicable to the physical characteristic of chromosome count, considered by Gardner as that one characteristic more constant than any other? No! Upon close examination of these data on chromosome counts in animals and inplants, it would seem quite appropriate to conclude that the currently popular imagined transformational pattern of phylal relationships, called the monophylogenetic scheme, is not based on sound scientific data and is most illogical.

## DNA QUANTITY PER CELL

But at this point some geneticists might hold that the number of chromosomes is not so important as the amount of DNA per cell. A most reasonable question is whether the amount of DNA per cell correlates with differentiation and organ complexity. A large number of researchers have reported on a positive correlation, but with especially interesting aspects with regard to the position expressed in this paper.

(1) Markert and Ursprung (1971) provide an excellent example of those who have examined carefully the idea that one might expect to find some simple correlation between the total amount of DNA in the cells of an organism and the variety of differentiated cells making up that organism. And they dutifully list a summary chart on DNA content. Then they state:

> This range of values fits in a rough way the relative complexity of these organisms. However, this generalization that complexity equals DNA content does not apply within the vertebrates as a distinct group.

These men who are specialists in developmental genetics see this fact as a genuine incongruity that requires explanation. Yet, on the next page they stated, "However, a direct examination of the karyotype of the cells of vertebrate orfanisms fails to support the conclusion that polploidy counld account for the difference in amounts of DNA. And further, they are quite dubious about the possibility of polyteny (strands of DNA remain attached side-by-side to one another so as to produce a giant cable) as an explanation for the variable amounts of DNA in vertebrate cells. These statements are more or less born out by my 2n counts of chromosome charts, if DNA proportionality to chromatin content is acceptable, which is quite well established by empirical research. But what scientific conclusion about affinity of groups of vertebrates is gained? To be precise, absolutely no scientific basis for affinity between or among vertebrates is gained.

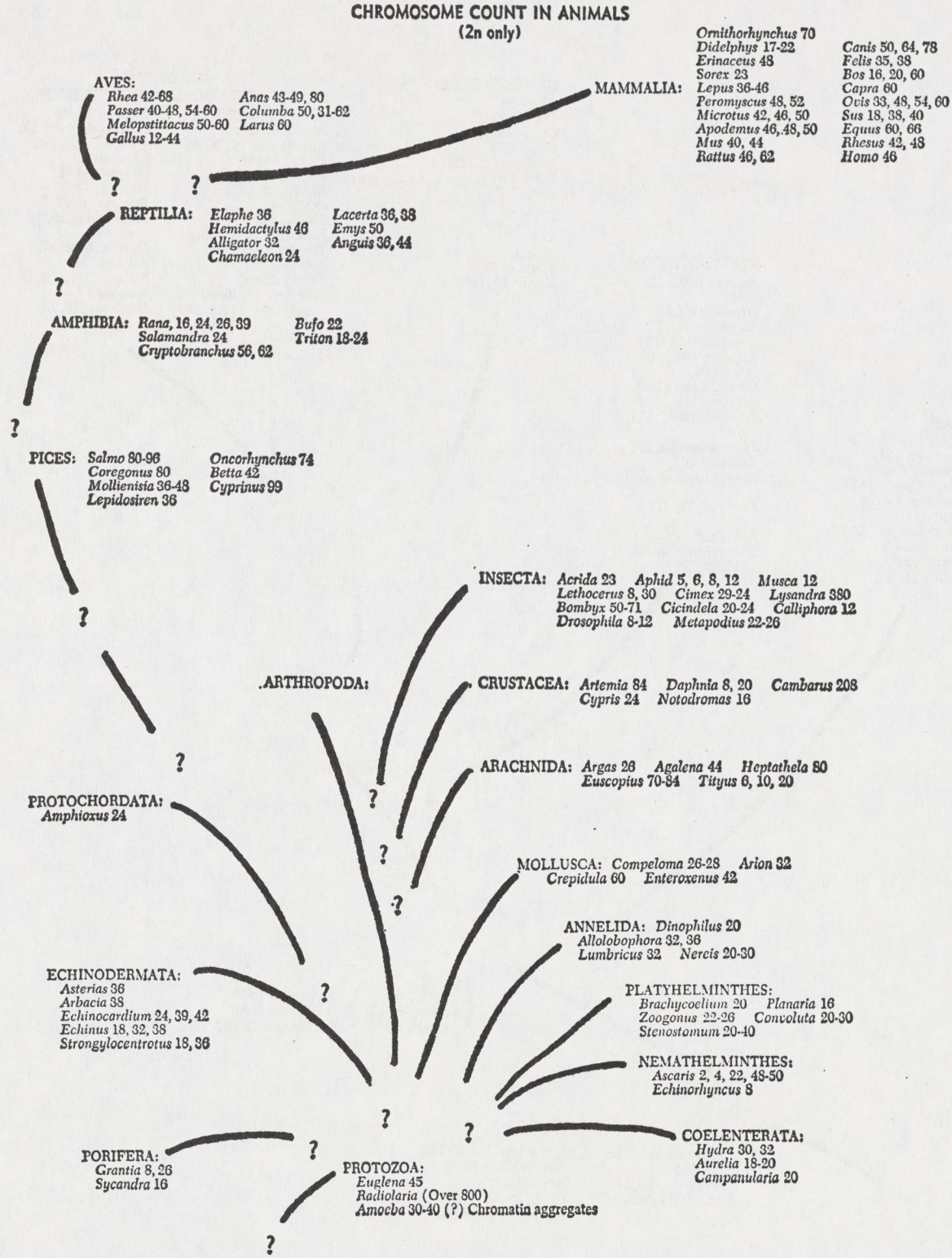
(2) Often those who try to build monophylogenetic trees refer to gene changes as a source of new traits. Certainly some explanation for new traits, such as wings in place of forelimbs, feathers in place of scales, eyes in place of sensor spots, etc., must be provided by the evolutionists who construct monophylogenetic trees, that is, if their scheme shall have real empirical basis. But note very well that gene changes as one type of mutation fail utterly in any empirical sense as source of new traits. ALL known results of gene mutational changes result ONLY in changes in already existing physical traits.

Even homeotic mutations do not result in truly new traits, but by definition merely "confer the properties of one body segment on another" in morphogenesis as is well brought out by Markert and Ursprung (p. 171ff). Furthermore a similar failure is shown in the same chapter in their discussion of transdetermination as a source of new traits. These authors have shown in most conclusive manner that genes affecting morphogenesis as mutants are degenerative and malfunctional. Thus in this area too absolutely no useful scientific basis for affinity between groups of orgamisms is gained.

(3) Interestingly enough, writing on DNA content in relation to phylogeny of selected boreal forest plants El-Lakany and Dugle (1972) reported use of calculations of relative amounts of DNA from photometric readings in terms of Feulgen Absorption Units (FAU) to arrive at the conclusion "that, within a single geographic area, DNA content is reduced with phylogenetic advancement". That is a very interesting conclusion in light of the fact that both Stebbins (1966) and Mirsky and Ris (1951) arrived at the same conclusion from their data. As shown in the attached reproduction of four figures from the El-Lakany paper all of his examples provide basis for notation of decrease, for reduction which means loss with respect to over-all chromatin material in the genome. In their Figure 1 on page 429 they show reduced DNA content in the family Pinaceae. In Figure 2 on page 430 they show reduced DNA contents in certain Herbaceae plants. In Figure 3 on the same page they show reduced DNA content of representative species of Lignosae plants. And in their Figure 4 on page 431 they show reduced DNA content in the genus Rosa.

According to these authors the more "advanced" plants have less DNA than less "advanced", and they suggest that there "appears to be an inverse relationship between phylogenetic advancement and the amount of DNA". How does loss fit with the concept of "progress" so commonly associated with evolution in the nineteenth century as biologists built their trees? Again the reader encounters such terms as, "suggested", "may", "inferred", "pro-

## CHROMOSOME COUNT IN ANIMALS
(2n only)





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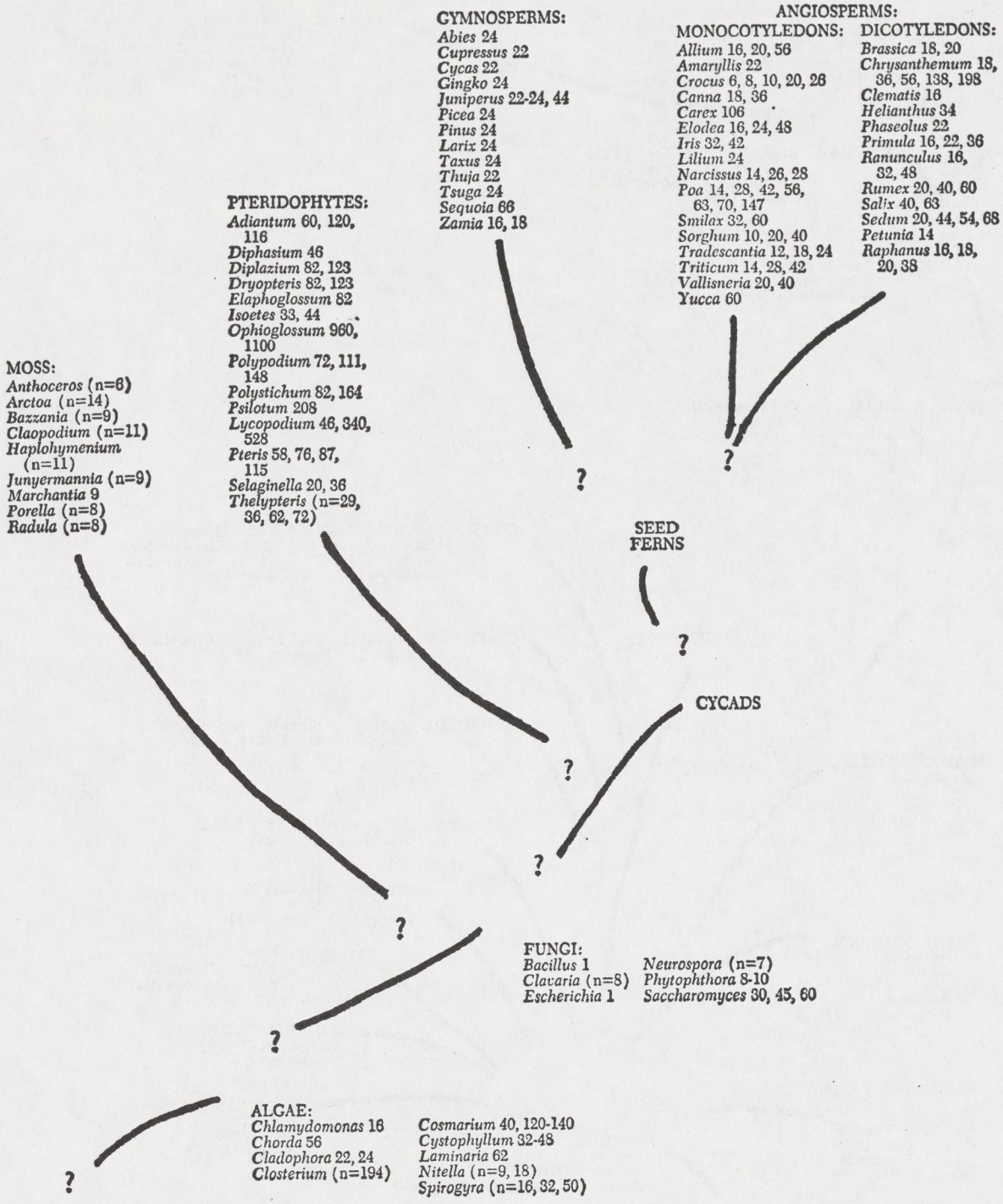
Sparrow, Underbrink, and Sparrow. "Chromosomes and Cellular Radiosensitivity," *Radiation Research,* Vol. 32:915-945, 1967.

## CHROMOSOME COUNT IN PLANTS
(2n, except as indicated)





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Ornduff, R. Editor. *Index to Plant Chromosome Numbers for 1966.* Utrecht, Netherlands: International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy, June, 1968.

Darlington, C. D. and A. P. Wylie. *Chromosome Atlas of Flowering Plants.* Second Edition. London: George Allen and Unwin Ltd., 1955.

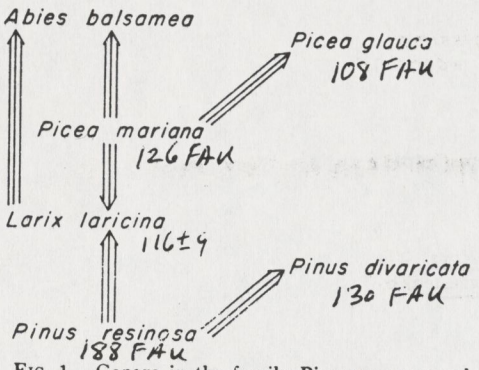



FIG. 1. Genera in the family Pinaceae arranged in phylogenetic sequence suggested by Chamberlain (1935). Positions of *Picea glauca* and *Pinus divaricata* were suggested by Wright (1955) and Shaw (1914). Arrow direction indicates reduction in DNA content.

p. 429

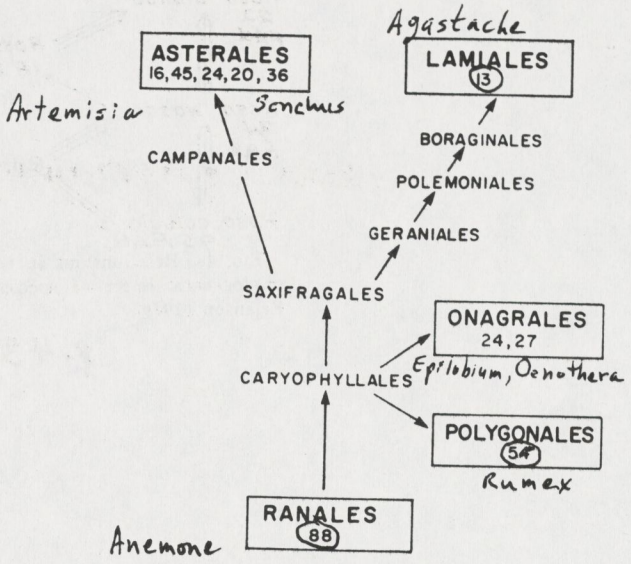



FIG. 2. Hutchinson's classification system of Herbaceae plants (adapted from Hutchinson, 1969). The numbers are approximate DNA contents of representative species as listed in Table 1.

p. 430

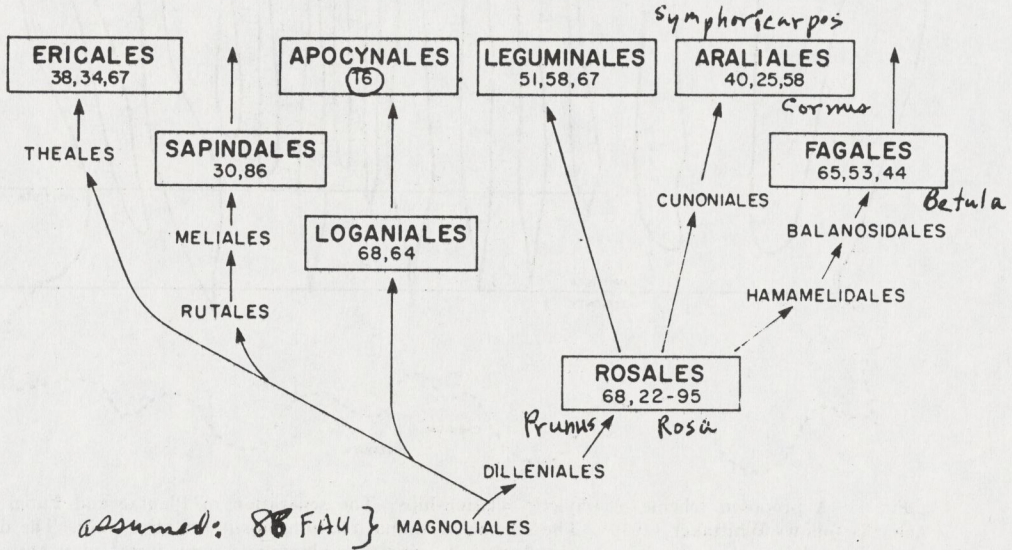



FIG. 3. Hutchinson's classification system of Lignosae plants (adapted from Hutchinson, 1969). The numbers are approximate DNA contents of representative species as listed in Table 1. For details of relationship within the genus *Rosa* see Fig. 4.

p. 430

per El-Lakany and Dugle

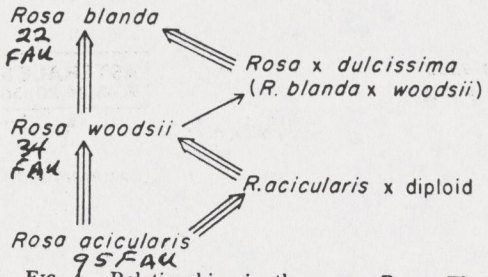



FIG. 4. Relationships in the genus *Rosa*. The phylogenetic order of species was suggested by Erlanson (1929).

p. 431

per El-Lakany and Dugle

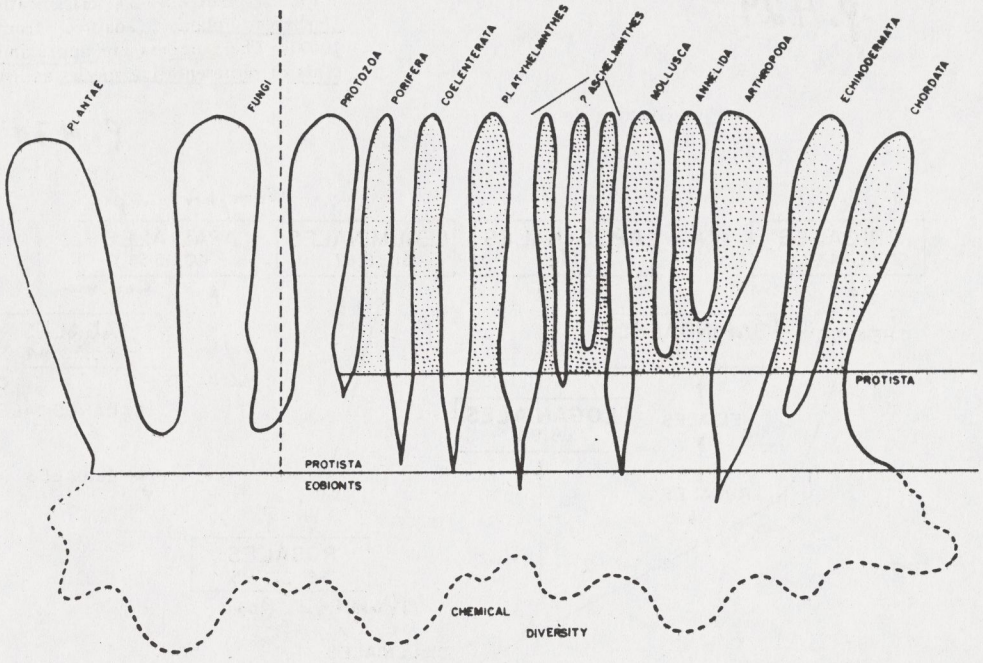



FIG. 3. A proposed scheme of phyletic relationships. The separation of Plantae and Fungi from Animalia follows Whittaker (1959). The scheme of animal relationships is tentative only. The depths of the clefts between groups suggest the degree of separation obtaining; some forms may have been distinct from the eobiont stage, others separated as protists and still others became distinguishable long after the protist stage was passed.

p. 121

per Nursall

bably", and "assumed". Now we are back in the same problem in dealing with the Stebbins work. Sheerly ad hoc hypothesizing is all that is afforded to try to explain these data. Concepts of polyploidy, aneuploidy, and/or translocations in NO way are useful to gain anything like a rigorous scientific basis for stating affinity between groups of organisms. Again, absolutely no scientific basis has been obtained for affinity between groups.

(4) And DNA content has been analyzed even in a "living fossil." Such is the result of a study of the "Nuclear DNA Contents of Coelacanth Erythrocytes" (Thomson, 1973). He and fellow researchers concluded,

> The DNA content in Latimeria was higher than would be expected in the ancestor of all tetrapods and we conclude that in the course of evolution from the original dipnoan-crossopterygian-amphibian stock, increase in the coelacanth genome has occurred. Thus increased cellular DNA contents have arisen many times, independently, in this whole assemblage.

From a rigorous scientific methodological viewpoint these are merely assertive statements without any firm empirical basis whatsoever. There is absolutely no empirical ground for statements about the coelacanth being the ancestor of all tetrapods. And the variable DNA cell content reported in no way is useful to establish affinity between organisms involved in the research.

Quite clearly there is an extensive literature that has accumulated on the DNA content per cells and the supposed "evolutionary" significance of such information. Beginning at least with Mirsky and Ris (1951) and continuing through the 1960's with Stebbins (1966) and others, and continuing in the 1970's with Ohno, Pederson, and others. Yet the whole gamut of reports by these biologists leaves the reader without any empirical findings of any actual affinity of groups of organisms monophylogenetically. Indeed there is much use of such words as "might", "could", "would", "should", "expect", and so forth in almost every single research report. But NO empirical facts of affinity. Diversity of DNA content is well established, but that is all.

In closing this section, then, from the standpoint of rigorous application of true scientific methodology, studies of DNA content per cell are totally useless as affording any scientific, reproducible basis of affinity of organisms of different major groups. No "progress", ONLY reduction and loss is established, that is, if any kind of transmutational changes have ever occurred.

## SIMILARITIES BASED ON PROTEIN ANALYSES

There are those who expect a certain "genetic insight" to derive from our growing knowledge of the molecular structure of proteins. Evolutionists have been quick to try to utilize such information for the erection of newer phylogenetic relationships; even to the construction of "protein clocks" (Fitch and Margoliash, 1967). Clear differences have been detected in representative species and this is best illustrated by the protein molecules hemoglobin and cytochrome c (Dickerson, 1972).

The sequence of cytochrome c has now been determined for more than 30-40 different species, ranging from man to yeast. Evolutionists hold that the similarities in the various cytochrome c molecules may be used to emphasize the relatedness of all life forms, and the differences are taken as a measure of the so-called "evolutionary" divergence of one species from another. Many biologists "hope" in this way to develop "family tree" showing the changes of the protein with even a time scale to indicate when one species supposedly split off from another species.

But when such scientists as Dickerson and others, who offer their ingenious "protein clocks" to shore up or prop up their monophylogenetic trees, begin to write about the "evolution" of proteins, they plainly move beyond all limits of application of cause and

effect, i. e., testing and experimentation. No statements of conclusive nature are possible. Hence these men use purely circomstantial similarities as evidence as "hopeful" ground in which to plant their monphylogenetic trees.

## A NEW, INGENIOUS SPECULATION

And now another effort of ingenious and imaginative proportions to take up the challenge to present a wider view than the classical, limited monophylogenetic explanation must be given consideration (Margulis, 1970). Dr. Lynn Margulis is concerned about the great gap between cells with true nuclei and without, or the significant gap between eukaryote and prokaryote cells.

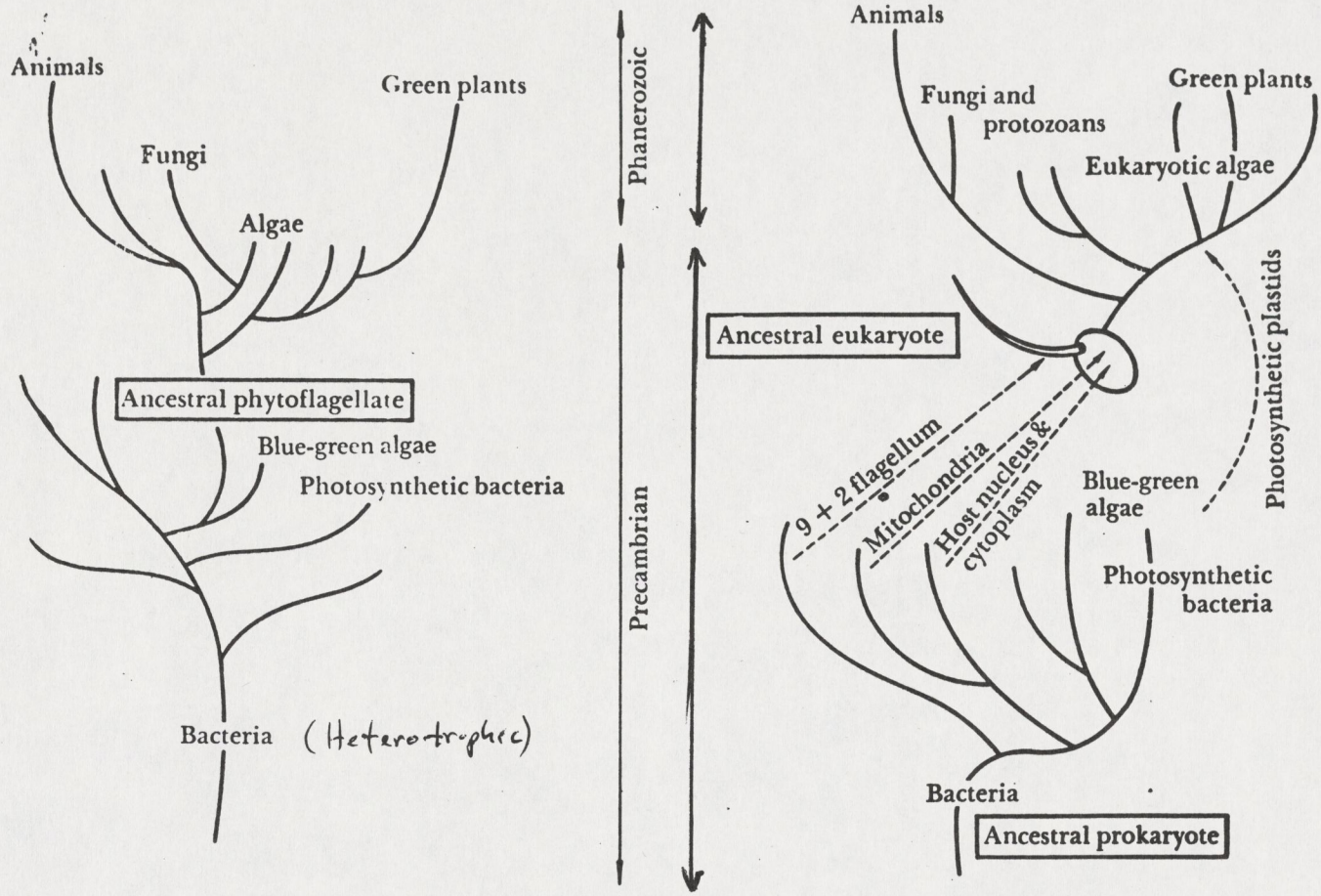
Dr. Margulis takes issue with the classical dichotomy of animal andplant cells. (See her Figure 1 attached.) She follows a modified "line-up" of four kingdoms of living things. (modified after Whittaker, 1969). In opposition to the classical view of phylogeny, she has proposed in recent years an alternative "serial symbiosis". (See her Figure 2 also attached.)

By speculatively combining concepts of mutations, selection, and symbiosis of certain organelles, Dr. Margulis presents a synthesis under the sub-title, "Summary of the serial symbiosis view" (Margulis, 1971), that eukaryote cells have had multiple ancestors. Or as she has stated her thesis: "a very specific series of symbioses led to the formation of new types of cells: our direct cellular ancestors, the eukaryotes." Her view is "a continous narrative" by her own words, and this constitutes an extensive exercise in deductive reasoning and interpretation .

Repeatedly she uses such words as, "believe", "hope", "claim", "suggest", "probably", and "argument" or "argued" -- the latter appears over and over again. Thus her writing comprises sheerly speculative assertions as far as affinity of groups is concerned. She is in the same untenuous position as Dr. Stebbins and all his "followers". Dr. Margulis, like Dr. Stebbins, is not writing in a truly scientific manner, as judged against the criterion of testibility, which is so central to all carefully designed scientific research.

Her use of the term, "hypothesis", is highly suspect from the standpoint of rigorous application of scientific methodology. She actually is referring to some specific conjecture or speculation about origin of true cells. She is NOT stating some concise answer to a problem that then might serve as a frame of reference for careful scientific study about affinities between groups of organisms. That would be a true scientific hypothesis; and yet, of course, she can formulate no scientific hypothesis about the origin of true cells, anyway. Such beginnings are forever past any application of cause and effect, i.e., testing by experience by some properly trained scientist. And such a clear assertive position can be taken in condemnation of all writings about spontaneous origin of protein components of life, of living cells (Fox and others, 1970).

What are the actual, precise aspects of scientific methodology? I have provided such in the attached chart. The multiple aspects may be conveniently referred to under the headings of "empirical" and "theoretical". Now how well do the imaginative narratives of planners of monophylogenetic trees fit true, actual scientific methodology? How well do the proferred data of such planners fit the criterion of repeatability, of testibility, as far as any claims of affinities of groups of animals or groups of plants is concerned? An irrevocable, conclusive judgement is that all monophyogenetic trees are built out of figments of men's imaginations and NOT as a consequence of careful inductive, experimental establishment of any real genetic affinity between major groups of plants and major groups of animals. ALL monophyogenetic trees are built solely upon the one basic assumption that the degree of relationship depends upon or is a function of the degree of similarity, whether at macro-, micro-, or sub-microscopic level. And no true




Classical View

Symbiotic View

p. 230

per L. Margulis, 1971

# ASPECTS OF SCIENTIFIC ACTIVITY

EMPIRICAL

Observations (initial observations included)

Descriptions

Classification

Calculations

Problems

Hypotheses

Analogy

Inductive reasoning

Generalizations -- laws

Predictions

Deductive reasoning

Experimentation

(testing)

(Experimental assumptions)

---

THEORETICAL

Theoretical model

Theoretical assumptions -- imaginary aspect or event

Postulates

Theorems

Conceptual scheme

(Theory)

| FOUNDATIONAL, BASIC ASSUMPTIONS OR PRESUPPOSITIONS |
|---|
| a. Objectivity of study<br>b. Cause and effect<br>c. Testability of ideas<br>d. Objects independent of observers<br>e. Uniformity of natural environment |

J.N.M.

Criteria for a good theory in physical science:

Three qualifications have already been cited:

(1) A fruitful theory correlates many separate facts, particularly the important prior observations, in a logical, preferably easily grasped structure of thought.

(2) In the course of continued use it suggests new relations and stimulates directed research.

(3) The theory permits us to deduce predictions that check with experience by test, and it is useful for clearing up puzzling difficulties and solving practical problems.

The history of science has shown that a good theory frequently has, in addition to the three attributes above, one or more of the following three:

(4) When the smoke of initial battle has lifted, the more successful of two rival theories often turns out to be the one that is simpler in the sense that it involves fewer basic assumptions or hypotheses.

(5) A theory is more readily acceptable to contemporary scientists if its postulates or assumptions are plausible.

(6) Successful theory is flexible enough to grow, and to undergo modifications where necessary.

--From Chapter 8, "On the Nature of Scientific Theory", in Foundations of Modern Physical Science by Gerald Holton and Duane H.D. Roller. Reading, Mass.: Addison-Wesley Publishing Company, Inc., 1958.

scientific theory is involved since no prior observations of affinity of groups of organisms can be made "before the fact" of erection of monophylogenetic trees.

And paleontologists have written in a "festschrift" for George Gaylord Simpson, just this past year (Schaeffer and others, 1972) about the dubious value of claims of affinity between past organisms on the basis of "succession" in rock strata. These men state:

> The notion of ancestry and descent is, of course, implicit in the concept of phylogeny and is a logical concomitant to the entire idea of organic evolution. But there exists a large information gap between what we know must have happened and knowledge of what actually did happen. The idea that the fossil record provides documentation for the actual course of phylogeny has followed as an almost axiomatic corollary to the adoption of evolutionary theory as the key to understanding the diversity of life. In this view the only drawback that has kept the fossil record from answering most questions in systematics is its notorious incompleteness. Proponents of this view have failed to grasp an essential point: an actual phylogeny is not capable of outright discovery; ... (Emphasis in original)

Or on an earlier page,

> But for the vast majority of invertebrate and vertebrate records any attempt in this direction (use of chronoclines) is not realistic. We simply wish to point out that it is dangerous to assume at the outset that a chronocline is a pure reflection of an ancestral-descendant sequence, no matter how complete the record may seem to be.

Similar clear criticism is manifest in the important argument by Karl Popper when he studied the question, "Is There a Law of Evolution?" (Popper, 1960). Within the context of his refutation of historicism, he concluded:

> But can there be a law of evolution? Can there be a scientific law..... I believe that the answer to this question must be No", and that the search for the law of the "unvarying order" in evolution cannot possibly fall within the scope of the scientific method, whether in biology or in sociology. My reasons are very simple. The evolution of life on earth, or of human society, is a unique historical process. Such a process, we may assume, proceeds in accordance with all kinds of causal laws, for example, the laws of mechanics, of chemistry, of heredity and segregation, of natural selection, etc. Its description, however, is not a law, but only a singular historical statement. .... it is clear that any law, formulated in this or in any other way, must be tested by new instances before it can be taken seriously by science. But we cannot hope to test a universal hypothesis nor to find a natural law acceptable to science if we are for ever confined to the observation of one unique process. (Emphasis in original)

Truly, then, there can be no real "historical" geology. As Popper wrote in his Preface, "There can be no scientific theory of historical development serving as a basis for historical prediction..... My proof consists in showing that no scientific predictor -- whether a human scientist or a calculating machine -- can possibly predict by scientific methods, its own future results. Attempts to do so can attain their result only after the event, where it is too late for a prediction; .." (Emphases in original) Thus all interpretations of the fossil record are after the fact, and not scientific. Therefore monophylogenetic trees are sterile mental exercises; such a status was recognized by scientific reviewer Philip Morrison as quoted in the Introduction.

All this leads to the conclusion that two scientists in the 1960's may very well have been on the better path. Each man stated clear reasons why a polyphylogenetic view of relationships of distinct groups of animals and plants was the more scientifically sound. Or as one Canadian zoologist summarized his paper on origins of major groups of animals:

> It is suggested that the major groups of animals arose polyphyletically, over a period of time, from a geographically widespread variety of protozoan eobionts which were evolving to explore the variety possible within a limited physico-chemical framework. (Nursall, 1962)

This suggested situation is illustrated in his article in Figure 3, attached. In clear contradistinction to the imaginations of Stebbins and Margulis, Nursall carefully recognized that all major groups are distinct and separate with no known affinities based on scientific evidences. Such a polyphyletic position had been propounded by an experimental biologist two years before and stated in this succinct manner:

> The difficulty of placing viruses, bacteria, certain "algae", sponges, and so on, in a fitting place in any taxonomic scheme based on a monophyletic hypothesis may stem from the possibility that the discontinuities are real and represent the existence of separate lines of descent from independent instances of neobiogenesis (establishment of primitive organisms) at different times in the history of the earth down to the present. (Keosian, 1960)

## CONCLUSIONS

On the basis of critical consideration ofbases for contended affinity between major groups of organisms as found in recent literature under the headings of 1. chromosome size and number, 2. DNA quantity, 3. protein complement, and 4. other cellular organeles, as measured against application of scientific methodology, a polyphylogenetic view or interpretation of the SAME empirical data seems worthy of serious consideration.

There is sound empirical basis for a polyphylogenetic interpretation, as compared with the favored position campaigned for by Stebbins and even Margulis, that can be gained from close examination of breeding records of animals and plants, and further close examination of the fossil record.

Also the conclusion is plain that textbook authors and professional biologists, who interpret empirical data through an "exclusive" monophylogenetic viewpoint, are doing so in a selected indoctrinare attitude far removed from careful examination of real data according to rigorous application of cause and effect premises and careful practice in agreement with cardinal, limiting principles of scientific methodology. No origins can be studied scientifically; no scientific theory can be promulgated about origins.

Therefore, specialists in phylogeny in so-called evolutionary history are duty bound to research and write in multiple conjectural fashion of the contrast between a monophylogenetic interpretation of facts and polyphylogenetic interpredations of the same facts. Furthermore, science teachers and professors, who use the results of research specialists, should be duty bound in academic freedom and responsibility to present BOTH monophylogenetic and polyphylogenetic interpretations. Each conceptual framework is offered by proponents as an interpretation of possible relationships of major groups of animals and major groups of plants.

In a word, this paper is a call for immediate re-examination of all data at the macro-, micro-, and sub-microscopic levels from the polyphylogenetic viewpoint. Also this paper is a call for immediate introduction of polyphylogenetic interpretations into new textbook material, along side of the long dominant monophylogenetic interpretations, at all levels of American educational efforts.

If such a two-way treatment of polyphylogenetic along with monophylogenetic interpretation of possible relationships of major groups of animals and major groups of plants is practiced by textbook authors, and if such is followed by teachers and professors, then selected indoctrination of another generation of bright, independently thinking students regarding phylogeny might be avoided.

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OPINION

# Quantitative and qualitative: numbers and reality in the study of living organisms

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It is not often that one can ride one's hobby horse in an international journal. The ride will be provocative. I begin by admitting bias: critics will say by displaying a prejudice. As a zoologist interested in animals, how they are constructed and what they do; how they behave and fit into the communities in which they live; why they are distributed in particular ways, and how they might have evolved, I find some of the modern trends in freshwater biology both unhelpful and dull.

In a maligned (or praised) little book—it depends on outlook, science not always being the objective pursuit that many imagine—Cannon (1958) lamented the intrusion into the subject of "a type of biologist ... who measured things" whose activities prefaced "that invasion by mathematics that has robbed biology of so much of its charm". I fear that Cannon would have found *Freshwater Biology* a dull journal as it seldom includes a biological paper that is not, at least to some degree, quantitative. While his singling out of measuring was unfortunate, measurements being of great importance in biology, the sentiment expressed must surely evoke sympathy among those interested in animals and their biology rather than in numbers and formulae, especially when, as is sometimes the case, these do little more than delude us into thinking that a phenomenon has been explained.

My aim is not, however, to denigrate the use of quantitative data in biology: these are essential in fields as diverse as fisheries research and genetics. Nevertheless, the mania (on the part of some it is nothing less) to express biological events in numerical terms and to support the simplest facts with a statement of their statistical significance has become so widespread as to obscure the fact that a true understanding of many biological phenomena (even in ecology where numbers are so easily generated) often demands qualitative rather than quantitative knowledge. Without the former, numbers are indeed often meaningless. Mathematics may be synonymous with the ordered structure of the physical world: it cannot explain everything in biology.

The benefits notwithstanding, the invasion of mathematics has had more serious consequences than robbing biology of its charm. New recruits to some branches of the subject are now so indoctrinated as often to believe that quantitative work is scientific: qualitative not. Quantitative data are dubbed as 'hard', qualitative information is dismissed, sometimes pejoratively, as 'descriptive', 'observational' or 'soft'. In reality the situation is often quite otherwise. Quantitative studies in biology are usually easier to conduct than qualitative investigations. As Schumacher (1973) points out "quality is much more difficult to 'handle' than quantity, just as the exercise of judgment is a higher function than the ability to count and calculate. Quantitative differences can be more easily grasped than qualitative differences; their concreteness is beguiling and gives them the appearance of scientific precision, even when this precision has been purchased by the suppression of vital differences of quality."

Similarly, while descriptive studies are not infrequently disparaged, real description is essential. All too often numerical speculations or calculations are either uninformative, erroneous, or, even worse, misleading, just because the vital descriptions on which such

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PROEBSTEL

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# MOLECULAR CHANGES AT SPECIATION

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KEY WORDS: mitochondrial DNA, population structure, gene genealogy, founder effect, phylogeography

## INTRODUCTION

The remarkably rapid growth of molecular genetics during the past two decades and concomitant advances in DNA technology have had an enormous impact in systematic and evolutionary biology (76, 89, 92, 110, 129). The ability to compare DNA sequences (either directly or indirectly) has resulted in a wealth of new, high resolution genetic markers appropriate for defining patterns of variation at all levels in the evolutionary hierarchy. Discoveries in molecular genetics have also fundamentally altered and expanded our understanding of genome structure and dynamics and of patterns and mechanisms of gene regulation. These discoveries, in turn, have fueled speculation about possible implications for evolutionary process (84, 127).

In this review I examine the consequences of speciation for patterns of molecular genetic variation within and among populations. I consider the following questions: (*i*) Does speciation leave a distinctive signature on patterns of molecular genetic variation? If so, can we use variation in DNA sequences (or allozymes) to gain insights into either the geography of speciation or the evolutionary forces that have been operating? (*ii*) Can we use estimates of genetic distance to make judgments about species status or to date speciation events?

Inferring process from pattern in evolutionary biology is notoriously difficult, and the use of molecular genetic variation to illuminate processes of



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speciation is no exception. There is little doubt that molecular markers can be used to document patterns of variation at high resolution, but these patterns are sometimes ambiguous and often cannot provide definitive evidence for a particular population history.

Although clearly a major focus of evolutionary biology, the study of speciation has never emerged as a coherent discipline. It falls at the interface between population biology and systematic biology and does not fit comfortably into either domain. Systematic biologists define relationships among species (the units produced by past speciation events) and then infer process by examining the geographic distributions of sister species and the nature of the differences between them. An alternative approach is to consider speciation as a problem in population genetics, extrapolating from evolutionary dynamics within populations and establishing criteria for identifying the role of traditional evolutionary forces (mutation, recombination, drift, selection, gene flow) in promoting or inhibiting genetic isolation or cohesion. In order to reconstruct phylogeny, systematists document character state distributions of presumably homologous characters across nonanastomosing lineages. In contrast, population geneticists traditionally have relied on observations of frequencies of Mendelian markers (allelic variants) within and between populations of interbreeding individuals. Data from molecules, especially DNA sequences, clearly demonstrate that these approaches are complementary and that there is an obvious and direct connection between ancestor-descendant relationships within populations (genealogies) and phylogeny (5, 7). These data may ultimately allow a synthesis of population genetic and systematic (phylogenetic) approaches to the study of speciation.

## MOLECULAR MARKERS IN POPULATION GENETICS AND SYSTEMATICS

### *Protein Electrophoresis*

Protein electrophoresis, introduced to population biologists in the 1960s, provided the first easy access to an array of genetic markers that could be used to define amounts and patterns of variation within and between species. The great strength of protein gel electrophoresis is that, with relatively little investment of time and money, it is possible to characterize patterns of variation for many independent nuclear gene markers (109). Allozyme data are especially useful for documenting genetic differences among individuals or populations and for defining patterns of genetic exchange (or lack thereof). They have been used effectively to study population structure (both current and historical) (36, 39, 68, 69, 90, 130), to delineate species boundaries (65, 66, 105), and to characterize patterns of introgression (72). Allozymes have also been used to reconstruct phylogenies of closely related species, but



considerably more controversy surrounds this application (24, 35, 137). First, there is clearly substantial "hidden variation" (41); bands with identical electrophoretic mobility cannot be assumed to represent identical alleles. Second, problems arise in defining characters and character states and in establishing reasonable criteria for ordering character states. It is impossible to determine genealogical relationships among alleles at single loci based on comparisons of electrophoretic mobility.

### *DNA Restriction Site and Sequence Data*

Evolutionary and systematic biologists have increasingly come to rely on data from DNA sequences. At the level of conspecific populations or closely related species, most published comparisons of DNA sequences have been derived from analyses of restriction fragment patterns or restriction site maps (53). Although indirect, this approach provides estimates of DNA sequence similarity or difference averaged over many thousands of base pairs (e.g. entire organelle genomes). Following the introduction of the polymerase chain reaction (PCR), which permits selective amplification of specific sequences from large numbers of individuals (e.g. population samples), direct sequencing of DNA has become the method of choice for many (but certainly not all) applications (75). The great advantage of DNA data is that it can be used to extract detailed information on gene genealogies. If recombination rates are sufficiently low, a short length of DNA sequence (a gene) will have a shared evolutionary history, and a sample of alleles (from one or more populations) can be traced backwards through a series of coalescence events to a common ancestral gene (79; see Figure 1). Knowledge of the genealogy of sampled genes provides population geneticists with new opportunities for evaluating the importance of selection, drift, and gene flow in maintaining variation within populations and determining population structure (79, 134, 135).

Restriction site maps and DNA sequences also have obvious advantages for phylogenetic analysis (110, 137). Characters are virtually limitless and character states easy to define. Sequence comparisons provide exceedingly high resolution and therefore are especially attractive for tracing population histories and reconstructing phylogenies of closely related species.

Chloroplast DNA (cpDNA) and animal mitochondrial DNA (mtDNA) have received far more attention than most nuclear gene sequences, both because they are easy to isolate and purify and because of their relatively simple sequence organization (4, 40, 71, 107, 119). Organelle genomes are most often uniparentally inherited and nonrecombining. These characteristics make them ideal markers for phylogeny reconstruction because their sequences record the history of a lineage uncomplicated by recombination. Animal mtDNA exhibits a high rate of sequence divergence and therefore has become

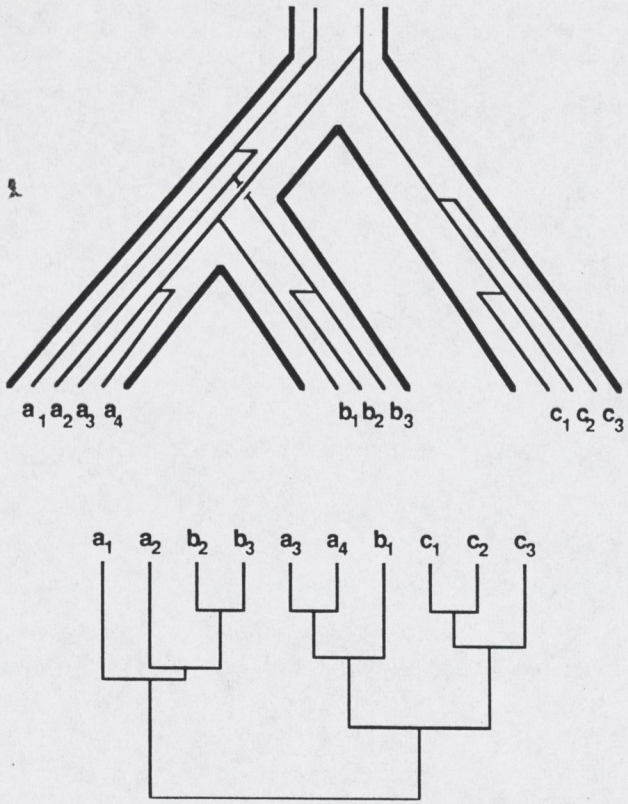


*Figure 1* Genealogy of alleles in three populations (species), showing random sorting of ancestral polymorphisms. In the upper part of the figure the allele genealogy is superimposed on the pattern of dichotomous branching of populations leading to three extant populations (A, B, C). The lower figure shows the same allele genealogy but with alleles clustered by most recent common ancestor rather than by population. Some alleles from population A ($a_3$, $a_4$) are more closely related to alleles from population B and C than they are to other alleles in population A. Only population C appears to be monophyletic with respect to this genetic marker.

a favorite tool for studies of population structure, hybrid zones, and closely related species (4, 7, 71, 157). In contrast, the slow average rate of cpDNA evolution has meant that plant biologists have had greater difficulty identifying molecular markers appropriate for studies at the level of speciation (e.g. see 15, 119, 136).

It should be noted here that we can distinguish between tokogenetic (birth) relationships among individuals within populations and phylogenetic relationships of species or higher taxa. Some cladists (115) have argued that hierarchical phylogenetic methods are not appropriate for analysis of relationships within species, because of reticulation (interbreeding). However, these authors acknowledge that phylogenetic methods can be applied to clonally inherited molecules [mtDNA, cpDNA, short (nonrecombining) segments of nuclear DNA] (49, 115). Debates about the appropriate use of terminology or methodology should not obscure the essential point—that it is necessary to distinguish clearly between allele phylogenies and organismal phylogenies and to understand the correspondence between them.



Both differential introgression and random sorting of ancestral polymorphisms can lead to discordance between gene trees and species trees (71, 110, 114, 121, 139, 141, 143, 159). Hybridization between species (or gene exchange between populations) may result in incorporation (possibly fixation) of alleles from one species in the gene pool of the second species (58, 73). This pattern of introgression may be limited to one or a few markers—mtDNA in the case of populations of house mice (58, 67) or voles (145) in Scandinavia; use of these markers for phylogenetic analysis will obviously give a different view of population histories than would markers that show no evidence of introgression.

A more general (and perhaps more serious) problem is that, when there is allelic variation within species, an allele phylogeny will not necessarily have the same topology as the species phylogeny. If polymorphisms persist through speciation events, the probability that the gene tree and the species tree have the same topology may be quite small. This is considered in more detail later in the review.

## *Neutral Theory of Molecular Evolution*

The neutral theory of molecular evolution (82) explains both polymorphisms within species and accumulation of amino acid or nucleotide substitutions over time in terms of mutation and random drift. It has provided an extremely important frame of reference for both molecular population geneticists and molecular systematists, and even its most severe critics agree that it is "the most widely held theory of molecular evolution" (64).

If alleles are strictly neutral, then variation within populations is a function of mutation rate ($u$) and effective population size ($N$); spatial patterns of variation are determined by these parameters and by the dispersal or migration rate ($m$). If population size and population structure are not constant over time, observed levels and patterns of variation will depend not only on current values of $N$ and $m$, but also on long-term effective population size and past gene flow (110, 133). Therefore, differences between observed and "expected" values may provide important clues to population histories, including the nature of recent speciation events. Expected values are those calculated using neutral theory and observed (or assumed) values of $u$, $N$ and $m$. For example, Nei & Graur (111) attribute the low levels of heterozygosity in

many species (compared with expected values) to population bottlenecks during recent periods of glaciation. Similarly, the genetic distances among mtDNA haplotypes in red-winged blackbirds, American eels, and hardhead catfish are far lower than expected based on estimates of current population sizes (8). To explain the discrepancy, Avise et al (8) suggest either that mutation rates are dramatically reduced in these species (considered unlikely) or that long-term effective population sizes are significantly smaller than current population sizes.

Population bottlenecks and founder events are often invoked as critical elements in speciation processes (see below). The decline in genetic variability during a population bottleneck depends both on the size of the bottleneck and the rate of population growth (38, 113). If the bottleneck is brief, with population size increasing rapidly, the reduction in heterozygosity is small (although many or most rare alleles will be eliminated). Only if small population size persists for many generations will the reduction in heterozygosity be substantial (17). Population bottlenecks also lead to a rapid increase in genetic distance between populations, although this effect gradually disappears once the populations returns to the prebottleneck size (38, 110). Note that this result (based on a strictly neutral model) does not imply that founder events or population bottlenecks accelerate the rate of molecular evolution. The increase in genetic distance is a result of changes in gene frequencies and does not reflect a change in the rate of accumulation of new mutations (157). However, if most mutations are very slightly deleterious, rather than strictly neutral, the rate of evolution is inversely proportional to population size (82, 116, 117). In small populations, slightly deleterious mutations are effectively neutral and may drift to fixation; in large populations, these mutations are consistently eliminated by selection. Therefore, under the model of slightly deleterious alleles, population bottlenecks are expected to increase the rate of evolution (150).

Different genetic systems will respond differently to fluctuations in population size and to migration rates (150). Comparisons between organelle genes and nuclear genes may be especially instructive. Because organelle genes are generally homoplasmic (invariant within individuals) and inherited uniparentally, the effective number of organelle genes is one fourth that for nuclear genes when the sex ratio of breeding individuals ($N_m/N_f$) is 1:1 (28). As a consequence, in comparison with nuclear genes, the time to fixation or loss of neutral alleles is shorter for mitochondrial or chloroplast genes, the expected haplotype diversity at equilibrium is lower (assuming equal mutation rates), the extent of population subdivision is greater, and the loss of variability during a population bottleneck is more extreme (28, 150, 157). In both *Daphnia pulex* and *Drosophila mercatorum*, comparisons of mtDNA and allozyme data reveal greater population subdivision for mtDNA than for



nuclear gene markers (45, 51). In the case of strict maternal inheritance (mtDNA in most animals and cpDNA in many plants) the fourfold difference is reduced or eliminated when $N_m/N_f<1$ (e.g. when males have harems). In contrast, male-biased dispersal will increase the difference between organelle and nuclear genes in their tendency to show population subdivision (28). Within the nuclear genome, X and Y chromosome markers will behave differently from autosomal markers, in general exhibiting greater sensitivity to founder events (150).

Even the most dedicated supporters of the neutral theory acknowledge that alleles at some loci are likely to be under strong directional and/or balancing selection. The frequencies of neutral alleles at one locus will be influenced by the impact of selection on linked loci. In particular, directional selection leading to the fixation of a new variant at one locus can result in a loss of neutral variation at linked loci (81, 96). The magnitude of this "hitchhiking effect" will depend upon the strength of selection (the rapidity with which the new mutation sweeps through the population) and the rate of recombination between the selected and neutral loci. For organelle genomes that lack recombination, hitchhiking effects may be of considerable significance (93). For example, recent selective sweeps could explain the lower than expected genetic distances among mtDNA haplotypes observed in the three wide-ranging vertebrate species studied by Avise et al (8). Even if relatively few variants are under direct selection at a given time, linkage and hitchhiking effects may extend their sphere of influence across significant portions of the genome (2).

## *Molecular Clocks*

According to the neutral theory the rate of gene substitution is simply equal to the neutral mutation rate per locus (82). As long as mutation rates are constant over time, nucleotide substitutions will accumulate in clock-like fashion, and the number of substitutions (and other measures of genetic distance) will be proportional to time since lineage splitting. The reality and constancy of "molecular clocks" (160) are obviously of enormous interest to students of speciation, since they potentially provide a framework for dating speciation events. Substitution rates vary among genes (due in part to variation in selective constraints) and also among evolutionary lineages (explained by generation-time effects or by differences in the efficiency of DNA repair) (29, 89, 110).

Molecular clocks must be carefully calibrated, using divergence times based on the fossil record or on known vicariance events. A number of protein electrophoretic clocks have been proposed, each with a characteristic value of a constant $k$ (where $t = kD$, $t$ being time of divergence and $D$ some measure of genetic distance). Unfortunately, values of $k$ vary by a factor of 20, suggest-

ing that protein electrophoretic clocks can at best give a very rough estimate of absolute times of divergence (6, 77). Calibrations of a mtDNA clock in a number of different vertebrate lineages suggest an initial average rate of divergence of 1% per million years per lineage (31, 131, 157). In primates, the relationship between time and amount of sequence divergence appears to be linear up to about 10–15% sequence divergence. In fact, there is significant heterogeneity in rates, among different coding regions, between synonomous and nonsynonomous substitutions, and between transitions and transversions (107). Thus, mtDNA sequences potentially harbor several different molecular clocks, each ticking at a characteristic rate. Unfortunately, it is not at all clear that clock rates are constant across lineages (107, 154). If we cannot extrapolate across lineages, it may be difficult (impossible) to obtain calibrations for organisms with poor fossil records.

Gillespie (61–64) has documented that rates of molecular evolution are often more variable than expectations based on a simple Poisson mutation process, leading him to suggest that patterns of both protein evolution and silent site evolution of DNA are not consistent with neutral theory. He proposes that molecular evolution is episodic (61) and is best explained by models invoking natural selection. Takahata (140) acknowledges that the molecular clock is "overdispersed" but suggests modifications of neutral theory to account for the observations. Regardless of which interpretation is correct, attempts to apply molecular clocks to comparisons of closely related species may be especially risky.

For much of what follows, it is convenient to start with the neutral theory as null hypothesis, as long as we do not fall into the trap of uncritically accepting it as truth (108).

## THE IMPACT OF SPECIATION ON MOLECULAR GENETIC VARIATION

Evolutionary biologists have traditionally classified speciation events on the basis of the geographic context in which initial divergence occurs (34, 97, 99, 156). Allopatric, parapatric, and sympatric models of speciation describe situations in which divergence occurs in geographically isolated populations, in contiguous but nonoverlapping populations, or between subpopulations at a single locality. In fact, these situations represent points along a continuum defined by the amount of gene flow ($m$) between diverging populations (57). More recently, Templeton (146–149) stressed the need for a mechanistic taxonomy that can provide a population genetic framework for understanding speciation processes. He also pointed out that both ancestral population structure and type of split (geographic subdivision) influence the probability of particular modes of speciation and the extent of genetic differentiation among sister species (146, 147).



An appropriate context for understanding the impact of speciation on patterns of molecular genetic variation would seem to be an amalgam of the traditional and mechanistic taxonomies outlined above, augmented by an explicit phylogenetic approach. The important components of this framework are (*a*) ancestral population structure, (*b*) nature of the sampling event(s) giving rise to daughter species, (*c*) role of natural selection (consequences of "selective sweeps"), and (*d*) genetic architecture of speciation.

### *Population Structure*

For any pair of sister species (or monophyletic group of species), the genetic structure of the common ancestral population(s) will be an important determinant of current patterns of variation. Genetic structure refers both to the total amount of variation and to how that variation is apportioned within and among populations. For DNA restriction site or sequence data, population structure can be characterized using an explicitly genealogical approach to calculate expected times to common ancestry for alleles from the same or different populations (79, 110). Taking advantage of the phylogenetic information that may be contained in DNA sequences, the genetic structure of a species can be clearly portrayed by superimposing a gene genealogy on a distribution map. Avise (5, Avise et al 7) has coined the term "intraspecific phylogeography" to describe this approach and has emphasized the utility of animal mtDNA for analyses of population structure.

The genetic structures of a wide variety of animal and plant species have been documented using as markers variants detected by protein electrophoresis (69, 90, 130). All possible structures have been found—ranging from high levels of polymorphism to virtual monomorphism, from homogeneity of allele frequencies across the entire range of widely distributed species to remarkable population subdivision on a very small spatial scale. Similarly, geographic surveys of mtDNA restriction site variation have revealed striking genetic structuring of populations in some animal species and absence of variation or homogeneity across vast distances in other taxa. For example, in American eels (10), red-winged blackbirds (14), monarch butterflies (40), crested newts (155) and sea urchins (120), there is relatively little sequence divergence among the observed mtDNA haplotypes and no evidence of geographic differentiation. In contrast, distinct phylogenetic assemblages (differing by 2–9% in mtDNA sequence) are geographically localized (often parapatrically distributed) in pocket gophers (9), field mice (11), grasshopper mice (125), desert tortoises (85), American oysters (124), and several species of sunfish (26). The observed "phylogenetic discontinuities" often correspond to current barriers to gene flow or to historical barriers inferred from regional geology and paleoclimatic reconstruction. Rarely do species show large amounts of sequence divergence among mtDNA haplotypes in the absence of geographic structure. Genetic structure revealed by

ntDNA comparisons is not always reflected in patterns of allozyme variation; oth oysters (32) and field mice (12) appear relatively homogeneous in allele equencies for nuclear gene markers. It may be unwise to extrapolate from bservations of mtDNA variation, given the difference between organelle and uclear markers in extent of population subdivision, especially in animals ith male-biased dispersal. Clearly genetic structure depends both on the ology of the organism and on the properties of the genetic marker.

Observations of current population structures can be used to infer ancestral pulation structure, but because "the demographies of populations have been markably dynamic and unsettled over space and recent evolutionary time" ), there are obvious problems with this approach. Population structure rtainly varies among congeneric species (2, 36, 39). However, data from tant populations at least provide insights into the range of population ructures commonly encountered in particular groups of organisms. Using ese data, we can begin to define how the amount of polymorphism within pulations and the extent of population subdivision interact with mode of eciation to produce patterns of variation in descendant lineages (species).

## *ow Is Variation Partitioned at Speciation?*

the process of speciation, a single lineage is split into two (or more) dependent lineages, each of which potentially carries a different sample of variation that existed in the ancestral species. For molecular markers that "neutral" with respect to speciation, the geography of the partition and the ount of gene flow at the time of divergence interact with the ancestral pulation structure to determine the extent of genetic differentiation (genetic tance) and the phylogenetic relationships of the descendant lineages (114, 6; see Figure 2). The demographic histories of the diverging lineages bsequent to the initial split) also influence amounts and patterns of varia- n.

It is difficult to construct a single, hierarchical classification scheme of mpling events. At one extreme, founders of a lineage leading to a new cies may be drawn from a single local population. The founder population y be a relictual population isolated by a vicariance event or a group of persing individuals (a single female?) that successfully colonize previously ccupied habitat. Sympatric speciation events may also involve founders wn from one local source area; in this case gene flow between sub- ulations persists during at least the initial stages of divergence. In all three dels, the genetic variation present in the founder population will be a mple of the variation found in a single local population; the extent to which sample reflects overall levels/patterns of variation in the ancestral species iously depends on ancestral population structure. If there is significant rogeneity among populations, the founder will represent only a small



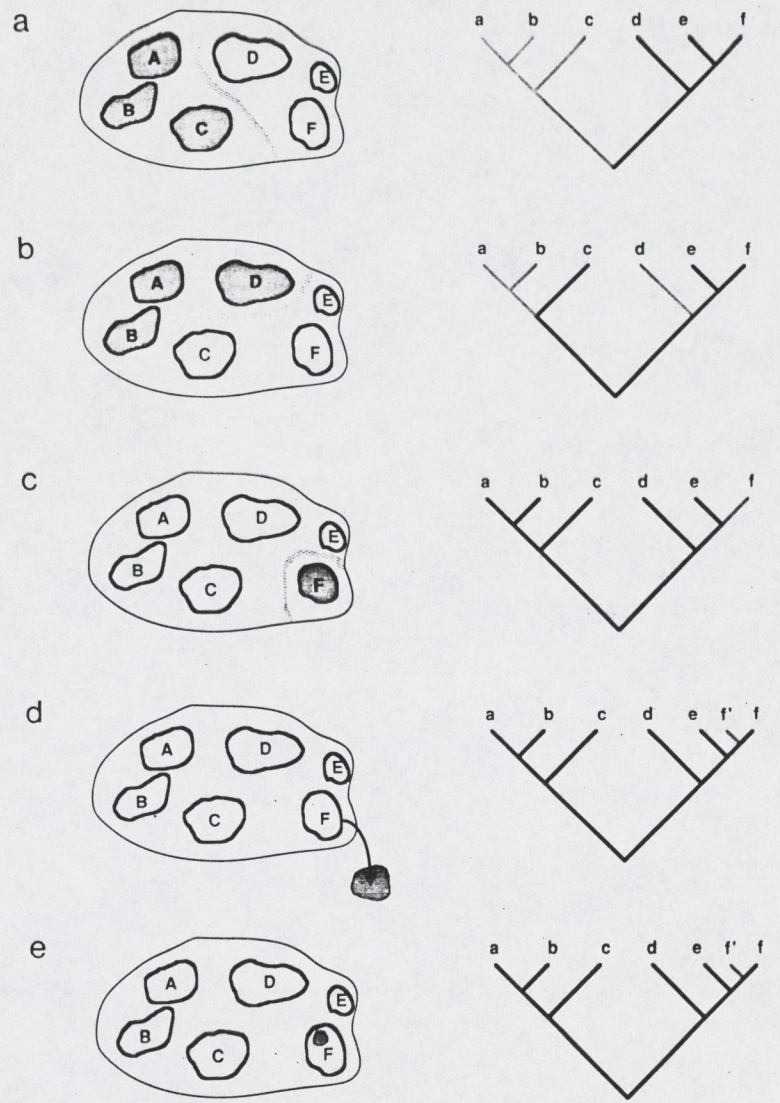


*Figure 2* Five models of speciation and corresponding gene trees, showing distribution of alleles in the two daughter species. For simplicity, each population is represented as monomorphic, the gene tree in each case is (((a,b)(c))((d)(e,f))) and speciation is assumed to occur instantaneously. The five models are: (*a*) speciation by subdivision with the partition congruent with existing phylogenetic discontinuity; (*b*) speciation by subdivision with partition not congruent with existing phylogenetic discontinuity; (*c*) divergence of peripheral population; (*d*) colonization of new habitat by propagule(s) from single population; and (*e*) local sympatric speciation. In each allele phylogeny the dark lines represent lineages occurring in one daughter species, the light lines represent lineages found in the other daughter species.

fraction of the variation found in the ancestral species. Individuals giving rise to a new species need not be drawn from a single population but, at least in theory, could be a random sample of the entire ancestral species (i.e. including representatives from throughout the range of this species). Perhaps more realistic are models that invoke partitioning of a single species into two (or more) major geographic subdivisions (Figure 2). Such models may involve either allopatric or parapatric divergence. Each daughter lineage derives from many founders and contains much of the variation found within a broad geographic area.

Apart from considerations of the impact of founder events, there have been few general discussions of the consequences of different modes of speciation for patterns of molecular genetic variation. For a given combination of ancestral population structure and type of split (sampling event), it should be possible to define expectations for (*a*) amounts of variation, (*b*) geographic patterns of variation, (*c*) genetic distances (between sister species) and (*d*) allele phylogenies.

Templeton (146) has explored the relationship between mode of speciation and expected amount of genetic differentiation between the daughter species. For an adaptive divergence model, with the ancestral population split into large subpopulations, he predicts that initial genetic distances may be quite large. This follows from his assumptions that adaptive divergence is most likely when the ancestral population consists of many small demes and that the split occurs "along geographical lines." Templeton suggests that speciation via genetic transilience or founder effect (which should occur most frequently when founders are derived from large, panmictic populations) will result in relatively low initial genetic distances, comparable to those seen between demes of the ancestral species. However, he cautions that there is no simple pattern between mode of speciation and genetic distance."

An alternative (and ultimately more powerful) approach is to examine expected gene genealogies when alleles are sampled from two recently diverged populations (species) (Figure 2). The simplest case is to assume that each population consists of $N$ individuals, that there is no selection or recombination and no gene flow between the populations (which diverged $t$ generations ago). Under these assumptions, the probability of obtaining certain types of evolutionary relationships (tree topologies) depends on the population size ($N$) and the time since divergence ($t$) (139, 143). When $t <$ , the probability that the allele phylogeny will accurately reflect the recent population history is low, but this probability increases with increasing $t$, the precise relationship depending on the number of alleles sampled from each of extant populations (143). When the ancestral population is polymorphic and extinction of lineages is random, it may often be the case that haplotypes found in different species are more similar than are some haplotypes found



within conspecific populations (i.e. interspecific coalescences will occur (going backwards in time) before some intraspecific coalescences). For example, analysis of *Adh* haplotypes in *Drosophila pseudoobscura* and *D. persimilis* indicates that the *D. persimilis* haplotype "falls within the cluster of *D. pseudoobscura* haplotypes" (128). If some type of balancing selection maintains variation within populations, polymorphisms may persist for millions of years (across several speciation events). This appears to be the case for polymorphisms at the major histocompatability complex loci in rodents and primates (59, 87, 100, 144) and at the self-incompatability locus in Solanaceae (80).

Clearly, both the nature of the divergence (speciation) event and the ancestral population structure will influence the probability of obtaining particular allele phylogenies. Neigel & Avise (114) have used computer simulations to explicitly model phylogenetic sorting of mtDNA lineages across speciation events. Founders were either chosen at random from the entire array of haplotypes present at the time of the split, at random from different halves of the array, or from opposite ends of the array. The latter two sampling regimes are equated with daughter species arising from geographically isolated populations, i.e. the ancestral species is assumed to have distinct genetic structure, such that geographically distant populations are characterized by haplotypes that are far apart on the mtDNA tree. Neigel & Avise (114) examined the "time to expected monophyly" (time until the allele phylogeny reflects the population history) and showed that it is dependent on both the geography of the original partition and the number of founder individuals for each of the daughter species. When the daughter species come from distinct geographic areas, there is a greater chance that at least one of the diverging lineages will appear monophyletic (lineage sorting will have occurred prior to speciation).

Population bottlenecks during speciation will decrease the time to expected monophyly, because $N$ will be very small and even when time since divergence is short, it will likely be $> 4N$ ($4N$ generations is the expected coalescence time for neutral alleles in a population—110). Bottlenecks force most (or all) lineages to coalesce in a short period of time, the pattern of coalescence depending on the severity and duration of the bottleneck. Therefore, all intraspecific coalescences will likely occur prior to interspecific coalescences (again going back in time), and allele and species phylogenies will be equivalent (79).

Can we increase our confidence that observed allele phylogenies provide an accurate reflection of recent populations history? Pamilo & Nei (121) argue that the consistency probability between gene trees and species trees cannot be increased significantly by increasing the number of alleles sampled from each population (species). They conclude that it is necessary to obtain sequence

from several independent (unlinked) gene regions. Thus, the apparent ntages of DNA sequence data for reconstructing phylogenies of closely d species may only be fully realized when a number of unlinked genes xamined (42, 79, 121, 159; but see 141). Most published studies have ved comparisons of only one gene (usually one organelle genome).

## nder Effect Speciation

ng-standing debate in evolutionary biology concerns the genetic con- nces of founder events and their implications for the origin of species 37, 123). The debate centers not only on the possibility that founder s are triggers for speciation in certain lineages, but on the nature of the ic and demographic processes that can provide the appropriate trigger. n & Templeton (37) outline three distinct models of founder effect ation: (*i*) Mayr's original model of "genetic revolution" (98), in which ation occurs in peripheral populations, results in substantial loss of ic variation and involves much of the genome; (*ii*) Carson's founder- models (available in several varieties—see 123) in which a population (and associated relaxation of selection) follows immediately on the heels bottleneck, resulting in minimal loss of genetic variation; and (*iii*) leton's genetic transilience model (147), in which founders derived an outcrossed and highly variable ancestral population experience a brief neck (an altered genetic environment), again with minimal loss of ion. Carson & Templeton (37) suggest that there is "little chance of ation if a significant drop in levels of genetic variation occurs." In st, Barton & Charlesworth (17) reject the argument that founder events to lead to speciation will involve only minor loss of variation (models 2 ), asserting that "population bottlenecks small enough to cause peak will inevitably cause a substantial and prolonged loss of variability at l loci." Therefore, inferences about founder effect speciation from ns of molecular genetic variation depend critically on details of the l.

tured prominently in the debate about founder effect speciation are the iian *Drosophilia*, which have been the inspiration for Carson's founder- models. The observation that recently derived Hawaiian species are no ariable at allozyme loci than many continental *Drosophila* suggests to n & Charlesworth (17) that founder events have not been important in tion, whereas Carson & Templeton (37) see the relatively high zygosity as in conflict with Mayr's model but precisely the "pattern ed under the founder-flush and genetic transilience models." Barton lso views the relatively high mtDNA haplotype diversity (and sequence ence among haplotypes) in *D. silvestris* and *D. heteroneura* (50) as ce against founder effect speciation.



Neither the founder-flush nor the genetic transilience modes of speciation will leave an unambiguous signature on patterns of molecular genetic variation, although loss of mtDNA variation without accompanying decline in heterozygosity at nuclear gene loci may be indicative of a severe but very brief population bottleneck (118). Intense and prolonged population bottlenecks will have a major impact on variation in mtDNA and nuclear gene markers. In some taxa genetic variation in one species represents a "depauperate subset" of variation found in a more widely distributed sister species (91). In other cases, there is almost complete absence of variation in one member of a clade, whereas congeners have "normal" levels of heterozygosity (101). Such patterns suggest that population bottlenecks have been associated with speciation. In contrast, the remarkable persistence of MHC polymorphisms in primates (e.g. shared polymorphisms between humans and chimpanzees) argues against any significant bottlenecks in these lineages (142). A direct correspondence of population bottlenecks with speciation is virtually impossible to prove. A bottleneck subsequent to lineage splitting would erase any characteristic signature left by the speciation event.

## Speciation by Subdivision

Vicariance events partition the range of a single species into two or more subdivisions and may ultimately lead to allopatric divergence of populations and to speciation. The consequences for patterns of genetic variation depend on the initial population structure and the nature of the partition. For species in which genetic variants are geographically localized—e.g. grasshopper mice (125), desert tortoises (85), sunfish species in southeastern United States (26)—speciation may involve further divergence between what are already distinct phylogenetic assemblages. Comparisons of mtDNA haplotypes in field mice indicate that *Peromyscus maniculatus* is paraphyletic with respect to *P. polionotus* and that the latter species is monophyletic (11). *P. polionotus* is restricted to the southeastern United States, whereas *P. maniculatus* is widespread throughout North America but does not overlap with *P. polionotus*. The observed pattern is, as one would expect if *P. polionotus* is a "peripheral isolate", recently diverged in allopatry (Figure 2). The *P. polionotus* clade harbors a diversity of haplotypes equivalent to that found within each of several genetically distinct geographical assemblages of *P. maniculatus*, suggesting a simple vicariance event without a significant bottleneck. In this case, geographic distribution and pattern of molecular genetic variation together provide strong support for a particular model of speciation.

It is not obvious that we should always expect the partition that occurs during speciation to be congruent with existing phylogenetic discontinuties (which may have been produced by previous vicariance events). The outcome of speciation, in terms of genetic distance or gene genealogies, is therefore

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table, but neither daughter species may appear monophyletic (Fig- allopatric speciation (subsequent to a vicariance event) occurred in which common haplotypes were widely distributed, the daughter ould be expected to share ancestral polymorphisms until lineage sulted in monophyly with respect to genetic markers.

### *ic Speciation*

quences of sympatric speciation for patterns of molecular genetic depend on details of the speciation model. Virtually all scenarios for divergence start with a stable polymorphism affecting performance nt resources or in different habitats (52, 95). Speciation involves the of an association between this polymorphism and alleles at loci assortative mating (often mediated by habitat or resource selection). tric speciation occurs at a single locality within the range of a d ancestral type, the daughter species, having shifted to a new resource, can then spread. The outcome, in terms of patterns of genetic variation, may not be distinguishable from that produced by event—i.e. one species will contain a subset of the variation found er species, the precise pattern depending on the population structure estor. Alternatively, in cases of sister species sympatric over a broad host races of *Rhagoletis pomonella* (33)), current distributions may ultiple "speciation events" (host shifts). In *R. pomonella*, allelic and heterozygosity are as high in the derived apple race as in the hawthorne race (56). This would seem to argue against a single local owever, it is possible that the scenario of the apple race arising in the Valley in the 1860s is correct (33), but that repeated episodes of sion have introduced additional variation. Inferences about origins er complicated by the the apparent importance of selection in de- g allele frequencies at allozyme loci (56), producing latitudinal varia- llozyme frequencies in both host races.

n geographic patterns of variation are not consistent with sympatric For example, based on mtDNA haplotype analysis, the normal and enotypes of whitefish found sympatrically in northern lakes belong to nonophyletic assemblages that are primarily distributed allopatrically is suggests that the sympatric pairs are the result of secondary contact s that differentiated in allopatry, rather than originating through ce in situ.

### *ion by Hybridization*

ar markers have proved especially informative in examining the rigins of polyploid plants and unisexual animals. The markers have ed both to establish the identity of maternal and paternal parental



species and to provide information on origins. Low levels of sequence variation within certain unisexual lizards—e.g. *Cnemidophorus tesselatus* and *C. neomexicanus* (48), *C. sexlineatus* (47)—suggest that these species are of recent origin. Lack of differentiation between the cpDNAs of polyploid and diploid representatives of the *Glycine tabacina* complex (54) suggests that not much time has elapsed since the hybridization events that gave rise to the polyploids. Indeed, recent origins appear to be the rule. Furthermore, in a number of different systems, allozymes, cpDNA, or mtDNA have provided evidence for multiple origins of unisexual (55, 78, 106) or polyploid (54, 136) lineages. Obligate parthenogenesis in *Daphnia pulex*, although not of hybrid origin, is also clearly polyphyletic (46). Finally, molecular markers have been used extensively to document species formation through introgression in plants (126).

### *Impact of Natural Selection*

If speciation is accompanied or followed by episodes of directional selection, resulting in the fixation of new alleles (e.g. one of the daughter species occupies a "new" niche or habitat), hitchhiking will result in loss of variability for markers closely linked to the loci under selection (79, 81). The impact of natural selection will depend on the number of loci under selection and the relative magnitudes of selection coefficients and rates of recombination. If few loci are involved, only a small proportion of the genome will be affected. If many loci, distributed across all chromosomes, are involved, the loss of variation could be pervasive and the molecular genetic consequences could mimic those produced by a population bottleneck. If selective sweeps are very rapid, variation may be eliminated in the region surrounding the locus under selection (i.e. linkage disequilibrium will persist throughout the course of the selective sweep). If selection coefficients are small and divergence very gradual, the amount of neutral variation at linked loci may remain close to that expected at equilibrium.

Some genetic systems appear to lack recombination entirely (e.g. animal mtDNA) and will be particularly sensitive to periodic directional selection (93). In the Drosophila nuclear genome, recombination is reduced near the base and tip of the X-chromosome, and genes that map to these regions have reduced levels of variation within species (but not reduced levels of divergence between species) (2). This is the pattern expected if directional selection and hitchhiking are important in determining patterns of molecular genetic variation. At this juncture, we do not have sufficient data on the role of directional selection during speciation or on rates of recombination in organisms other than those favored by geneticists. It is clear, however, that the impact of directional selection can mimic that of a founder event (for a linked group of markers) and render suspect interpretations of recent popula-

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tion histories based on assumptions of neutrality. Analyses of patterns of variation for several unlinked molecular markers will be necessary if we are to have confidence in interpreting these patterns.

## GENETIC ARCHITECTURE OF SPECIATION

The genetic architecture of speciation refers to the number, effect (large or small), and chromosomal distribution of genes that contribute to reproductive (genetic) isolation. Templeton (148) distinguished three alternative architectures: (*i*) many segregating units (each presumably of small effect) (type I), (*ii*) one or a few major segregating units, with many epistatic modifiers (type II), and (*iii*) complementary pairs of loci (type III). He argued (149) that only in cases of speciation by slow adaptive divergence are many genes likly to be involved and that more often speciation involves a type II architecture (especially in founder event speciation). These views are by no means universally accepted. Based on both theory and observations (from experimental crosses and hybrid zones), Barton & Charlesworth (16, 17) suggest that reproductive isolation usually depends on changes at many independent gene loci and that "there is no simple relationship between genetic architecture and the likely mode of speciation" (16). Using cline theory, estimates of the number of genes responsible for isolation are 150 for chromosomal races of the grasshopper *Podisma pedestris* (18, 19) and 50–300 for the hybridizing frogs *Bombina bombina/B. variegata* (138).

Patterns of differentiation (or introgression) for molecular markers depend on the underlying genetic architecture of speciation. If only a few major genes are involved, differentiation of neutral markers may only occur (or persist) for a small segment of the genome (regions closely linked to the few genes under selection) (19). If many genes, each of small effect, are involved, divergence will be more uniformly distributed across loci. In a hybrid zone, the flow of neutral alleles across the zone will be retarded to the extent that they are linked to loci that affect hybrid fitness and/or positive assortative mating (20, 21, 23, 72). Many hybrid zones show clear evidence of differential introgression, i.e. there is substantial variation in the extent to which molecular markers (allozymes, rDNA, mtDNA, Y-chromosome markers) penetrate hybrid zones (72). The tendency for mtDNA to introgress more extensively than nuclear gene markers is usually attributed to its segregating independently of all nuclear genes (22, 71). However, in no single hybrid zone have sufficient markers been examined to clearly define the underlying genetic architecture. With techniques borrowed from molecular genetics (identification of restriction fragment length polymorphisms either by traditional surveys using cloned probes or newer PCR based random amplification techniques), it will be possible not only to shed light on genetic architecture but ultimately to map



genes responsible for components of the mate recognition system or for post-zygotic barriers (86, 122).

In a few cases, information on genetic architecture is available. For *Drosophila* there is convincing evidence for a major effect of the X chromosome on post-zygotic isolation and this "rule of speciation" may well extend to other groups of animals (44). The host races of *Rhagoletis pomonella* are consistently differentiated at six loci coding for soluble enzymes (out of a much larger number surveyed) (56). These six loci map to three discrete regions of the genome, suggesting that differentiation of the host races may depend on a limited number of genes. This is consistent with a model of recent sympatric origins.

## INFERENCES FROM GENETIC DISTANCE

### *Genetic Distance as an Indicator of Species Status*

The taxonomic status of allopatric populations is difficult to determine when we employ a species concept based on genetic exchange or field for recombination. In these situations a phylogenetic species concept (115) can be more consistently applied. However, if the evolution of genetic isolation were simply time-dependent and if there existed a reliable and universal molecular clock, genetic distance would be a good predictor of species status. In *Drosophila*, there is a significant correlation between genetic distance (based on allozyme data) and the extent of either pre- or post-zygotic isolation measured in the laboratory (43). Using this relationship and an estimate of the degree of isolation required to prevent fusion of sympatric populations, Coyne & Orr (43) calculated a threshold value of *D* beyond which populations of *Drosophila* appear to be distinct species. It is intriguing that this value is much lower for sympatric pairs than for allopatric pairs, suggesting that species status is achieved approximately twice as fast in sympatry as in allopatry.

In other groups of organisms, genetic distance is not a good predictor of degree of genetic (reproductive) isolation. For example, in sea urchins on either side of the Isthmus of Panama there is "no correlation between genetic divergence and strength of reproductive isolation" (88). In pocket gophers (genus *Thomomys*) speciation is "unrelated to the level of genetic differentiation between populations" (68).

Thorpe (152) plotted the distributions of genetic identities (based on allozyme data) for conspecific populations and congeneric species in a wide variety of taxa. He concluded that "if allopatric populations of dubious status have genetic identities below 0.85 it is improbable that they should be considered conspecific, while nominate species with I values above 0.85 should be considered doubtful if there is no other evidence of their specific

status" (152). This suggestion has met with some approval, although support is certainly not unequivocal (13, 24, 102, 110). One obvious flaw is that birds were intentionally excluded from the analysis, because "their speciation processes seem to differ fundamentally from those of most other organisms" (152). This assessment was based on the observation that many avian sister species have values of I significantly greater than 0.85.

In fact many species pairs in a variety of groups of animals and plants show little genetic differentiation (1, 25, 60, 70, 74, 83, 102, 132, 153, 158). One of the most striking recent examples is an analysis of mtDNA variation in cichlid fishes from Lake Victoria (103). The "species flock" in Lake Victoria comprises some 200 species and yet appears to harbor little genetic variation (only about 2% of the surveyed nucleotide sites are variable in the 14 species examined). In contrast, subspecies of sunfish often differ by 6–10% in mtDNA sequence (26). In field crickets, closely related but clearly distinct species share mtDNA haplotypes, whereas geographically isolated populations of single species may have haplotypes that differ by 1–2% (R. G. Harrison, S. Bogdanowicz, unpublished data). Such observations suggest that extreme caution must be used in assessing species status on the basis of estimates of genetic differentiation. This is not unexpected, given the diverse genetic architectures and population histories that can lead to the evolution of barriers to gene exchange.

### *Can We Date Speciation Events?*

One of the most appealing characteristics of electrophoretic, restriction site, or DNA sequence data is the possibility that they can provide reliable molecular clocks for estimating absolute divergence times between species. In fact, clock calibrations for allozyme data are remarkably variable (6), due in large part to the lack of independent estimates of the times of speciation events. Clocks based on animal mtDNA may be more reliable, although there is still considerable debate about whether rates are the same in all lineages (e.g. see 154).

However, for recent divergence events there are problems other than those associated with clock rates. Even if substitutions accumulate at a constant rate, lineage sorting due to sampling of alleles (haplotypes) at speciation and subsequent random lineage extinction may introduce significant errors, especially when little time has elapsed since speciation. Obviously, this is only a problem when the ancestral population (from which the daughter species are "sampled") is polymorphic. In such cases, it is clear that the divergence of gene sequences must precede population splitting and therefore current measures of sequence divergence can only overestimate the time since speciation (110, 143). Another way to think about the problem is to acknowledge that



the amount of divergence at the time of speciation is not zero (151) and to apply a correction based on current levels of intraspecific variation (110, 112, 157). However, this correction assumes that levels of variation in the daughter species accurately reflect the amount of variation found in the ancestral population. Furthermore, the net number of nucleotide substitutions ($d$) has such a large variance when $d$ is small that estimates of divergence times will be very unreliable (110, 143). This problem will be compounded if population bottlenecks have occurred. In addition, there may well be variation in the estimate of time since divergence based on comparisons of different molecular markers. This could reflect differences in the level of polymorphism at the time of speciation (151), or it could be a result of differential introgression or natural selection.

Finally, there is the possibility that speciation events themselves accelerate rates of divergence and therefore estimates of genetic differentiation will be proportional to the number of speciation events rather than to time. An early test of this hypothesis (3) compared rates of protein evolution in two groups of fishes of supposedly equal age, but with very different rates of speciation; it found no evidence that speciation accelerates protein divergence. This study has been criticized, primarily because the groups being compared do not in fact appear to satisfy the assumptions underlying the test (94). More recently, Mindell et al (104) presented evidence based on allozyme data in *Sceloporus* lizards and concluded that "punctuational change is at least a viable explanation" for their observations. This question obviously deserves more careful attention.

## CONCLUSIONS

With recent advances in DNA technology, it is clear that we have arrived at a critical juncture in the application of molecular markers in systematic and evolutionary biology. DNA sequences can provide remarkably detailed views of patterns of variation within species and phylogenetic relationships among species. The challenge is to understand what these patterns reveal about evolutionary history and about evolutionary process.

In studies of speciation, evolutionary biologists must first clearly define how particular modes of speciation influence patterns of molecular genetic variation. Building on the work of others (5, 7, 79, 110, 114, 121, 139, 141, 143, 146, 148–150), I have attempted in this review to provide an introduction to major issues in molecular studies of speciation and an assessment of the problems encountered in resolving these issues. Debate over the nature and consequences of founder event speciation emphasizes how difficult it can be to infer process from pattern. It is evident that speciation events do not

leave unique signatures and that it will be impossible to "prove" that a certain series of historical events has occurred. However, from patterns of molecular genetic variation, together with detailed knowledge of the biology of organisms, it will be possible to eliminate certain models or at least assign high probabilities to some scenarios and low probabilities to other scenarios. For example, it may be possible to show that recent bottlenecks or sympatric origins are unlikely explanations, based on both haplotype diversity and spatial distributions of haplotypes (e.g. see 142).

Especially important is the need to generate sequence data from several many) unlinked genes, and to compare genealogies (phylogenies) based on hese independent markers. Because a set of completely linked characters e.g. an organelle genome) behaves as a single unit in random lineage xtinction and introgression, an allele phylogeny based on a single gene may ot reflect population history. However, unlinked markers will sort independently at divergence events and by using many such markers we can ncrease our chances of obtaining the "correct" species tree (121). Data from nlinked markers may also allow us to discriminate between reduction in ariation due to selective sweeps and reduction in variation due to population ottlenecks. Such data should also allow evaluation of the importance of ifferential introgression as a confusion in constructing phylogenies and ating divergence events. It is essential both to build on the already substantial data base from cpDNA and animal mtDNA and to embark on a rogram of characterizing and comparing nuclear gene markers.

The ultimate frontier in studying the genetics of speciation is to be able to ap (and eventually clone) genes responsible for prezygotic and postzygotic rriers to gene exchange. Producing detailed RFLP maps for species of terest is a first step in this process. Techniques for generating markers and r analyzing the results of crosses are now available (86, 122). The task is a rmidable one, but provided with the necessary resources, evolutionary ologists should now be able to make significant progress in clarifying the netics of the speciation process.

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# THE THEORY AND PRACTICE OF BRANCH AUTONOMY

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KEY WORDS: carbon allocation, hydraulic architecture, source-sink relations, stress isolation, scaling

## INTRODUCTION

The past 15 years have seen a surge of interest in modularity in plants; that is, in the implications of the fact that plants are composed of repetitive modules that may in some ways behave as in independent units (32). Much has been written about the importance of modularity in plant population biology (e.g. 33, 34, 113, 114), and also about the advantages and disadvantages of independence or interdependence among separate but connected modules (7, 31, 69). The interest in modularity is mainly at two scales. At the smallest scale, interest has focused on the "nutritional unit" (1) or "physiologically independent subunit" (111, 112), comprising a unit of foliage, the section of stem to which it is attached, and the subtending axillary bud. At the opposite end of the spectrum, research has focused on clonal herbs (3, 81, 82, 113), in which each module (ramet) contains all of the structural parts and physiological processes necessary for independent existence. However, this upsurge of interest in modularity has also led to renewed speculation about other, intermediately scaled, functional units that may also behave semiautonomously (112).

For woody plants, considerable interest has focused on the degree of autonomy of individual branches[1] on a tree or shrub (107, 112, 117).

[1]We define a branch as a unit attached directly to the main stem of the tree; it includes both leaves and woody tissues.



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# SOME FUNDAMENTAL PROBLEMS IN BIOLOGICAL CLASSIFICATION

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When Dr. Hull first invited me to address this Symposium, he suggested that I present a paper on the various schools of taxonomy as such. This seemed to me to be a very wide field to cover within 25 minutes. So I decided to narrow it somewhat. What I intend to do is to discuss one major area of difficulty in the theory of each of the three main contemporary schools of thought in North America. By the three schools I mean, first, the advocates of a purely historical phylogenetic systematics in the sense of Hennig (1950, 1966); secondly, the evolutionary school, whose prominent recent exponents have included Simpson (1961) and Mayr (1969); and, thirdly, the school of numerical or phenetic taxonomy, which received its main impetus from the well-known book by Sokal and Sneath (1963). I will confess at the outset that I am an adherent of the Hennig school, but it is not my purpose in this paper to act as advocate for one school against the others. Rather I want to introduce a critical note into our discussions at this Symposium, by suggesting that members of all schools would benefit from some self-criticism and re-examination of some of their theoretical postulates. The areas of difficulty which I will discuss are the following: for phylogenetic systematics, there is the problem of how to extend systematic presentations to include fossil organisms; for evolutionary systematics, there is the problem of how to measure rates of evolution; and for numerical taxonomy, there is the problem of the validity of the unit character hypothesis.

First, the problem in phylogenetic systematics. In the well-known book on phylogenetic systematics by Hennig (1966), there is very little discussion of how to incorporate fossil organisms into systematic presentations. The difficulty to which I wish to draw your attention was raised briefly on page 191, in a discussion of the consequences of assigning categorical rank by age of origin as Hennig proposed. Hennig writes as follows: "...each terminal twig in the

phylogenetic tree of the fossil group would be not only a species, but at the same time a representative of a higher category, such as the 'ordinal stage'. This would be absolutely correct and meaningful in a system that includes both fossil and recent forms, because if each fossil species had modern descendents they would be representatives of separate orders. The fact that the fossil species occurs as the sole representative of a high category means that it became extinct without further splitting up. This is 'absurd' only insofar as it contradicts ideas associated with our more or less typological way of thinking of the higher categories."

While accepting Hennig's defense of his view against a charge of absurdity (the word "absurd" is much abused; to say that a theory is absurd should mean that it is internally inconsistent), the fact remains that the kind of systematic presentation Hennig then had in mind would be very much open to a charge of conceptual redundancy because of a proliferation of monobasic group names. All species which originated and became extinct in the Triassic would, if orders are defined as groups originating in the Triassic, be considered to represent monobasic orders, families, and genera. For species which became extinct in earlier periods, the redundancy would of course be still greater. And what does it mean in any case to refer a Triassic species to a family, if our definition of family is a group originating in the Late Cretaceous. If a species became extinct before the Late Cretaceous, then it never gave rise to any family in this sense. At this point in the argument most of Hennig's critics would conclude that they had shown the impossibility of using a time criterion for ranking taxa. But this does not necessarily follow. To understand the source of this conceptual redundancy, we must compare the structure of our conceptual model, the Linnaean hierarchy, with the real structure of phylogenies.

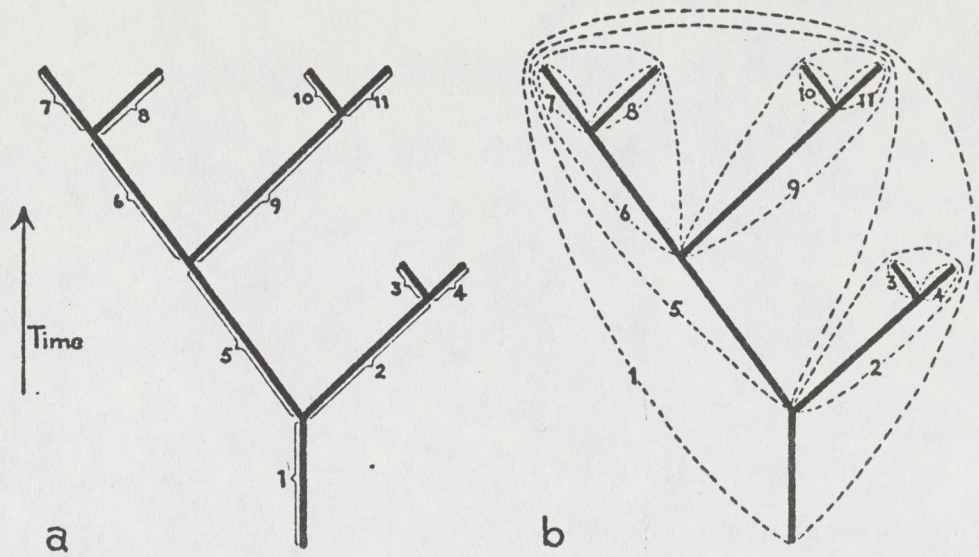


Fig. 1. Two methods of applying names to a given phylogeny: (a) the naming of time-extended species (each number indicates a nameable species); and (b) the naming of monophyletic groups (each number indicates a nameable monophyletic group).

Now phylogenies form what Gregg (1967) calls truncated hierarchies, in which the end-points (representing the extinction of terminal species) are not all equidistant from the beginning of the phylogeny (representing the origin of the ancestral species). There are two distinct principles upon which we can apply names to a particular phylogeny. Either we can name each time-extended species, delimited by two successive processes of speciation in the case of non-terminal species; or we can name an encaptic hierarchy of monophyletic groups, each consisting of an ancestral species and all its descendents. Terminal species will be named also according to the latter principle, but non-terminal species will not. The relations between these two forms of presentation are examplified by Fig. 1

The notion that a series of monobasic group names is needed for extinct species (whether terminal or otherwise) arises as follows. In the

traditional Linnaean hierarchy, species occupy a fixed category level in relation to all other taxa. But if we identify the supraspecific categories with age classes, then our conceptual model, the Linnaean hierarchy, becomes inappropriate because the species category is not one of a series of age classes. There are species at all levels in the real course of phylogenies.

In order to overcome this formal problem, I believe that phylogenetic systematics must recognize that their identification of categories of taxa with age classes necessitates changes in the traditional form of the Linnaean system. Indeed it is arguable that the Linnaean names for categories above the species level (that is, genus, family, order, etc.) should be abandoned entirely, as these names were intended as terms of Aristotelian (essentialist) logic and are therefore inappropriate to modern classifications. I cannot explore the ramifications of this question further in this short talk, but I urge all of you who are concerned with classification of fossils to study and comment on the presentation in Hennig's (1969) recent book on fossil insects, in which Hennig has renounced the use of the Linnaean category names and substituted a numerical indication of subordination levels.

I turn now to evolutionary systematics. At the outset I must say that I find the available expositions of the theory of evolutionary systematics far from satisfactory. It seems to me that a logically consistent evolutionary systematics would differ from Hennig's phylogenetic systematics only in that the taxa would be classified in evolutionary grades instead of in age classes. The definition of the taxa as such would be no different. I will try to explain my meaning with a figure (Fig. 2).

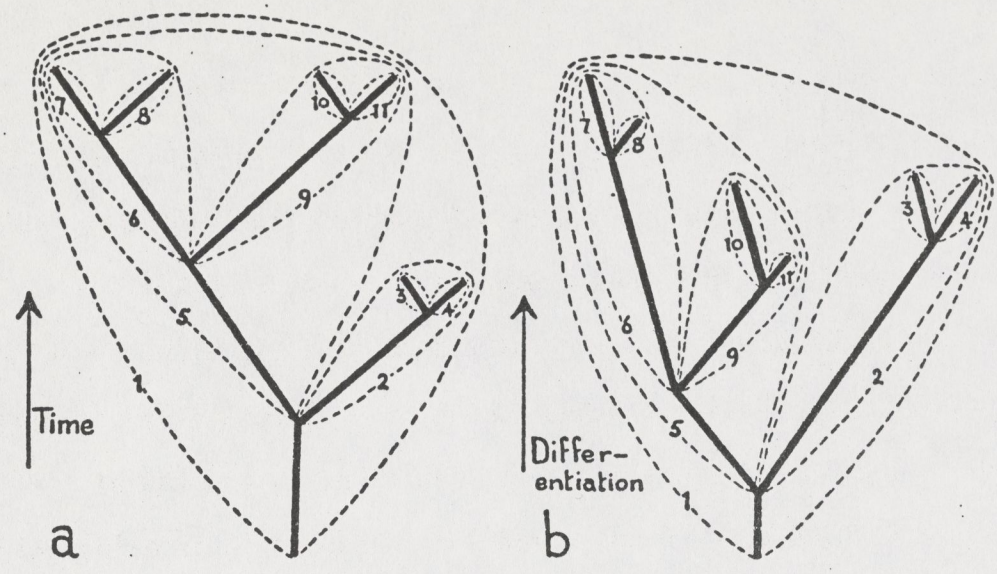


Fig. 2. Two representations of a given phylogeny: (a) with time as the vertical axis, and (b) with evolutionary differentiation as the vertical axis. The numbered monophyletic groups (taxa) are the same in both cases.

For the purposes of this comparison, I have assumed that a satisfactory measure of evolutionary differentiation has been found, and that a comparison of classifications in evolutionary grades and age classes has therefore become possible. The figure presents such a comparison. You will note that the branching (cladistic) sequence is exactly the same in both parts of the figure. No change in the parameter used as the axis can possibly affect this sequence. I feel it necessary to emphasize this point, since many published discussions give the impression that in classifying organisms a choice has to be made between clades (monophyletic groups) and grades. In fact the choice is between age classes and evolutionary grades; if our intention is to represent the systematic structure of a given phylogeny in a hierarchical manner, then the taxa to be named are the same monophyletic groups (that is ancestral species and all their descendents) in both cases.

The central problem of evolutionary classification is that no

satisfactory measure has yet been found for our intuitive concept of evolutionary differentiation (or anagenesis). Evolutionary grades can at present only be defined impressionistically, and there is no general standard against which we can judge the merits of particular authors' interpretations. Haldane (1949) proposed a unit of evolutionary change, the darwin, for "an increase or decrease of size by a factor of e per million years, or, what is practically equivalent, an increase or decrease of 1/100 per 1000 years". This provides a standard for describing particular series of measurements, but does not overcome the problem of non-congruence (in other words, that radically different values in darwins can be obtained from different series of measurements of the same organisms). One obvious approach to this problem is to select a particular series of measurements as an indicator of evolutionary change, as Haldane has done with Simpson's data on the tooth cusps of fossil horses. However Haldane's approach will break down if the range of taxa considered is made too wide, because of inapplicability of the chosen series of measurements. Furthermore, the use of such an external-morphological parameter as an indicator of evolutionary change is open to the criticism that, since the chosen indicator bears few relationships to other attributes of the taxa, the resulting classification can only be of limited interest. To construct a general evolutionary classification we should be able to answer questions like whether ants or ostriches are at a higher evolutionary level than horses, and our chosen indicator should have extensive relationships to other attributes of the taxa to be classified. We will not be able to filfill these demands by measuring horses' tooth cusps, or any other external-morphological parameter. Ultimately we may hope that some technical breakthrough in the field of genetics will make feasible the routine compilation of detailed maps of the nuclectide structure of chromosomes. Since the nucleotide triplet structure of DNA and RNA molecules is comparable in all organisms, and determines many of their other attributes, such a breakthrough would make it possible to test

various mathematical descriptions of nucleotide structure as general indicators of the evolutionary differentiation or organisms. In the meantime it remains scarcely possible to construct a general evolutionary classification of all groups of living things in any detail.

Next I turn to numerical or phenetic taxonomy. One of the reasons why I find much of the work of numerical taxonomists unconvincing is their failure to deal to my satisfaction with what I call the problem of the metric base. Sokal and Sneath (1963) were evidently aware of this problem, and to cope with it they postulated the existence of unit characters, defined as characters which are not logically subdivisible. This definition suggests that we should consider the concept of unit characters within the framework of Wittgenstein's theory of logical atomism. In the Tractatus logico-philosophicus Wittgenstein (1921) indicated that every meaningful statement must be logically reducible to elementary (atomic) statements based on experiences ("pictures of reality"). Only these elementary atomic statements are not logically subdivisible. Unit characters then are the predicates of those elementary statements whose subjects are organisms. The main difficulty with Wittgenstein's theory was that nobody ever succeeded in finding convincing examples of absolutely atomic statements and in demonstrating how more complex statements are built up from these. Later in life Wittgenstein abandoned this theory. Nowadays you will be hard put to find any philosopher who believes in absolutely atomic statements. The consensus is that there are only relatively atomic statements which may be treated as atomic in certain contexts but not in others. Sokal's and Sneath's postulate of absolute unit characters thus conflicts with the views of modern philosophers, and thus is not something which biologists should accept uncritically. If, as I expect, the unit character hypothesis must eventually be abandoned, what metric base for calculations of resemblance between organisms can be found? Will it be possible to arrange characters in a hierarchy and then treat particular levels in this hierarchy as

relatively atomic? I have no answer to these questions, but I wish to suggest that this is something which phenetic taxonomists should be seriously studying.

The recent development of numerical taxonomy has been of some philosophical interest as an exploration of the consequences of the theory of polytypic definition advanced by some recent philosophers, such as Beckner (1959). According to this theory the list of defining attributes of a class can never be completed, but pssession of any one of numerous "sets" of attributes is sufficient for membership. This represents a radical break with the long tradition, going back at least to Socrates, of conceiving classes as extensions of single defining attributes. Advocates of the theory of polytypic definition have supported their position by pointing to the failure of attempts to define biological taxa in terms of single "essential" attributes. However, an alternative explanation of this failure is that taxa are not classes of individuals, but systems at a higher level of organization. This latter view is obviously appealing to many biologists, and finds philosophical support in an important new book by Löther (1972). Numerical taxonomists should be seriously considering whether the theory of polytypic definition is in fact correct, and, if it is not, what consequences follow for the application of numerical methods.

In conclusion I will make some general comments on the current controversies between the different schools of taxonomy. Many polemical articles have appeared in recent years, some unfortunately written by persons who have not taken the trouble to understand properly the views they are criticizing. I would commend to you the opinion, which I believe originates from Karl Popper, that, if criticism is to be useful, then the views criticized must be presented in their strongest possible form. We will not contribute to the advance of science if we try to discredit an opponent's views by misrepresenting them. It is in my opinion a wrong concept to think of the different schools of taxonomy as competitors, for the reason that there is no one optimal classification to be

preferred over all others for all purposes. Indeed we may hope that the controversies of recent years will eventually give way to mutual respect, when each school has put its own house in order. In this brief talk I have tried to demonstrate that there are problems in need of further examination in the theory of all schools. None has yet built an unassailable fortress. We may expect modification in the positions of all in the years ahead, perhaps even at this Congress.

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[Lance Johnson Thesis]

Scientific
Preface null hypothesis
proven or disproven
accepted or rejected
not rejected
type 1 - 2 error

SINEs - phages incorporate in genomes evolve into types

Transposons

retroposons

Darwinism or Punctuated Equilibrium Gould/Eldredge

not incompatible

Darwin said can speed

Mayr - peripheral

or

THESIS

# PHILOSOPHICAL PROBLEMS IN THE EVALUATION OF GENETIC ENGINEERING

Submitted by

LANCE T. JOHNSON

Department of Philosophy

In partial fulfillment of the requirements

For the Degree of Master of Arts

Colorado State University

Fort Collins, Colorado

Fall 1998

Colorado State University

September 25, 1998

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY LANCE T. JOHNSON ENTITLED "PHILOSOPHICAL PROBLEMS IN THE EVALUATION OF GENETIC ENGINEERING" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS.

Committee on Graduate Work

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Adviser:

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Department Head/Director:

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**LANCE JOHNSON**

**PHILOSOPHICAL PROBLEMS IN THE EVALUATION OF GENETIC ENGINEERING - M.A. THESIS**

## ABSTRACT

After a brief introduction to the biochemistry and techniques of genetic engineering in Chapter 1, I discuss several ethical "models" that are used to consider the moral import of genetic engineering, as well as their strengths and weaknesses. In Chapter 3, I discuss special concerns in the philosophy of ecology. This includes the autonomy/provincialism debate in the philosophy of biology; the importance of ecology as a bridge between biology and the physical sciences; and the use of models in ecology, with particular emphasis on the utility of case studies for applied ecology. In the final chapter, I discuss some difficulties in assessing the potential effects of GEO releases into the environment. In keeping with my recommendation in Chapter 3 for the use of case studies in applied ecology, I explain the design of a case study and demonstrate it with a case study for the introduction of genetically modified canola (*Brassica napus*). I then proceed to evaluate the case study, followed by an evaluation of : virus-resistant plants (a special concern); genetically engineered microorganisms, another special concern; and Rissler and Mellon's (1996) tiered approach to risk assessment for genetically engineered crops. This last issue identifies two potential ethical difficulties with GEO assessments, neither of which seems to have been addressed in the literature. Finally, I conclude by examining some problems in my own analysis, and I briefly discuss the significance of these problems.

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# PREFACE

Humans have always wondered about the world they live in, where they "fit" into the world, how that world is ordered, and how the world was created. This is part of the branch of philosophy known as metaphysics, and it is possible to argue that this capacity for wonder, and to further structure such wondering through *reason*, is the single best attribute that separates humans from the rest of creation.

Although methodologies in the search for knowledge about the world have differed in the 2000 years since Plato and Aristotle, objectivity has always been admired. Some basic tenets of objectivity are that things in the world exist independently of the mind (the contrast with subjectivity), and that things exist in the sensible world and are observable or verifiable. In striving to achieve an objective methodology, scientists structure both experiments and observations in such a way that (1) they are limited to only certain alternatives, that have *explanatory* value to the scientist; and (2) the experiments and observations are largely free from creative or otherwise subjective interpretation, with the possible exception of newly-emerging paradigms, e.g. quantum mechanics in the first half of the twentieth century. The obvious example is the "null hypothesis", which according to the scientific method will be either accepted or rejected following a properly structured experiment, in the absence of alternative explanations. Objectivity is universally applied to all of the sciences, although there are certainly "softer" sciences (e.g. sociology and psychology) in which it is harder to definitively prove one or another result of an experiment, or explain away alternative reasons for the results.

In the history of science, there are two people who have had such an extraordinary impact on both science and worldviews that "revolutions" are commonly attributed to them: Nicholas Copernicus and Charles Darwin. Copernicus (and after his death, Galileo) was the first to claim that the planets in our solar system revolved around the sun, rather than an earth-centered solar system. This resulted in Galileo's enforced exile and recanting of this theory in front of the inquisition, and the theory was not widely accepted for nearly two centuries. Darwin, upon publication of The Origin of Species in 1859, caused a maelstrom in the scientific and theological communities alike that continues to this day.

As a result of recurring events like the Copernican and Darwinian revolutions, the seemingly large gaps between scientific endeavor and metaphysical theory are periodically bridged, and when this occurs large-scale changes in worldview soon follow. Copernicus' revelations in astronomy helped to pull away the authority of the Roman Catholic church, and advances in science soon followed. Darwin, by postulating both historical and biological evidence for gradual and divergent speciation from a common ancestor, as well as environmental effects on survival ("selective pressures") which influenced differential survivability of individuals within a population, upset biologists and theologians alike. He had a clear impact on theological authority derived from creationism theory, and he forever changed the impact that naturalists had on biology.

The Darwinian Revolution irreversibly changed science: new fields arrived (ecology, paleoecology, evolutionary biology); bridges were formed across disciplines, which Ernst Mayr has popularized as the evolutionary synthesis in its many and varied forms; and perhaps most importantly, biological methodology was vastly improved. In current biology, we can see how naturalists (mainly through observations), experimental biologists (through field and laboratory experiments), chemists and molecular biologists, and the "new" science of ecology all have specific roles and functions in understanding the workings of the biological world. Mainly due to Darwin's influence, ecology and evolutionary biology are virtually inseparable - the actions and relations of organisms and populations are always understood in an evolutionary context.

In seeking to arrive at some understanding of how introductions of novel transgenic organisms might affect the ecosystem, it will thus be necessary to understand Darwinian evolutionary thought, natural selection, and different notions of adaptation and fitness. These are some of the primary impacts predicted by ecologists.

In addition, the philosophical foundations of ecological science will offer tremendous insight into analyzing these potential ecological "invaders" (many ecologists predict that the effect of transgenic organisms will be analogous to invasions of exotic species.) Value in science, in particular predictive and explanatory value in ecology, will be examined in detail with an eye towards the application of those strengths and weaknesses of ecological thought to the introduction of transgenic organisms.

# INTRODUCTION

The science of genetics can trace its history back to Gregor Mendel, to whom is attributed "particulate theory", viz. the theory that there are inheritable particulates known as genes, through which inheritable traits are transmitted. The expression of the traits is known as the phenotype, which is contrasted with the genotype (the array of genes). Early in this century T.H. Morgan helped to formalize this understanding (with scientific evidence) as the "chromosome theory of inheritance", chromosomes being discrete physical entities in each cell nucleus upon which genes are located. The initial discovery of the mechanism for transfer of genetic information, deoxyribonucleic acid (DNA), is customarily attributed to Friedrich Mieschler in 1869, although some details remained to be worked out. In 1944, DNA was identified as genetic material, and in 1953 Watson and Crick made the now-famous discovery of the double-helix structure of DNA (see Weaver and Hedrick 1989).

Since the first *in vitro* work with recombinant DNA in the early 1970s, the technology of genetic manipulation has increased by leaps and bounds. The most optimistic forecasts predict that the entire genome of humankind may be mapped by 2005, an endeavor known as the Human Genome Project. Sheep and monkeys were cloned in 1997, likely paving the way for greater ease in mammalian cloning projects and even more ambitious projects.

Genetic engineering, a generic term for a variety of tasks falling under the broader heading "genetic manipulation" but producing specifically-designed and desired results, has become extremely advanced. From pigs that produce human insulin, to tomatoes more tolerant of frost due to the inclusion of the now-famous "ice-minus" bacteria, genetic engineering is rapidly becoming a household term, a part of our everyday existence. However, from the beginning, scientists and laypersons alike have voiced concern ranging from quietly urging caution to outright rage at the notion of humans who were so unassuming as to "play God" with other life forms.

These concerns came to a head with the controversies in Cambridge, MA in the late 70s and early 80s, and resulted in the well known Asilomar conference of 1975

(Krimsky, 1982). One result of the conference was a consensus among the researchers involved that prior to any explicit government regulation of their genetic engineering experiments, it would be far wiser to govern themselves to prevent (1) undue fear of a "Frankenstein Syndrome" in the backyards of the community (see e.g. Rollin 1995), and (2) overly stringent government regulation on the actual "hard science", which could include funding restrictions, excessively costly laboratory and field safeguards, and even the possibility of having individual experiments or entire laboratories shut down (Krimsky, 1982).

Finally, the result of generic safety concerns for all genetic engineering was that oversight responsibility would lie with the U.S. Environmental Protection Agency (hereafter simply EPA), and the U.S. Department of Agriculture (hereafter USDA) for initial field testing and releases of genetically engineered organisms (hereafter GEOs). Although the profit-driven impetus for most early genetic manipulation technology was found in the medical field (which is rapidly approaching the ability to perform gene therapy), much current research is in agriculture. Agricultural experiments include the creation of pesticide and herbicide resistant plants, cold and drought-tolerant plants, insect and fungus-tolerant (or -repellent) plants, animals that produce more meat or milk and less fat, animals that grow faster due to such chemicals as BGH (bovine growth hormone), etc.

Though many of these endeavors are noble and increase the potential for more environmentally-beneficent agriculture, e.g. by reducing the dependence on such chemicals as broad-based herbicides and insecticides, there are many additional primary and secondary effects that must be considered. Examples of primary effects include immediate effects on neighboring plant communities by migration of genetically-engineered plants or their *vectors* for genetic change (see Chapter 1), which could directly alter the community composition or even the larger ecosystem. Secondary effects could include: (1) long-term selection pressures on the insect or plant populations that are being targeted by incorporating traits for herbicide or pesticide resistance; (2) selection pressures on local wild relatives of the engineered species; and (3) even long-term effects on outside ecocommunity composition through interbreeding and/or migration of the engineered traits across species, which has been known to occur with both viruses and plasmids, two

popular vectors for genetic transfer between species. An additional secondary effect, or one which may be called (somewhat arbitrarily) a tertiary effect, are the unforeseen effects that could result over the course of generations for a transgenic population. For instance, gene mutations, pleiotropic effects, gene inertia, gene migration, epistasis, and genotypic or phenotypic plasticity could all occur in generations far removed from the original transgenic organisms. Again, such tertiary effects could be completely unforeseen and/or occur in unintended species or populations, which themselves might not even be in the immediate geographic area, making such effects further unpredictable.

In my investigation, I intend to discuss the basics of genetic engineering, and then present the difficulties in evaluating the potential ecological risks of environmental introductions of transgenic organisms. Much of this difficulty lies with the lack of predictability in evolutionary biology and ecology, but also with the related problems of assessing natural selection in natural populations. I will conclude with a further attempt to evaluate the potential risks of such introductions according to some basic tenets of ecology, and much of my discussion will follow Philip Regal's (1986) presentation of several models for that type of assessment.

In Chapter 1, I will introduce basic genetics as well as the tools for genetic engineering, with a focus on the vectors most commonly used for transmission of genetic information from one species to another. I will also introduce the areas of viral ecology and plant genetics, as I feel that these two areas are most important for the determination of possible environmental consequences of GEO introductions. I will include by introducing some of the potential environmental impacts of engineered microrganisms and plants, particularly the potential for "weediness" in engineered crops that readily reproduce with wild relatives.

Chapter 2 will be a discussion of the theory of evolution by natural selection. I will focus on (1) a brief history of Darwinian evolution, to help form a conceptual framework for further discussion; (2) the various mechanisms of natural selection, especially selection pressures and the importance of understanding fitness within both a genic selectionism model and a genotypic, or individualistic, selectionism model; (3) the role of adaptation in natural selection, from both a genetic and phenotypic perspective; and (4) some of the

difficulties in recognizing natural selection at all levels, including but not limited to *phenotypic plasticity, pleiotropy, epistasis, genetic inertia, stabilizing selection, intermediate selection during speciation, local vs. total extinctions,* etc. Again, I will tie all of this in to ecology and evolutionary biology (both of which are mutually inseparable), and the risks of GEO introductions to the environment, leaning heavily on Regal's (1986) presentation of several models in which to form predictive models for GEO releases.

In Chapter 3, I will begin by presenting some major concerns in the philosophy of science, including theory formation, explanatory power, values in science, and methodology. I will then tie these into a discussion of particular concerns for the philosophy of biology, which I claim is sufficiently similar to ecology (especially regarding their common philosophical difficulties) that it will assist me in presenting a conceptual framework for understanding the limits to the predictive power of ecologists. I will then utilize several theories in ecology which may help to determine the potential dangers from GEO introductions. These include Optimality Theory in its various forms, different interpretations of the notions of both "niche" and "equilibrium theory", and I will follow Shrader-Frechette and McCoy (1994) in recommending the use of case studies.

I will conclude with the difficulty of these assessment techniques, how they may or may not be resolved, some of the weaknesses of the young science of ecology and how recognizing those weaknesses can only strengthen it as a discipline, and the need to identify what values are underlying both the criticisms and opportunistic benefits touted for genetic engineering. For example, if a genetically-engineered strain of plant migrates outside its intended domain, and proceeds to interfere with a rare and endangered wild plant or insect in the outlying ecological community, what is to be done? Or even more difficult to address, what if the viral vector used to transmit the novel DNA to the host organism suddenly springs forth a new and terribly virulent strain with unforeseen qualities, as viruses in nature (and especially those in new environments) are wont to do?

Clearly, genetic engineering offers innumerable benefits, many of which have little or no sacrifice for human populations. Indeed, some would argue that the newfound ability to predict a fetal disease via amniocentesis, for even a *single* fetus, far outweighs any damage that might be done to the fetus, or to future generations. However, analogy to

potential environmental consequences of outright human meddling in the natural world from that of fetal genetic testing, above, would necessarily have to be ignorant of the damage done to ecosystems in Australia by the seemingly-innocuous introduction of the rabbit, or the effects on songbird populations in Hawaii by the otherwise-virtuous mongoose - both of which seemed like harmless human activities at the time! (Similarly, I could mention the way that kudzu vine has overrun the SE U.S., but that is not such a dramatic example.)

Further, but so much further that it is beyond the scope of this thesis, is the dramatic change in the role that humans now play in the world. Many people who currently side with the likes of Jeremy Rifkin against the entire project of genetic engineering do so out of a belief that there is something inherently wrong with a scenario in which humans are rapidly gaining the power and the potential to literally *design* their environment -- *right down to the species level.* By the intentional introduction of genes with known and desired traits into foreign hosts, we humans are no longer artificers who fine-tune their environment over long periods of time via conventional breeding; we can now design the environment we live in, collectively known as earth. The limits to genetic engineering are strictly technological, and there is good reason to believe that many of those will be overcome, and that it is strictly a matter of time. This is precisely the fuel that fans the flames of the Rifkin-led opposition to genetic engineering and is something that the global community of *Homo sapiens* will need to resolve, as the scope and intensity of genetic engineering continues to increase in the future.

# ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following people: Scott Gollwitzer, for providing me with a constant source of inspiration and helpful advice; Bill Williams, who first led me down the path to understanding the need for a coherent environmental ethic; Kenley Dove of New York's Purchase College, a true paradigm of scholarly excellence in philosophy; Holmes Rolston, III, who has been a patient and exceedingly helpful advisor throughout my studies at Colorado State University; and Bernie Rollin, who first attracted me to the ethical consequences of genetic engineering in his excellent seminar three years ago.

Finally, I wish to thank my mother, Betsy Tripaldi, for being the driving force behind me when the writing process became really difficult, and of course Carrie Lee, for her amazing patience and understanding as I struggled to finish this project.

READING GUIDE:

1. I have deleted the old Chapter 2 (evolutionary theory) and the old first part of Chapter 3 (general philosophy of science).
2. I have added the following:
   - Chapter 2 is completely new (a more thorough moral investigation)
   - Chapter 3 is ONLY a discussion of philosophy of biology/ecology as it directly pertains to this thesis
   - Chapter 4 has an added section, entitled "Problems in the Analysis", where I attempt to show some of my own weaknesses and defuse them, in context…

3. That is about it. The Table of contents should speak for itself, and is also a *de facto* reading guide.

Thanks, Lance Johnson
9/25/98

The thesis was approved on 3/18/98, pending certain specified provisions, which I have now accomplished. I am now presenting the revised thesis to the Committee members, so that you can individually approve the revised version. I will be pleased to meet with the Committee as a whole, if desired. I do need to have the final copy into the Graduate Office by Nov. 5.

Thanks, LS

# CHAPTER 1 : GENETIC ENGINEERING, ITS APPLICATIONS TO PLANTS AND VIRUSES, AND ENVIRONMENTAL CONCERNS

## GENETIC ENGINEERING

The potential release of genetically engineered organisms into the environment poses both a philosophical and scientific problem. In this first chapter, I will enlist science in the service of philosophy to enumerate the current technology of genetic engineering, so that it will be more easily and generally understood on a basic level when I illustrate the further scientific and philosophical difficulties in assessing the potential for ecological perturbations.

In order to fully assess the ecological implications of environmental releases of genetically engineered organisms (GEOs), it is first necessary to understand several key concepts. First, the mechanisms for the transmission of genetic information, and what it means to transfer genetic information between genomes. Second, the different levels of genetic manipulation currently available, including changes in both the RNA and DNA of various organisms. This includes an understanding of mutations on a molecular level, as this will be important in later evaluations of the potential evolutionary consequences of unpredicted events like intergeneric gene flow/gene transfer, or even relatively simple events like unpredicted mutations in recombinant populations. Fourth, an understanding of the biotechnology currently available for plants, as well as some of the advantages and disadvantages of current methods. I will then entertain a limited discussion of viruses, their role in genetic manipulation, and outline some of the current topics surrounding the natural selection and mutation of viruses. I will conclude with a brief introduction into anticipated ecological effects of populations of genetically engineered organisms, particularly those designed for a better differential of survival in the face of particular selective pressures.

## I. The Structure of Genetic Information

A gene can be described as "the genetic information *transcribed* into a single RNA molecule, which is in turn *translated* into a single protein" (Nicholl 1994,10; emphasis added). I have emphasized the terms 'transcribed' and 'translated' as they are essential steps in the mechanism for actual transfer of genetic information from DNA, its most basic form, to proteins, the expression of that genetic information in a living organism. Genes are located on chromosomes, which are found in the nucleus of nearly every cell in an organism's body, and may be singular (haploid) or double (diploid) in constitution. The region of a chromosome where a particular gene is located is the *locus* of the gene.

Since the essential steps of genetic engineering occur at the molecular level, my discussion will be limited to an understanding of the important events in transmission of genetic information - *at the molecular level.*

Deoxyribonucleic acid, or DNA, is a molecule consisting of four nucleotides (adenine, thymine, cytosine and guanine) + a sugar molecule + a phosphate molecule. It occurs naturally in a double-helix structure, right-handed in direction, and replicates itself *identically* during each cell division. DNA differs from RNA (ribonucleic acid) in two main ways: (1) DNA is found on the chromosomes in the nucleus, whereas RNA is found in an organelle called the nucleolus (which is in turn found within the nucleus) and on the organelles known as ribosomes, located in the cell's cytoplasm; (2) RNA also has four nucleotides, but it has uracil instead of guanine. These nucleotides are triplet codes with three bases coding for each amino acid. Emery (1984) noted that by 1966 all 20 amino acids, the basic building blocks of proteins, had been discovered and named.

The mechanism of gene expression involves four basic steps: (1) Replication of the DNA; (2) the activity of restriction endonucleases and site-specific nucleotide formation; (3) transcription; and (4) translation.

DNA usually has information stored in one strand called the coding strand, although the potential exists for information storage in both strands. The noncoding strand of the DNA, after unraveling, produces a messenger RNA (mRNA) molecule containing the same sequence as the coding strand of DNA, except that uracil is substituted for thymine in the mRNA. This process, known as *transcription*, is initiated by the enzyme "RNA polymerase". The mRNA then migrates to the ribosomes, the location of the codon/anticodon recognition event (Nicholl 1994,18). A codon (sequence of three nucleotide bases, e.g. AUG) carried by the mRNA is "recognized" by the anticodon (e.g. UAC) in the transfer RNA (tRNA), and then inserted into the ribosome. In this instance, the amino acid methionine would be synthesized, and would be the first amino acid in a polypeptide chain that would eventually form an enzyme or protein.

The so-called "recognition event", requiring both mRNA and tRNA (with associated amino acid residues), as well as ribosomes with ribosomal RNA (rRNA) and ribosomal proteins, is a complicated process known as *translation* (see Emery 1984,23-25, and Nicholl 1994,17-19). Ribosomal RNA is RNA that is merely part of the structure of the cell organelles known as ribosomes, which are the site of all protein synthesis.

## II. Genetic Manipulation

All of the genetic manipulation that will be the focus of this investigation will fall under the heading of recombinant DNA, defined by Nicholl as "a DNA molecule made up of sequences that are not normally joined together"(1994,160). This may or may not include interspecific or intergeneric genetic material, but those specifics are dealt with on a case-by-case basis, and are termed *transgenic* organisms. First, it is essential to describe some of the mechanisms available for recombinant DNA technology, including cleavage of the DNA, splicing mechanisms, and vectors for DNA transmittal.

Restriction endonucleases are enzymes used to cleave DNA at sequence-specific sites (so-called type II restriction endonucleases). In bacterial cells, common sites for recombinant DNA experimentation, there are two types of site-specific enzymes: cleavers

and methylators. Emery describes why they are called restriction enzymes with the following example:

> If DNA from one strain of *E. coli* is introduced into a different strain, the former is fragmented by restriction endonucleases from the latter and loses its function. The host's DNA is not so attacked because the sites vulnerable to its own enzymes are protected by a process of methylation. (1984,36)

When the DNA has been cleaved, the complementary bases are either "sticky" or not sticky, sometimes called "blunt". This results in different "joining strategies", which are very important for the genetic engineer. Some restriction enzymes are known to have "sticky" ends, and hence if the foreign DNA as well as the host DNA are both cleaved by a sticky restriction enzyme, the "cohesive [sticky] ends will come together via hydrogen bonds, which is known as annealing....the two molecules can then be 'sealed' and stabilized by the joining enzyme *DNA ligase*..."(Emery 1984,38).

An alternate method for "blunt" ends produced by a restriction enzyme is as follows. First, add to each end of the molecule a synthetic DNA linker, which is "designed to contain sequences recognized by a restriction enzyme which will produce staggered cohesive ends...[and] methylation may be required to first protect the DNA itself from attack by the restriction enzyme" (Emery 1984,39).

A third method is to add complementary bases to the cut ends of both the foreign DNA and the vector DNA. An enzyme, *terminal transferase*, is added which

> specifically add bases (deoxynucleotides) to...a DNA molecule, and...it is possible to add so-called *homopolymer tails* (e.g. GGG,CCC, etc.)...[to the foreign DNA and the vector DNA]. When the two are mixed complementary base pairing will occur and the DNA molecules will join together and can then be ligated. (Emery 1984,39)

The most common recombinant DNA (or rDNA) vectors are plasmids, bacteriophage, and cosmids. Nicholl describes four essential features required of vectors:

> Ideally they should be fairly small DNA molecules, to facilitate isolation and handling. There must be an *origin of replication*, so that their DNA can be copied and thus maintained in the cell population as the host organism grows and divides. It is desirable to have some sort of *selectable marker* that will enable the vector to be detected, and the vector must also have at least one unique

> restriction endonuclease recognition site, to enable DNA to be inserted during the production of recombinants. (1994:51; original emphasis)

Plasmids are organelles found in bacteria which are "stably inherited in an extrachromosomal state....tend to confer antibiotic resistance on their bacterial host....and are a circular duplex of DNA with a limited number of specific restriction sites" (Emery 1984,43). Plasmids are partially described by their copy number, which is the number of copies of itself the plasmid produces upon replication in a host cell. Generally, low copy number plasmids show a close relation between replication of the plasmid DNA and the host cell chromosomal DNA replication. There are two types of plasmids: conjugative and nonconjugative. Nicholl notes that

> Conjugative plasmids can mediate their own transfer between bacteria by the process of conjugation, which requires functions specified by the *tra* (transfer) and *mob* (mobilizing) regions carried on the plasmid. Nonconjugative plasmids are not self-transmissible, but may be mobilized by a conjugation- proficient plasmid if their *mob* region is functional. (1994,52; original emphasis)

Plasmids, because of their convenience and availability, are probably the most common rDNA vector.

Bacteriophages, often shorted to simply phages, are viruses that literally eat bacteria and are dependent on bacteria for their propagation. Generally, phages have a "head", a "tail" used for attaching to the bacterial cell wall, and a "body" made up of the central core of DNA surrounded by a protein coat. Phage activity incorporates the following steps. First, the tail attaches to the bacterium, and injects its DNA into the host cell. The phage DNA circularizes by its two ends joining together. The phage then exhibits one of two possible replication pathways: virulent phage exhibit only the *lytic phase*, in which the phage DNA replicates independently of the host DNA, then the progeny particles burst the host cell and invade other bacteria. Temperate phage exhibit mainly the *lysogenic phase*, in which the phage genome integrates into the circular bacterial chromosome, then replicates along with it. Many temperate phage are functional "lytics", meaning they can enter the lytic phase under suitable conditions (from Emery 1984, and Nicholl 1994). Some phages (notably the M13 phage) are single-stranded

DNA, and called filamentous phage; M13 infects only *E. coli* bacteria that have certain interior protein constructs, and much of its mechanical adsorption technique remains unknown (Nicholl 1994,59-60).

Cosmids are an artificial construct of plasmid DNA packaged in a phage particle, otherwise known as a hybrid (short for hybrid plasmid/phage vector). Since hybrids "lack phage genes, they behave as plasmids when introduced into *E. coli* by the packaging/infection mechanism of Lambda ( λ ) phage" (Nicholl 1994,66). Cosmids combine the efficient mechanism for introducing rDNA into a host cell along with a much higher cloning capacity, but these advantages are offset by less ease of use and less ability to utilize the cloned sequences.

There are other examples of vectors, particularly those utilized for incorporating rDNA in eukaryotic organisms. These include various forms of yeast plasmids, plant Ti plasmids, SV40 and adenoviruses, and plant Ti plasmids will be discussed later in the chapter (see table below).

| Cell Type | Vector Type | Genome | Examples |
|---|---|---|---|
| Plant Cells | Plasmid | DNA | Ti plasmids of *Agrobacterium tumefaciens* |
| | Viral | DNA/RNA | Cauliflower mosaic virus/Geminiviruses (DNA); Tobacco mosaic virus (RNA) |
| Animal Cells | Plasmid | DNA | Various types, many are hybrid vectors utilizing part of the SV40 genome |
| | Viral | DNA/RNA | Baculoviruses, SV40 virus, pappiloma & Vaccinia viruses(DNA) - retroviruses (RNA) |
| | Transposons | DNA | P elements in *Drosophila melanogaster* |

(Table above is modified from D.S.T. Nicholl, An Introduction to Genetic Engineering, 1994,69.)

## III. The Molecular Basis for Mutations

There are two main types of mutations: large chromosomal rearrangements, including inversions/ duplications/ deletions of large segments of chromosomal DNA; and nucleotide base changes, a/k/a point mutations, which are the most common and most of interest. Of the latter, there can be either a deletion or insertion of additional bases, or a substitution of bases. Of particular interest are "frameshift mutations", which Emery describes as one or two bases added or deleted, which changes the "reading frame for all

subsequent codons in the gene" (1984,29). Conversely, if three (or any multiple of three) bases are changed, it will only change one or a few amino acids in the protein product, often resulting in no visible change.

There are two forms that base substitution may take. Transitions are the replacement of one purine for another purine (e.g. adenine for guanine), or one pyrimidine for another pyrimidine (e.g. cytosine for uracil or guanine). Transversions are the replacement of purine for pyrimidine, or vice versa. A common cause for base substitutions are copying errors induced by mutagenic agents, so that the wrong base is incorporated into the DNA during replication.

Emery gives three potential effects of base substitutions: (1) Changes in an amino acid-specifying codon ( a "missense" or "silent" mutation) usually has no effect, but can (a) reduce or kill the activity of the protein product, (b) increase activity of the protein product, or (c) indirectly affect biological activity via changes in the molecular shape of the protein or simply making it more vulnerable to catalytic breakdown. (2) The generation of a new "stop codon" within the gene would lead to premature termination of translation, and the protein may or may not have normal activity (a "nonsense" mutation.) (3) The elimination of a normal stop codon, which would continue translation activity until the next stop codon is reached, resulting in an abnormally elongated protein (Emery 1984,29-30).

An additional type of mutation occurs in tRNA anticodons, and are known as "suppressor mutations". These overcome the effects of gene mutations which code for proteins, and many researchers have high hopes for the future use of such directed mutagenesis in gene therapy.

Daniel Dennett, in his excellent 1995 text Darwin's Dangerous Idea, stresses throughout that Darwinian evolution was so dangerous for two reasons. First, it upset the formerly universal notion of an orderly world *designed* by some form of Mind, viz. God. Second, and perhaps more importantly, evolution occurs by the accumulation of *randomly occurring, inheritable variation*. In the early years of genetics, it was believed that mutations were hence the greatest source of variability, although it was later determined that genetic recombination through sexual selection was by far the greater

mechanism for the production of variability. Furthermore, Mayr (1994) is a major proponent of the individual (or phenotype) as the unit of selection, and if this true, mutations can be seen to have an enormous potential effect on the fitness of each individual. This will be further discussed in the next chapter.

## GENETIC ENGINEERING IN PLANTS

There are several reasons for including a special discussion on genetically engineered plants. First, large-scale commercial crop plantings will present a large, and potentially risky, example of ecological introductions of GEOs; unless only sterile plants are used, there will be no way to completely prevent gene flow between crops and wild relatives. This will be discussed in more detail in Chapter Four. Second, plant applications of genetic engineering technology are by far the single greatest source of the utilization of this technology, and Snow and Palma (1997) note that as of May 1996, APHIS (Animal and Plant Health Inspection Service - a branch of the U.S. Department of Agriculture) has authorized over 2000 field trials of 44 different genetically engineered plant species. Varieties of genetically engineered squash, soybean, potato and cotton have already been approved for commercial release, along with some tomato varieties. Finally, plants have proven to be both easy and extremely difficult to genetically engineer (depending on both the plant species and the types of traits the researchers desire to express), and the further steps that are required for propagation of genetically engineered plants may themselves engender new and different ecological risks upon release in the field. This might vary from increased ability to propagate in some instances, to inclusion of bacterial and viral genomes, each of which is capable of further spread in a natural fashion. Again, this will be discussed in more detail in the final chapters, but it is important to keep these considerations in mind.

## *AGROBACTERIUM*-MEDIATED GENE TRANSFER

The tumor-inducing ability of two closely related species of soil dwelling bacteria, *Agrobacterium tumefaciens* (the cause of crown gall disease) and *Agrobacterium rhizogenes* (the cause of hairy root disease) has long been known and understood. The bacteria invade a wound on the plant, and the tumor-inducing ability is due to genes on plasmids within the bacteria. A "small, discrete portion of plasmid is transferred to and maintained in the plant cells during tumor formation, known as transferred DNA (or T-DNA)"(White 1989,5). *A. tumefaciens* has plasmids known as Ti, or tumor-inducing plasmids, while *A. rhizogenes* has Ri plasmids, for root-inducing. There are three known T-DNA genes which control the biosynthesis of two phytohormones, by encoding the enzymes for phytohormone synthesis. This leads to the incorporation of *Agrobacterium* genes into the plant genome, allowing the constitutive synthesis of the phytohormones, which then alter the developmental program of the transformed plant tissue White 1989,5). The tumor cells synthesize amino acids called opines, leading to the "opine hypothesis" - the bacteria create a niche in the plant tumor, as only the bacteria can utilize the opines. Opines are also used as a "genetic marker", i.e., they are used for selection of the vector during culturing in the laboratory.

Although much of this seems like very thorough knowledge, there is much that is not known. Plasmids have different regions, one of the important regions being the virulence or *vir* region. The functions of only two of these genes, *virA* and *virG,* are known; the remainder are thought to be inducers, regulators, or somehow involved in "nicking" the gene (White 1989,6). The *vir* region contains genes essential for gene transfer, though not themselves transferred to the plant genome, and they are closely related in all plasmids; as they are not transferred, they are not affected by removals or mutations, which may have evolutionary and technological significance (White 1989,8). In addition, there are several regions containing T-DNA, which is known to be transferable across plasmid types, and White notes that "T-DNA regions from various Ri and Ti plasmids appear to be mobilized to plant cells by a conserved mechanism", which

seems to be important regarding the evolutionary significance of the entire Ti plasmid mechanism (1989,6).

## GENE MARKERS

The synthesis of opines was mentioned earlier as an easily utilized genetic marker that was a byproduct of the use of *Agrobacterium*-mediated DNA vectors. But what exactly is a genetic marker? Walden notes that a genetic marker will

> bring about in the plant cell a phenotypic change which demonstrates that foreign DNA has entered the cell and that it is not only expressed but also being passed onto progeny. Moreover, the development of dominant selectable markers functional in plant tissue allows the direct selection of transgenic cells by their ability to grow and proliferate under selective conditions. (1989,27)

Additional genetic markers include resistance traits for various antibiotics, including neomycin, streptomycin, methotrexate, hygromycin, and bleomycin (Walden 1989,28-32). These are additionally useful because they can be cultured within *Escherischia coli* or other enteric bacteria cheaply and easily.

## THE TRANSFORMATION MECHANISM

There are two elements required for transformation of the plant cells: (1) a plasmid vector containing foreign DNA in a border (i.e., *trans*) sequence so that it functions as a T-DNA region (thus allowing recombinant DNA manipulations to be done in *E. coli*, before transfer to *Agrobacterium*); (2) actual transfer to a plant, "mediated by a strain of *Agrobacterium* that contains a full complement of *vir* genes"(White 1989,11). Integrative vectors recombine into a Ti plasmid, while binary vectors usually do not. Both vector types allow the use of both plant-expressed gene vectors (to select for transgenic cells) and bacteria-expressed gene markers, to select for the vector DNA (usually via antibiotic resistance) in *Agrobacterium* and *E. coli* cultures.

However, after transformation it is still necessary to perform plant inoculation. This is usually done with plant tissue explants, a segment of plant tissue maintained in

tissue culture. First, the plant tissue is infected with bacteria on a wound, and nursed with proper tissues and compounds for the growth and division of the infected cells. The cells are then moved onto a special medium to eliminate unwanted bacteria and select *for* transformed plant cells with a given antibiotic resistance, and that also have phytohormones to induce the growth of the desired tissue type (e.g. roots, shoots, etc.).

F.F. White lists four factors that can affect the stability of foreign DNA integration with *Agrobacterium* vectors: (1) a high variation in T-DNA copy number, which is unpredictable; (2) unlinked T-DNA elements can end up in a transformed plant cell, which is also unpredictable; (3) with the use of integrative vectors, rearranged sequences at insertion sites may result, with random results; and (4) though single insertions are "stably inherited in normal Mendelian fashion, they are not always *expressed*"(1989,17-18; emphasis added).

## ALTERNATIVE METHODS FOR DNA TRANSFER IN PLANTS

DNA can be directly introduced into plant cells, via (1) transformations of plant cell protoplasts, or (2) electroporation, in which electric shock creates micrscopic pores in the plant cell wall large enough to allow passage of RNA or DNA molecules (see White 1989; Walden 1989; Jones 1985). However, the DNA incorporation process is poorly understood, and all methods require further plant regeneration techniques, which are themselves a current technological limitation to DNA transfer in plants (White 1989; Walden 1989).

RNA and DNA plant viruses can also be vectors. Introduction of the viral genome is easily done through "rub inoculation" on a plant wound, high levels of transgenic product often result from the explosive nature of viral replication, and the timing of inoculation can help influence the gene expression in the plant. However, there are three distinct potential disadvantages: (1) the production of undesirable disease symptoms cannot always be controlled; (2) the release of infectious agents into the environment (possibly accelerated by the rapid nature of viral evolution); and (3) the unpredictable integration of the virus genome into the genome of the host plant (White 1989,24-25).

## VIRUSES AND VIRAL EVOLUTION

Viruses, small clumps of parasitic RNA or DNA, are unique for several reasons. They are considered by some biologists not to be "living" organisms (primarily due to a lack of autometabolism), yet they embody nearly every facet of a living organism, and accordingly viruses are considered to be living organisms by current scientific consensus. Most scientists refer to them as being capable of natural selection and evolution, yet in nearly every way they differ from most living organisms in the way in which they embody this ability, if they can be truly said to "embody" it at all.

However, because they can only be viewed under an electron microscope (they cannot even be cultured on media to form a colony visible to the naked eye, as can bacteria), it is hard to understand the mechanisms of natural selection in bacteria. Indeed, even the origins of viruses are debatable, although Morse indicates that the most popular hypothesis is that of viruses as "foot-loose genetic elements...that 'escaped' from cells"(1994,3). Similarly, most evidence leans toward viruses having polyphyletic origins (more than one ancestor), especially given "the likelihood of gene exchanges between viruses and host cells, and even between different viruses coinfecting a host cell"(Morse 1994,6).

Prior to the rapid advances in molecular biology in the 1960s and 1970s, there was a pervasive feeling that Darwinian evolution did not really apply to viruses. This is due in part to the long-standing tradition of "top-down" evolutionary biology, starting with a long history of field observations and only fairly recently extending to genetic knowledge (see Chapter 2 for an extended discussion of the evolutionary synthesis). This is further compounded by a lack of information regarding biological interpretation and the ecological context of natural selection, the absence of viral fossils, and the typically stable morphology of viruses, which is a common evolutionary "marker" for most organisms (Morse 1994,8).

Some of this evolutionary thinking is due in part to specifically contextual viral biology. Morse notes that "replication of RNA viruses, especially, can have high error rates...yielding, from a single parent, populations of genomes containing many variants

centered around a consensus sequence"(1994,9). Continuing along this line of thinking, Morse quotes a fellow virus researcher, H.M. Temin, as follows:

> the high rate of virus genetic variation allows mutation-driven evolution....A consequence of the high rate of mutation and recombination in retrovirus [RNA-based] replication is that many variant viruses will be present in any retrovirus population. Any variant with a relative advantage will increase, and successful variants can become 'frozen' as proviruses. In addition, retroviruses have a very high frequency of phenotypic mixing. This phenomenon allows retroviruses to undergo multiple mutations and recombinations before they are subject to selection (analogous to the effect of recessive mutations in diploid organisms). (from Morse 1994,8)

Viral evolution is a very interesting forum for the discussion of the evolutionary synthesis. The evolutionary, or *neoDarwinian* synthesis, united genetic and phenotypic perspectives on natural selection as the driving force for evolution. It is important to note that Darwin arrived at his theory of natural selection prior to a thorough understanding of genes as a "blueprint for life". It is remarkable that his theory is capable of being synthesized with genetic theory, and that is part of the reason that "evolution by natural selection" is currently such a powerful paradigm in biology. The synthesis was primarily a result of refuting the Lamarckian perspective that acquired characteristics could somehow be transmitted to later generations, although in many ways the synthesis opened a whole new can of worms, especially with regard to the question: "What is the properly understood unit of natural selection?" Many reductionistic geneticists have taken the gene to be the unit of selection, while others feel that the organism is more properly considered the unit of selection. This divergence of philosophies regarding natural selection has created further rifts between biology subdisciplines, a topic to which I will return in Chapter Three.

The final issue to confront thus becomes the question "Do viruses act according to natural selection?" To fully understand this question, it is necessary to first perceive natural selection as a two-step process, following Mayr (1994). First, the genome of the population must produce variation, without which there could be no change; this occurs independently of the selection process. Step two is the weeding out, or selecting, of the most-fit individuals (the somewhat complicated notion of fitness will be discussed in the

next chapter). Step two, alone, cannot produce variation; without step one, there appears to be only stabilizing selection, which can also occur with the production of variation.

A key problem with addressing natural selection in viruses, writes Morse, are the "difficulties of studying viruses under natural conditions and the limitations of demonstrating natural selection in nature"(1994,16). A similar difficulty, though not limited to the study of viruses, is that natural selection can occur in nature which is far less than the detection limits presently available for laboratory observation. Further, because of this possibility, after selection occurs it may be masked by further variations by as little as one or a few parent organisms, as was discussed earlier.

Finally, for viruses it must be inquired whether or not the host organism might influence the stability of the viral genotype and/or phenotype. To this end, variations in the level of virulence of all manner of viruses have been extensively studied (see Morse 1994,17-21, and references therein), and there is much evidence for excessive virulence as well as stability in longterm, coevolved viruses types (some are so stable they can be used as population markers for migration studies of their host organisms!) However, without a knowledge of the mechanisms and/or regulatory factors involved for evolving toward a lack of virulence, it is nearly impossible to assert if such a population trait is merely temporary (like some of the adaptations of Galapagos finches, as asserted by Peter Grant in his work - e.g. see [Boag and Grant, 1981]) or even the result of plasticity, which could in turn be on the part of the host cell or the virus, of course.

In Chapter Four I will illustrate some of the particular concerns that engineering traits for virus resistance in plants entails, including some evidence that such genetic modification has already led to evolutionary changes in the target viruses. This is an area of acute concern, as well as a fledgling scientific discipline, and therefore requires extraordinary scrutiny if ecological harm is to be avoided.

## POTENTIAL ECOLOGICAL EFFECTS

I will now briefly address some potential ecological effects of environmental releases of GEOs. This will serve the added purpose of (1) clarifying my focus on plants and microorganisms as the engineered organisms of primary ecological concern; and (2) illustrate some of the philosophical and scientific problems to follow.

Genetic engineering, especially in its agricultural aspects, is far more powerful than conventional breeding practices for two primary reasons. One is the availability of an enormously greater gene pool (and, hence, *trait* pool), due to the ability to translate traits from vastly different organisms through molecular manipulation. Second, its simple novelty translates into a power over the processes of trait manipulation that is essentially limited only by imagination and technology. Whole genes, parts of genes, or even reconstitution and reassembly of the gene in an organism can have incredible effects on the phenotype of that organism. New combinations can be designed, whereas conventional breeders are limited to genes already present, and their subsequent expression and heritability.

In their excellent text The Ecological Risks of Engineered Crops, Rissler and Mellon also note that (1) organisms with new genetic constitutions may not require the same conditions for genetic expression as their relatives, a form of *constraint*; (2) many new transgenic plants possess traits that are obviously advantageous, including frost/drought tolerance, herbicide/pesticide resistance, and pest/disease resistance; (3) most transgenic organisms have "little known precedent in evolution"; and (4) plant and microrganism "pharmaceutical factories" [chemical-producers] will have unknown effects on soils, nutrient cycling, decomposers and other consumers in their natural environments (1996,5).

The ecological risk assessment faces several difficulties, one of which is hinted at above (the lack of a natural history for transgenic organisms). In addition, knowledge about gene flow between certain crop species and their wild relatives is incomplete at best, and in many areas of the world is entirely unknown. Furthermore, the constraints which help determine whether or not such gene flow occurs are largely unknown, so that

a transgenic crop may now possess (or evolve) the ability to interbreed with wild relatives. This would then lead to the potential for "weediness" in the wild relative, particularly if it received a trait designed to combat particular selective pressures, e.g. those described in the preceding paragraph.

For bacterial or viral transgenics, there is the added potential for interspecific gene transfer (in addition to nearly all of the factors cited above.) Many bacterial cells are limited to what they will infect, but often the mechanisms for such infectious behavior is unknown, and so may be "masked" by its new traits (particularly traits for enhanced survival). However, viral cells are often virtually unlimited in what they can invade, and if they are not, then the mechanism is unknown. In many ways viruses may pose the greatest potential risks, particularly since the effects may not be witnessed for two or more generations of the host organism.

It is my hope that the preceding discussion will help pave the way for an understanding of the philosophical issues that plague the intended use of the new recombinant DNA technology. In the following chapters I will outline these difficulties, both scientific and philosophical, with the intention of further illuminating if, and how, ecologists may attempt to predict the potential for ecological perturbations following GEO releases.

## SUMMARY

Since the first manipulations of recombinant DNA in 1972-3, the field has advanced by leaps and bounds. Much is now known about both the processes and mechanisms of genetic information transfer, as well as ways to control interspecific genetic transfer with vectors. Many of the critics of genetic engineering have pointed out that interspecific gene flow also occurs in nature, and that beneficial traits like herbicide resistance will become epidemics once found in wild or weedy populations. In addition, the likelihood of adverse evolutionary impacts from such stochastic events is also potentially high, and is not something to be taken lightly.

In the next chapter, I will examine the Darwinian Revolution, from Darwin's own construction of evolutionary theory to current conceptions of it, given the advances in genetics, molecular biology and ecology in the last fifty years alone. I will include alternative theories like Gould and Eldredge's "punctuated equilibria", and even notions of the gene as the unit of selection. All of this is important, both for the ways Darwinian thinking has changed the history and philosophy of science, and for the impact that evolutionary thinking has on some of the proposed methods for ecological appraisals of the consequences of introducing genetically engineered organisms into the environment.

## CHAPTER 2: THE MORAL DIMENSION OF GENETIC ENGINEERING

In this chapter, I will introduce some of the moral concerns related to biotechnology, and I will attempt to evaluate the relevance of those concerns with respect to conventional morality as well as environmental impact.

I have broken down some of the moral positions on biotechnology according to the outlook of the various individuals or groups that are being discussed. It might be argued that "scientific naysayers" no longer exist, and that their viewpoint has been subsumed by more moderate discourse on risk assessment. That may well be the case, but these individuals have already contributed to the extant dialogue concerning genetic engineering, and as such it is important to acknowledge their moral outlook. Rifkin and others who maintain that genetic engineering is intrinsically wrong have contributed inordinately to the social debate about biotechnology and its applications, and it is important to consider that viewpoint in some detail.

Finally, there are those who wish to emphasize the process of genetic engineering when considering the moral relevance of new biotechnology products, rather than the product. In many ways, this approach is an insidious form of the "intrinsically wrong" model, as nearly all of these proponents view biotechnology as unnecessary and needlessly dangerous. However, most of these individuals are scientists who realize the potential gains from genetic engineering, as well as some of the current benefits (e.g. a cheap, readily available supply of insulin for humans suffering from diabetes.) Realizing that the potential health benefits from genetic engineering are theoretically almost endless, people like John Fagan and Mae-Wan Ho would never argue against biotechnology as being "intrinsically wrong". Instead, they wish to attack the risk assessment process, and circumvent social and professional agreement on necessary and sufficient risk assessment protocol. I will first describe some aspects of arguments from these individuals who wish to attack the genetic engineering process rather than evaluate each biotechnology product on its merit. However, I think that it is clear in the end that evaluating the product is the key to moral evaluation of genetic engineering.

I. The "Scientific Naysayer" Model

Some scientists, for various reasons, have vigorously asserted that for all intents and purposes, genetic engineering is a benign technology. It is true that such claims are largely a reaction to the individuals and groups that claim biotechnology is a disaster waiting to happen, and certainly this reaction is understandable. However, in the course of defending the safety of genetic engineering, these scientists have misused and abused a number of scientific theories to "illustrate" just how "safe" it is. It might be noted that scientists are objective and methodological, and need not be concerned about the moral implications of their work; I will address this claim in the following chapter, and illustrate why this is not the case. I have named the awkward defense of genetic engineering, by scientists, the "Scientific Naysayer Model". It is unfortunate that this misuse of scientific theory occurs, and particularly when it is justified by a recourse to another socially-problematic aspect of science: evolutionary theory. In the following pages, I will attempt to defuse some of these ill-founded claims, some of which are formed from a bridge between ecological theory and evolutionary theory – the concept of evolutionary optimization. This is an important issue in the philosophy of biology and ecology, and it will also help to frame both Chapter Three and Chapter Four of this thesis.

Allan Campbell, in a 1991 chapter about genetically engineered microorganisms (GEMs), establishes that he clearly feels that the risks of GEMs are greatly overstated. Indeed, Campbell presents what is essentially a "straw man" argument of sorts (albeit in a scientific guise), by (1) placing all emphasis on competition as a limiting factor for GEM success in the environment; and (2) assuming that natural populations of microorganisms are already at a saturation density in relation to other microbial populations(1991, 32). I consider this to be a straw man argument for three reasons: (a) placing a singular emphasis on competition in the microbial world is utterly simple, as it is generally considered to be only one of many determining factors in the success of invading species; (b) the concept of "saturation density" is so difficult to quantify for microbes as to be impractical, except in the smallest habitats or for use in very general models; (c) the

"saturation density" is a largely mistaken and outdated concept, as are other steady-state views in ecology. It is important to note, however, that if Campbell intends to utilize such concepts to facilitate practical difficulties in microbial ecology, that is a completely acceptable methodological alternative – but it will also affect the resulting attempts at risk assessment.

In the same 1991 paper, Campbell makes a severe error, again resulting from an oversimplification of natural selection theory. Campbell asserts that

> Escape from small-scale laboratory experiments can be of concern only if the engineered organism is selectively superior to its natural counterparts. Neither for pathogens nor for nonpathogens do scientists know how to engineer such selective superiority. (1991, 33)

Unfortunately, the natural world does not comply with our theoretical models, and there are numerous theoretical alternatives to Campbell's claim:

1) There may be no "natural counterparts" to the GEM, and it may be able to exploit a new niche[1] in the non-laboratory habitat.

2) Similarly, the GEM may not have a selective advantage *per se*, but it may nevertheless be able to exploit a niche in the new habitat.

3) The GEM, with its novel traits, may have neutral selective value initially – but may have positive selective value in the future, providing it is able to maintain its numbers;

4) The GEM may have periodic selective advantages, for example at different seasons or for different host organisms (where applicable).

5) The potential for reproduction (including conjugation), and therefore recombination of genetic material with extant microbe populations, may result in one of four principal outcomes:

a) A new selective advantage for the GEM-hybrids;

b) No selective advantage for the GEM-hybrids, but maintenance of the novel trait in the microbial gene pool;

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[1] The astute reader will probably note that the concept of an ecological niche is also the product of the old, balance-of-nature paradigm in ecology. However, it is also unfortunately true that the conceptual utility of the term "niche" simply cannot be denied, and so I will use it for the sole purpose of communicating a

c) Elimination of the novel traits from the gene pool; or

d) Disruption of existing niches and/or ecosystems.

Later, on the same page, Campbell raises the issue of the physiological tradeoffs that are likely to accompany GEMs into the environment. There are two main problems with this assertion. First, a chief advantage of recombinant DNA (rDNA), as compared to conventional breeding (artificial selection), is that there are generally no "tradeoffs" associated with the new trait. Of course, if the new trait is energetically much more costly – e.g. Campbell's example of a recombinant dandelion that produces twice as many seeds as the existing wild-type plants – then the acquisition and expense of energy for reproduction, in an environment with limited energy resources, may be a limitation on the fitness of the recombinant dandelion.

The second, more insidious problem, is Campbell's assumption of adaptive optimality in the existing dandelion population. For instance, the production of vast numbers of seed per individual plant may not be a constraint, but rather appropriate habitat for the germination of viable offspring may pose a definite limitation. Similarly, it can be assumed that seed dormancy is not a problem either (since long dormancy is a trait commonly associated with noxious weeds). Finally, if it is hypothetically asserted that the genetically engineered dandelion has no relevant selective differences from the native dandelions except more efficient seed germination, it can be reasonably expected that the GEO will establish itself, all conditions being equal.

From this hypothetical example, based in turn on Campbell's original example, it can be seen that the concept of evolutionary optimality forms a dangerous theoretical temptation for the ecologist attempting to form risk assessments. Philosophically, the assumptions that surround adaptive success in the environment have been heavily discussed in recent years, and are particularly illuminating for this endeavor.

Gould and Lewontin (1979) were among the first to criticize the assumptions surrounding evolutionary optimality, particularly as it related to what they termed the

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relatively simple notion of a particular spatiotemporal position in an ecosystem – even though there may be no such thing in reality.

"adaptationist programme". Biologists, when observing natural populations, have grown accustomed to assuming that natural selection has "shaped" that population, and that therefore the population is optimally adapted for that environment. Gould and Lewontin are clearly concerned that this methodological assumption had been misplaced, and had subsequently grown into an assumption that the population was optimally adapted to its habitat.

Parker and Maynard Smith (1990) instead argue that evolutionary optimization models have great heuristic value, but of course are limited – they are models, and models have discrete shortcomings, as I will discuss in the next chapter. In fact, optimization models are frequently utilized to help form a continuum between general and specific models: optimization models can test specific assumptions based on more general, theoretical principles. It is important to note that optimization models have been largely employed in the study of energetics – e.g., to analyze the energy efficiency of animal gaits (Parker and Maynard Smith 1990, 27). The authors are also very clear in their assertion that there is a definite, precise context in which the optimization theory is appropriate; it is the inappropriate uses of this approach that is dangerous (ibid.).

It seems evident that Campbell has misused the adaptive/optimization approach to GEM releases. Clearly, it would be difficult if not impossible to perform an analysis of energetics for GEMs, or even for genetically engineered plants – the emphasis of this thesis. The conditions for microbial life are now only barely understood, and much of what is known is based on laboratory and microcosm experiments, which have their own limitations for extrapolation of experimental data to an external environment. If Campbell intended only to assert that, e.g., waterborne microbes will not survive as airborne particulate matter, then such an assertion can probably be ascertained in controlled, small-scale laboratory experiments. Such evidence is not likely to be disputed in the scientific community. Unfortunately, Campbell's tendency to downplay the environmental risks of GEMs is similar to the strategy of an earlier scientist, Winston J. Brill.

Brill, writing in the journal Science in 1985 to oppose proposed biotechnology regulations, came under heavy fire from ecologists for several reasons. First, he was very unscientific in his choice of words – e.g., "seems very small", and "is very unlikely". These are obviously unquantifiable terms, which in itself is an excellent reason to then employ well-established risk assessment procedures (which Brill was writing to contest, ironically.) Second, Brill was roundly condemned for ignoring sound ecological principles, e.g. with regard to the weedy characteristics of obnoxious plant species (which I will return to in Chapter Four.)

Clearly, there are some major difficulties with the "scientific naysayer" model, and these difficulties are overwhelming. It is neither appropriate nor accurate for scientists to assume a public policy position in which it is stated that there are "no particular risks associated with GEOs, aside from risks that are associated with the unmodified organisms." Fortunately, thanks to the persistent efforts of ecologists and led by the Ecological Society of America, the voices of these scientists have long been stifled and a more appropriate approach has been implemented toward ecological risk assessment for GEOs. I will investigate some models used for such risk assessment in Chapters Three and Four.

## II. The "Intrinsically Wrong" Model

Jeremy Rifkin, author of the popular and influential text *Algeny* (1983), is commonly characterized as a lead proponent of the view that all genetic engineering is "intrinsically wrong". However, before proceeding to evaluate his conclusion, it will perhaps be fruitful to first review his reasoning, in order to understand how he has arrived at this conclusion.

Rifkin begins by weaving a convoluted social criticism of current paradigms in science, particularly the pervasive influence of Darwinian evolutionary theory. He then discusses an emerging paradigm that he perceives, "temporal evolution", and notes that within this paradigm biotechnology is inherently morally acceptable. Finally, he criticizes

such thinking as inevitable but myopic, and demonstrates his fervent stance against *all* biotechnology.

Rifkin is but one of many who feel that biotechnology is intrinsically wrong, but struggle to illustrate the reasoning behind that stance. In his criticism of evolutionary theory, and his later juxtaposition of Darwinian biologists and ecologists (e.g., p. 181 in *Algeny*), Rifkin demonstrates some grave misunderstandings of neoDarwinian evolutionary thought. Indeed, there are several particular shortcomings which deserve special mention: the influence and production of random variation, a normal process in every population; the very fact that species are not fixed and immutable, which is intrinsically related to the continuing debate over the definition of the term "species" in the philosophy of biology literature; the importance of geographic isolation for speciation to occur between two related populations; and more technical aspects – such as Kimura's "neutral mutation theory" – which are all in direct contrast to the efforts of Rifkin, and others, who wish to criticize Darwinian evolutionary thinking by first oversimplifying, and thus misrepresenting, its central tenets.

Perhaps more revealing, Rifkin places great emphasis on the economic context in which Darwin conceived his theory, while at the same time ignoring the contradictory religious context as well as Darwin's extensive training in geology. Rifkin claims that Darwin's emphasis on competition was a "natural" byproduct of the Industrial Age, a time of flourishing capitalism in Darwin's native Great Britain. Similarly, gradualism was a philosophical byproduct of the period, although it was also a result of Darwin's analogy to the process of artifical selection – in which change comes slowly, over many generations. Like most deconstructionists, Rifkin only utilizes that evidence which supports his thesis, and accordingly he ignores Darwin's many volumes of copious notes and observations – long an accepted form of science, particularly for field biologists and natural historians.

The results of Rifkin's selective writing are insidious, and they are important for that reason alone. In describing the scientific method in *Algeny* (pp.117-118), Rifkin asserts Darwin's failure on the grounds that it is "non-predictive, non-repeatable" science. This is a category error, viz. confusing a retrodictive theory with a predictive theory.

Furthermore, the process of natural selection does not fall victim to the same claims, and Philip Kitcher goes to great lengths in his text *Abusing Science* (1982) to show that the study of natural selection can be based on the verification of **testable hypotheses**. Again, this is representative of Rifkin's failure to comprehend developments and refinements in neoDarwinian thought in the past half-century, and I contend that it is unfair and misleading to his readers whom may be less scientifically literate.

Rifkin eventually proposes a new "temporal theory of evolution", basing it on advances in physics, and on more mysterious "advances" in biological theory: biological fields, biological clocks, and Rifkin's own misunderstanding of the genotype/phenotype debate in the "units of selection" debate amongst philosophers of biology. Ironically, this issue was completely ignored in his discussion of Darwinian evolution, even though he did devote some space to a brief discussion of the "evolutionary synthesis", from which the aforementioned units of selection controversy was born. Unfortunately, a discussion of Rifkin's "temporal theory" would necessarily be both lengthy and tangential to my current thesis, but for Rifkin it sets the stage for the final "leap of faith" that he develops throughout *Algeny*.

The premise throughout *Algeny* is that all cosmologies are both influenced by, and influences on, the natural world. Sumerian culture was deeply affected by the management of water resources, and their gods were all related in some way to water – e.g., Enki variously meant "water", "semen", or the god of fertility. Similarly, water was the source of the Sumerian economy, and economics is the point of departure for Rifkin's analysis. Trained as an economist, Rifkin seems dedicated to connecting all cosmology and economy, and then extending that connection to Darwinian thought. Surely, Darwin never intended his Theory of Natural Selection as a worldview, although he did recognize the religious and social implications of his theory. In fact, he was cowed somewhat by the influence of the Church of England, to the extent that he left man's relation to the rest of nature implicit in *The Origin of Species*.

Perhaps more troubling is the distinction between Darwinism as a cosmology, and neoDarwinism as a scientific tenet – a distinction ignored in *Algeny*. It may be true that

the emphasis on competition that is a key part of the public conception of Darwinian theory forms part of the prevailing worldview (and Darwin's, as well); but I maintain that there is an important difference between this "public conception" and the now highly refined scientific theory. Like most scientific theories, natural selection has withstood innumerable critiques, revisions and refinements – all of which serve to strengthen the theory, not weaken it. Some noteworthy examples include Gould and Eldredge'' (1977) proposal of "punctuated equilibria" as a replacement for Darwin's evolutionary tempo of gradualism; Kimura's aforementioned "neutral mutation theory", i.e., not all mutations have immediate selective value, either positive or negative; the units of selection controversy, competently argued by Elliot Sober, Ernst Mayer and others; and the issue of phenotypic plasticity, a possible explanatory mechanism for the variation within species witnessed in the finches of the Galapagos Islands (see e.g. Grant and Grant [1993]).

Lastly, Rifkin argues against biotechnology, "to save for humanity what is important and sacred about life" (1983, 252). However, there are four main problems with Rifkin's characterization of both life and biotechnology:

(1) Life, of course, still has mysteries – e.g., biologists have not yet reached a consensus on the proper definition of what constitutes "life".

(2) The technical limitations to biotechnology may never be overcome – e.g. the worst fears about eugenics and the engineering of intelligence, appearance, etc. will likely never be realized.

(3) Life, though manipulated to some degree, is not necessarily any less sacred.

> Example: If the discovery of DNA did not erase the mystery of life, why should its mystery be erased by the manipulation of DNA?
>
> Example: Similarly, the discovery and manipulation of the chemical triggers for programmed cell death, or *apoptosis*, has not decreased the "mystery" of life, either.

(4) Rifkin's use of the word "desacralization" – used in relation to "what is…sacred about life" - without an expression of his intended meaning, is virtually meaningless. I will return to this problematic word in Rifkin's writing in the following pages.

## III. Philosophical Problems with the "Intrinsically Wrong" Model

Statements about the intrinsic "value" or "goodness" (or conversely, "badness") of human activities has always run into philosophically difficult barriers. Who, or what, decides that an action has intrinsic worth? Further, who or what is privy to that knowledge? Or, is it perhaps a matter of social consensus – which is notoriously fickle, and may change over time? This is a highly problematic ethical domain, and as such I will only discuss it briefly; other texts, e.g. Rollin's *The Frankenstein Syndrome* (1995), have adequately discussed this moral aspect of genetic engineering.

It is my opinion that it is difficult to address the theological opposition to genetic engineering on moral grounds, as may be expressed by such statements as "It is wrong to disturb God's creation." It would seem that, if faith is indeed "properly basic" as some philosophers of religion contend, then moral extension of that faith may be equally "basic", and as such irrefutable. On the contrary, Rollin (1995) contends that regulating the application of genetic engineering is not a theological, but rather a social moral issue:

> Advances in knowledge and technology that fly in the face of religious tenets may appear morally problematic to adherents of those tenets – many religious people were offended by Newton's physics or Darwin's biology – but that in itself does not create a problem for our secular society in general or for its social ethic (24).

Rollin (ibid.) also mentions that although Rifkin (1983) fails to define what he means by genetic engineering as the "desacralization" of life, there seems to be some implicit notion that it is intrinsically wrong. In supposing this to be the case, Rollin notes that perhaps Rifkin feels that such practices are "fundamentally inimical to a good human life"(1995, 30). This possibility is an ethically sound view, notes Rollin, if it addresses the potential <u>consequences</u> of genetic engineering, and not the <u>process</u> itself:

> We cannot abandon or bury the science and technology we find unintelligible or frightening – though at various times most of us feel that urge. Rifkin notwithstanding, it will not go away or be abandoned. We must manage it. (1995,32)

IV. Product vs. Process

Like Rollin, I maintain that the ethical import – indeed, perhaps the ethical relevance – of genetic engineering is dependent on its particular consequences, or *products*, of the genetic manipulation (the *process*). For example, if a benign laboratory strain of a bacteria species is engineered to produce insulin for human diabetics, there will probably be no resulting hazards, environmental or pathogenic. This hypothetical organism is not likely be transformed into an "Andromeda strain". However, if naturally occurring organisms are engineered with traits that are likely to confer a potential adaptive, or *fitness*, advantage in the wild, then environmental disruptions are possible or even likely.

In the preceding pages, I have presented two extremes for the ethical consideration of genetically engineered organisms, or GEOs. Some authors, like Rollin (1995), have focused on genetically engineered animals and their attendant ethical issues – particularly animal welfare considerations. I feel that the greatest danger posed by biotechnology is that of ecological perturbations, because (1) they are largely irreversible, and (2) may have large, unpredictable effects on existing ecosystems. Naturally, such concerns focus on the outcome of the genetic manipulation, because that is the context in which the ecological risks will be perceived.

Previously, I criticized the "scientific naysayer" model, on the grounds that the representative arguments were oversimplifications and did not adequately represent potential ecological scenarios. I presented arguments against this position, and duly noted that there were many "unscientific", "unquantifiable" claims being made by people like Winston Brill (1985). Interestingly, there are also highly educated scientists who oppose all genetic engineering, and who demand that all biotechnology experimentation for commercial purposes should cease until an "adequate" regulatory structure is in place. Unfortunately, it is beyond the scope of this thesis to evaluate whether the current regulatory structure is in fact "adequate", and so it will be assumed that it is adequate, by and large. However, as these individuals are suspicious of the entire process of genetic

engineering, it will be fruitful to briefly examine the merits of their position, as it is a position of considerable social import.

Mae-Wan Ho, in an Internet publication entitled "Genetically Engineered Foods: The Hazards are Inherent to the Technology", begins with three questionable assumptions. The first is that the biotechnology industry is coming out with products "that nobody needs"(1997, 1). The next claim, based on political economics, is that biotechnology companies "displace and marginalise…the need for sustainable agriculture that could regenerate the environment"(ibid.). Lastly, Ho claims that the technology "is, according to existing knowledge, inherently hazardous to health and biodiversity"(ibid.). In making these value claims, Ho goes on to note that "geneticists have now linked the emergence of both pathogenic bacteria and antibiotic resistance to horizontal gene transfer"(ibid.), and offers this as evidence of the "inherently hazardous" nature of genetic engineering.

Unfortunately, Ho also fails to note that such horizontal transfer is a normal, natural process, and it is neither increased nor decreased by the occurrence of genetic engineering. It is true that some of the biological units of pathogenicity and antibiotic resistance, viz. bacterial plasmids, are also used in genetic engineering; as such, there is cause for concern about increasing the incidence of transmittance of those traits as a result of environmental releases of GEOs. However, this is an empirical matter, and the very methods that are currently utilized to assess such risks can also be utilized for recombinant organisms – this is the key weakness of Ho's position. Ho wishes to claim that there is an inherent danger associated with genetic engineering, and for that reason the process should be reconsidered and even banned outright.

Ho notes that recombinant techniques utilize "modified versions of precisely those genetic parasites, or vectors, that cause diseases including cancers and spread virulence genes and antibiotic resistances. Thus the technology will greatly increase the frequency of horizontal transfer of those genes that spread virulence and antibiotic resistances"(1997, 2). This is simply flawed reasoning, because increased presence of vectors has not yet been shown to equal increased horizontal transfer, although it does

increase the probability of horizontal transfer. Furthermore, this is also an example of processes that are open to empirical assessment, prior to making such inflammatory claims.

There are several examples of the rhetoric that Ho seems to favor, as compared to actual scientific evaluation. Ho notes that marker genes from transgenic organisms have been transferred to natural populations of soil and fungi, but he does not mention at what density or relative concentration those genes were found. Ho does not mention if those marker genes were determined to affect the viability (i.e., the fitness) of the recipient organisms. Lastly, he does not discuss whether there may have been temporal or climatic variation in the transfer of genetic material. These are all inherent concerns, with regard to the potential ecological effects of recombinant organisms on natural ecosystems; accordingly, I will return to this topic in Chapters Three and Four. To understand the problems with Ho's claims, it might be noted that if such horizontal transfer of genetic material occurred under particular conditions in a natural state, then the application of that recombinant organism can be avoided under those conditions in which such transfer is facilitated. Rather than use examples of horizontal gene transfer to justify an outright ban on biotechnology, it should be seen as a fruitful research area: one that combines basic plant and soil ecology data with applied genetic engineering research applications.

John Fagan is another scientist who wishes to see a halt on biotechnology applications, until a "reasonable regulatory structure" is implemented (1996,10). Interestingly, Fagan is guilty of employing many of the same rhetorical shortcomings displayed by W.J. Brill in 1985, except that their positions are diametrically opposed. Fagan uses expressions like "a reasonable probability"(1996,8); a "significant possibility"(1996,9);"significant risk"(1996,10); and "an appreciable possibility"(ibid.) to promote a ban on genetically engineered foods until exhaustive pre-commercialization human testing is performed.

Fagan's concerns can largely be grouped under two headings: Biotechnology products, due to their very nature as products of genetic tinkering, are simply one big "unknown"; second, genetically engineered food may contain unknown allergens and

toxins, and must be thoroughly tested for them. I have previously described a number of reasons for regulating the products of biotechnology, and not the process. Therefore, I will not discuss the first of Fagan's concerns any further. However, the concern over allergens and toxins in recombinant foods is a reasonable concern, and I will now discuss this aspect at some length.

Like most empirical assessments, risk assessment is a science that has undergone considerable procedural revisions in its history. Unfortunately, to describe the many facets of risk assessment is far beyond the scope of this thesis, but I will address some brief comments on it, in light of the concern expressed by Fagan and others. The U.S. Food and Drug Administration (hereafter simply FDA) is the agency responsible for food safety testing in this country. Their current protocol for recombinant foods requires that they be tested for known allergens and toxins, based on the parent or unmodified organism (the UMO). All allergens are proteins, and in an Internet document entitled "Foods Derived from New Plant Varieties" the FDA has noted that

> At this time, FDA is unaware of any practical method to predict or assess the potential for *new proteins* in food to induce allergenicity and requests comments on this issue. (1998, 9; emphasis added)

Similarly, the FDA earlier noted that regulation of allergens in recombinant food is to be based on the characteristics of the host plant and the donor plant (1998,8). With regard to toxins, the FDA states that

> The likelihood of activation of quiescent [metabolic]pathways or increased expression from active pathways is considered extremely low in food plants with a long history of use that have never exhibited production of unknown or unexpected toxins, *since the genetic changes that can lead to such events occur during growth and are induced with traditional breeding manipulations*. In the few cases where toxicants have been raised to unsafe levels in a commercial plant variety, the toxicants were known to occur in significant levels in one of the parent species. (1998,8; emphasis added)

In contrast, Fagan devotes six pages to the development of a highly convoluted testing scheme for potential allergens in recombinant foods. The crux of the differing positions is to be found in the two words employed by the FDA: "practical method". It is important to note that all risk assessment schemata are subject to several conditions:

accepted conventions of the scientific community; a reasonable cost to employ the assessment; and historical data.

Three problems are illuminated immediately, regarding Fagan's risk assessment scheme. First, his formula for evaluation is not conventional, and would require a large amount of both animal and human test subjects (engendering further potential problems) – for *unknown, potential* allergens and toxins. I think it is doubtful that the FDA would consider such an elaborate risk assessment procedure, although that remains to be seen. The FDA requested comments on new and potential allergens in the same report (1998,9). The cost-benefit aspect of such a complex risk assessment procedure is a difficult issue; after all, how much is a life worth, in the event of a new toxin causing the death of one or more individuals? However, based on this FDA report, it seems highly unlikely that any new toxins or allergens will be produced, much less one that might result in a human death. Finally, I would like to note that on the basis of historical data the experts from the FDA, as well as outside consultants that may have been utilized for this report, consider the production of new toxins or allergens highly unlikely. In my opinion the FDA is traditionally conservative regarding food testing, although I would also note that for truly novel products they are indeed open to criticism.

Finally, I would like to conclude this section by noting some of the empirical shortcomings that seem to be pervasive in Fagan's writing. In his proposal for extensive precommercialization human testing, he does not suggest how large and variable a sample of humans might be required for a "dependable" or "significant" test. These are real problems, and it is important to consider the consequences of his use of such imprecise terminology for applications of empirical procedures.

Fagan also mentions the well-known GEM-tryptophan incident, in which some 37 people died and 1500 were disabled from an unforeseen byproduct of L-tryptophan produced by a recombinant microorganism (Fagan 1996,10). This was clearly an empirical issue, as

> ....scientists have deduced that this [toxic] compound was generated within the [genetically modified]bacteria when internal tryptophan concentrations reached such

> high levels that tryptophan or its precursors began to undergo side reactions that led to dimerization[conversion of L-tryptophan to the new, toxic contaminant].(Fagan 1996,10)

Unfortunately, the company destroyed all of the remaining samples of the GEM, and there is some doubt as to whether the problem was related to the recombinant procedure, the purification procedures involved in producing the tryptophan, or both. What does remain clear is the fact that this entire situation is one that was amenable to existing empirical testing methodology. For example, the company (Showa Denko) could have experimented with producing variable concentrations of tryptophan in the GEM, while assessing some of the chemical byproducts of their procedures.

Is this a classic example of the hazards inherent to the process of genetic engineering? Fagan explicitly thinks so (ibid.), but I do not feel that he is justified in his conviction. There is an overwhelming amount of evidence that indicates that most hazards related to genetic engineering are based on the product, and can be adequately assessed by examining the molecular biology and, in some cases, the natural history of the parent and donor organisms. This is not to say that there are not particular problems related to environmental releases of GEOs, nor that such releases are equally easy to assess for environmental risks. Furthermore, evaluating the product may not always be successful for establishing health or environmental risks – but it is an example of utilizing the best available technology and information in the best possible manner. I wish to claim that our current social and professional consensus on risk assessment procedures is derived from a long history of reflection and modification, and as such it is worthy of considerable respect.

In the following chapter, I will examine some particular aspects of the philosophy of biology and the philosophy of ecology, and I will propose that for practical ecological applications the case study method will provide the most accurate information. Finally, in Chapter Four, I will apply the case study method to a genetically engineered plant, oilseed rape, which is being widely utilized in North America.

## CHAPTER 3: SPECIAL CONCERNS IN THE PHILOSOPHY OF ECOLOGY

Ecology is largely an offshoot of the scientific discipline of biology - it is one of the life sciences. To the degree that ecologists examine such phenomena as ecosystems and biogeochemical cycles, ecology may appear to be almost equally a part of the physical sciences, but at heart it is a biological science. Accordingly, I will discuss the following issues in this section: (1) The autonomy/provincialism debate in the philosophy of biology, which also has implications for ecology; (2) An update on the state of critical knowledge in ecology, much of which helps to shape the debate on future directions in ecological methodology; (3) A brief examination of some methods that prevail in ecology, and why that is the case; and (4) How to resolve some of the epistemic crises in ecology, including those involved in the troubling distinction between ecologist *qua* scientist and ecologist *qua* environmental manager.

After examining these issues, it should be clear what positive steps may be taken by ecologists (acting as both scientists and *de facto* shapers of environmental policy) to facilitate the careful release of genetically engineered organisms into the environment. Such assessment procedures, and their philosophical strengths and weaknesses, will be the topic of Chapter 4.

### AUTONOMY vs. PROVINCIALISM IN THE PHILOSOPHY OF BIOLOGY

Are organisms special entities, deserving of unique treatment in the philosophy of science? This is perhaps the key question in the philosophy of biology, and is followed closely by the complex issue of species definitions that I will allude to later in this section. To return to the question surrounding the philosophical status of investigations of organisms, it is first important to note that this is of *direct* interest to this thesis, particularly since I am examining the problematic issue of introducing genetically engineered *organisms* into the environment. Furthermore, since much of ecology deals with interactions between organisms and between organisms and their biotic and abiotic environments, this is an issue that is very critical for ecologists. Finally, it is also an issue

for ecologists because the young discipline of ecology falls squarely between the realms of biological and physical science - hence, debates about research methods are acutely important for ecology as it matures into an older, more mainstream science. One would hope that as ecology becomes more mature and theoretical (though I hesitate to use the two words interchangeably, I will nonetheless), it might tend to be less radically split by the divisive ideological debates that have plagued it in the past ten to twenty years. However, one aspect of this ongoing debate is that of the proper role for the ecologist to play in the political sphere, and I will return to that issue in a later section on values in ecology.

The autonomy/provincialism debate takes the following form: Are the differences between biological science and physical science such that they (a) require different methodologies, and (b) possess different epistemic values (e.g. confirmation, sufficient evidence, etc.) as a result of their respective conclusions? To further reduce this general question, should the notions of causality, experiment replication, elimination of alternative hypotheses, and even predictive value be applied equally to both areas of science? Autonomists, as the label implies, believe that biology in theory and practice is properly distinct from that of physical science. Provincialists, however, see biology as a "province" of sorts of the physical sciences, albeit one with deficiencies which may never be reconciled. But what seems most important to the provincialists is that biology needs to strive to attain the ideal (reductionistic) *methodology* of the physical sciences, and naturally the kind of deterministic finality of knowledge associated with those methods will follow.

Alexander Rosenberg (1985) is the premier advocate of this particular distinction, and it is to him that I owe both my understanding of the distinction and many of the arguments aimed at resolving or clarifying it, as well. Historically, the arguments in favor of provincialists have rapidly accrued with the many gains in molecular biology and biochemistry in the past 20-30 years, and the closest physical science analog to these fields is organic chemistry. As more is understood in these realms, due to advances in both instrumentation and laboratory techniques, more information is accumulated that allows for definite knowledge of how organisms behave, or to put it in *teleological* terms,

how organisms *achieve their goals, or ends*. Similarly, as more is known, then such behavior on the part of the organism can be reduced to nonteleological language, which is therefore more comfortable to reductionistic scientists who do not care for the explicitly metaphysical formulation of such teleological language. Before analyzing either stance, it is important to examine *why* teleological language is prevalent in biology in the first place.

Upon examination of the embryological development of any higher-level organism, it becomes immediately apparent that everything occurs as a fulfillment of a function. As early cell-differentiation proceeds to the more specialized division into organs and limbs, it has been noted that the cells seem almost *programmed* to achieve their end, i.e., a complete organism. Naturally, mutations and other maldevelopments occur, but research aimed at halting certain developments or intentionally grafting cell groups into the wrong areas can even be righted sometimes by the organism.

Rosenberg (1985,38-41) even utilizes the example of the uracil/thymine difference in RNA and DNA, respectively; this phenomenon can only be understood in a functional manner, since chemically speaking it does not matter which molecule is formed, except that thymine is a more energetically-costly molecule. Thymine acts as an internal mechanism to prevent a point mutation in the event that cytosine deaminates to form uracil - in this event, a "daughter helix" will have an incorrect base-pair at that site, and will change the coding instructions for RNAs constructed from that daughter helix. The thymine base, which possesses an extra methyl group, acts to prevent an internal DNA-repair mechanism which is incorporated to remove uracil resulting from deaminated cytosine in the DNA double-helix. This example clearly defies any form of explanation except in terms of a function.

The difference between these two examples is a crucial one, that of *explanation*. Simply put, autonomists employ a different form of explanation for what they observe in living organisms, which is called a functional explanation. This was hinted at in Chapter Two in my discussion of Gould and Lewontin's article (1979) on the "adaptationist programme" in evolutionary thought, viz. that every aspect of an organism was *de facto* assumed to be an adaptation. Similarly, for autonomists nearly every trait observed in

biology is perceived as an adaptation, and one that is there *for a function*. Admittedly, the adaptation may no longer serve a useful function, but it is assumed that it once did, and now it merely performs a function regardless of its (perceived) utility.

At this point in my discussion, it is important to note that the steps toward outlining this debate take two forms, which I will term "evolutionary" and "philosophical" justification. I will briefly discuss both forms, but I will not attempt to close this debate once and for all, as it is largely beyond the scope of this thesis.. The evolutionary approach is of interest because that is one direction that ecologists always take in examining ecological risks regarding extant populations, and the philosophical approach is directly relevant for reasons which should be obvious. Finally, I will conclude this section by presenting my stance on this issue, for the purposes of this paper.

In the interpretation of Darwinian theory by biologists, there is sufficient room for an autonomist or a provincialist account of evolutionary theory. For the autonomist, evolutionary changes are the underlying causes of biological teleology - that is, the evidence for such inherent teleology in living organisms can be found in the succession of evolutionary changes, or adaptations, that are part of the natural history of that taxa. However, the provincialist reading of Darwinian evolution would naturally stress the inherent randomness, the lack of algorithms of any kind, of the physical causes and events for which organisms are adapted in the first place. In Chapter 2, I discussed some of the implications for this aspect of Darwinian thought, which is also the thesis of Daniel Dennett's 1995 text Darwin's Dangerous Idea. The newfound "danger" in Darwin's idea was this randomness in nature to which all organisms were compelled to adapt, which in turn made Darwinian evolutionary processes inherently probabilistic. This is clearly a problem for the autonomist, but for a further reason.

Adaptive explanations are philosophically weak, insofar as they are *explanations* at all. Why was a particular adaptation "chosen" rather than another? Assuming that there are other equally valid alternatives for such adaptations as three- and four-chambered hearts in reptiles and mammals (to borrow an example from Rosenberg 1985), scientists can only explain the explanatory efficacy of the current adaptation, and not what makes it

superior to other possibilities (a point also made by Dennett 1995). Naturally, if in some instances there are not equally valid alternatives, then this becomes a moot issue, but in the millions of years in which evolution has occurred, that does not seem likely to be the case in many instances. The interesting part of this argument lies in the fact that since (in the great majority of cases) we cannot know the route which a particular adaptation has taken in being selected for - e.g., did it start as an "exaptation" following Gould and Vrba (1981)? - such explanation is quite unlike a causal claim in the physical sciences.

Perhaps it is this explicitly philosophical concern that becomes the actual hangup in the evolutionary aspect of the autonomy/provincialism debate: if we can *never* know for sure how an adaptation became prevalent in a taxon or even a particular population of a taxon, then that adaptation can never consist of a causal nature like that of the physical sciences. Rather than draw this problem out further, I will return to it in a roundabout fashion in my later discussion of models in philosophy; indeed, I will suggest that in some very important ways it may not be of any *import* for biologists to know the causal routes associated with adaptations, for the simple reason that explanation in biology is of a different nature than explanation in the physical sciences. As such, I am already framing the exact reasons why I am supporting an autonomist view of ecology, for methodological and explanatory reasons. Furthermore, such autonomist explanations are one of the reasons that ecologists have such a difficult time in determining what factor or factors will be most important in assessing ecological risk -- a fact that I will return to in Chapter 4.

The philosophical aspects of this debate center around the nature of laws in biology. Autonomists claim that there are special functional laws in the life sciences that are not equivalent to laws in the physical sciences, and this incommensurability is the heart of the autonomist position.

Rosenberg describes one possibility for outlining the logical shape which a "law" for a goal-driven, purposive system may take. Given:

1. A goal-driven system, *S*, like an animal or an organ
2. In a particular environment, *E*
3. With a goal or purpose, *G*

4. That can be brought about by behavior, *B*

An empirical, testable law of the form *T* can be derived, as follows: Whenever a system of *S*'s type in an environment *E*'s type has a goal of *G*'s type, behavior *B* occurs, because it brings about (or tends to bring about) goal *G*. (Rosenberg 1985,49)

This law has no causal statements within it, it assumes that contingencies can be accounted for (in theory if not in practice), and it encompasses strong teleological claims. Rosenberg (*ibid.*) utilizes the example of "intercalation" (limb regeneration) in cockroaches and amphibians to perceive how the positional values required for such regeneration may be explained. An obvious weakness of this "law" is the logical "stretch" required to move from a statement that organisms or organs have certain characteristic behaviors to the claim that they actually have *goals* and that the behavior is "*for the sake of these goals*"(Rosenberg 1985,49, original emphasis). Clearly, these particular claims are themselves not testable, and therefore the debate remains incommensurable - if the goals exist, then autonomy is the correct point of view; if not, then there is still room for a provincialist interpretation of biology.

Another possibility that Rosenberg presents is a 5-part definition for a so-called "directively-organized system", which utilizes component subsystems that can account for the apparent "goal-directed" behavior described in the previous law of form *T*. Rosenberg's description follows a 1979 article by Ernst Nagel. Using the same environment *E*, goal-directed system *S*, behavior *B*, and goal *G*:

1. The state of each of the component subsystems of *B* at a given time, *t*, together with the states of the others at that time, causes, in a purely physical way, the attainment of goal *G* at a later time $t + d$.
2. The state of each of these subsystems at *t* is instantaneously independent of the states of the others at *t*.
3. Each subsystem has only a restricted range of states.
4. If the state of one of the subsystems changes greatly enough at *t*, then, in the absence of changes in the other subsystems, at $t + d$, the whole system *S* will be caused not to attain goal *G*.

But:

5. The subsystems are so causally linked that whenever such a great change occurs in one of them at *t*, this change causes changes in the other subsystems at a later time $t + e$, which together with the initial great change at *t* causes the whole system to attain its goal at $t + d$. (Rosenberg 1985,53)

It is immediately important to realize that positing such an alternative formulation to teleology will allow for the replacement of such metaphysical views from science - if they can be sufficiently outlined and justified. Such a directively-organized system, in which all organisms must be one to remain alive, is simply a "strategy for analyzing teleological systems [which] involves the discussion of internal components of functional systems that conspire together, so to speak, to produce behavior that has the external appearance of teleology"(Rosenberg 1985:59). It may be fairly asked whether such an alternate expression is simply a desperate hope to avoid the existence of teleological, and therefore explicitly metaphysical, prejudices in the life sciences. In some ways, I would answer that this is certainly true, and may only represent a philosophical attempt to justify an alternative methodology for explanation of what are essentially the same aspects of goal-directed behavior.

This "directively-organized system" definition faces three immediate pragmatic obstacles, all of which will provide illumination for my autonomist position. First, if such internal component subsystems cannot be found or sufficiently articulated for a given system *S*, then such a nonteleological explanation will not suffice. This may be a methodological problem for practicing scientists, who simply may not be able to find such information on the basis of experimental limitations. Indeed, there are examples from genetics that can be used to argue both for or against the existence of such subsystems. Further, another problem may be posed by the existence of variations in the internal subsystems within identical teleological systems. This may occur in the context of variations within species in different environments, or it may occur within similar species in the same environment. The final problem, and the one which truly spells disaster for practicing scientists, is the question of incomplete descriptions of internal subsystems in a given system, *S*. Beyond the sphere of molecular biology, whose knowledge base is growing almost geometrically, such incomplete data is nearly always the case. Interestingly, recent advances in identifying the entire genomes of the *E. coli* bacteria and some yeast (*Saccharomyces*) species point to the possibility of future realization of this goal for provincialist biologists. Some headway is being made in the

ecological specialization area of energetics, but even this field remains highly theoretical and largely speculative (particularly in its explanatory strengths.)

So, what conclusion may be reached? Although the notion of "directively-organized systems" carries great appeal for most scientists, it remains plagued by empirical shortcomings, and may continue to be plagued for a long time. Further, to exhaustively categorize *all* of the necessary component subsystems for any system *S* may be impracticable, if not impossible. Third, the possibility of limitless exceptions for such subsystems, depending on almost equally limitless variables like climate, population density, season, brood number, age, food availability, etc. create a situation in which such a system would have far less explanatory efficacy than current models and accepted theories. Finally, there is a further issue surrounding the fact that just because teleology has been eliminated from the physical sciences, does that mean it is necessarily better to eliminate it from the life sciences? This is a metaphysical prejudice that is attached to physical sciences and is sometimes derisively termed "physics envy", particularly by ecologists who are dismayed by the overquantification of ecology. It is for all of the above reasons that I will subscribe to the autonomist view of ecology, with the added claim that <u>some</u> ecology can of course be quantified. Furthermore, I will continue this chapter by stressing that it is explanatory efficacy that is most important for ecologists, and that it is one of many values that are both implicit and explicit to the science of ecology.

I have attempted to illuminate the exact nature of the autonomy/provincialism debate in the philosophy of biology, as well as some of the steps that have been taken to justify each of the respective positions. Ecology is a science with largely biological roots (particularly community ecology), so it is important to enumerate that debate because it has very specific implications for the degree of "knowledge" that ecologists feel is justified in their studies. Those epistemic limitations are then carried into the sphere of environmental policy, where ecologists are expected to make decisions based upon the "best available data", which is itself often sorely lacking (see e.g. Abbott 1994; Andow 1994).

## ECOLOGICAL KNOWLEDGE

Every ecology textbook customarily has a number of definitions for such controversial words as "ecosystem", "niche", "community", and even "equilibrium" and "stability". However, the authors will normally go on to use a single definition, and explain why they feel that is the best definition. A word like "ecosystem" or "community" has two components - its spatiotemporal range and the actual relations between its constituent "members". These two components are the main reason for such definitional difficulty, and ecologists will customarily state what exactly they take to be the community or ecosystem of mention, and why. For instance, one ecologist may choose to use a watershed for the boundaries of an ecosystem in a study, although there are clearly other organisms of various types outside of that watershed that interrelate with organisms within that watershed. However, such easily defined boundaries have great practical value, also known as *heuristic* value.

Similarly, an ecologist may choose to define a community by a large or otherwise predominant plant species (a mixed-hardwood forest), a topographical feature (a wetland or ridge), or its climate, which may also be a function of altitude (e.g. a subalpine ecosystem above treeline.) Any of these broad community definitions may be further enlarged, or further delineated, by adding to it a prescribed spatial limitation, either in a unit like acres or by use of a cartographic mapping system's coordinates.

Interestingly, the scenarios I have just posed are all relevant to an ecologist undertaking some form of study, either of a natural history-type, a formal or modified experiment, or even some form of modeling technique. I point out this distinction for a very good reason: there are some clear differences between ecology in *theory* and ecology in *practice*, and this insight will prove very beneficial in the following pages. It is important to note that such a distinction is customarily unique to the life sciences, since most investigations in physics and chemistry occur in the laboratory under conditions that are for the most part controlled through the use of apparatus. (A notable exception is geology, which for its almost purely historical nature is outside the realm of this entire topic.) Scientists that are dealing with live organisms, and especially those that are in the

field and therefore assumed to be replicating "reality", are far more difficult to design controlled experiments for - there are too many variables, too many unknown initial conditions of varying import, and in the end there are often too many alternative explanations of equal value to the hypothesis and/or the null hypothesis.

Therefore, the theory/practice distinction in ecology takes on an initial form - the practicing ecologist attempting to observe and/or design experiments and models, and the ecologist that works toward evaluating and developing theories, both new and old alike. Before moving on to another format for this theory/practice distinction in ecology, it will perhaps be beneficial to examine some reasons for the difficulty in establishing working definitions in ecology (much less ecological *laws*!), as these reasons will be important when I present an example of the case study technique in Chapter 4.

As discussed in the section on provincialism vs. autonomy, there is really nothing that approaches the epistemological level of "laws" in the life sciences, including ecology. One reason for this was presented in the earlier discussion of the statistical model of covering-law explanation theory - since living organisms often lack a discrete "causality" (i.e., a distinct *cause* for their behavior), it is best to present such explanations in a statistical form. Interestingly, Darwinian evolution by natural selection is also presented in a *de facto* statistical form: "If enough organisms of species X have adaptations of form Y with a higher survival value than other members of species X, then over time species X will *probably* evolve so that all members include that adaptation Y, or evolve into a new species that has that adaptation Y."

Even the Lotka-Volterra predator-prey equations, which explain roughly what can be expected to happen in a given predator-prey relationship, cannot be presented as laws. There are simply too many exceptions and necessary conditions, so as to preclude wording the equations as laws. However, the difficulties related to the use of ecological terminology run a little deeper, and further investigation will prove useful in later sections of this chapter. I will focus on the terms "community" and "stability", and to a lesser extent "equilibrium". Interestingly, Shrader-Frechette and McCoy (1993), to whom much of my philosophy of ecology discussion is indebted, also focused on these terms,

but for different reasons. I wish to use "community" because it is now used to distinguish community (arguably, a/k/a conventional) ecology from such subdisciplines as population ecology, systems ecology, and microscopic subdisciplines like microbial and molecular ecology. Furthermore, community ecology (loosely speaking) and the now-controversial notion of "stability" and its related term "equilibrium" will all be used in present and future decisionmaking formats for environmental policy, and therefore these particular terms shape the political environment in which ecologists will be expected to contribute as experts. Finally, keeping to the patterns in this paper, I will limit my discussion to that of terrestrial ecology only, as that is what I have chosen to use for my examples throughout this thesis.

At the turn of the century, Frederick Clements pioneered the use of the term "community" as an assemblage of at least two plant species, commonly found together in such easily-defined environments as wetlands, meadows, forests, etc. Thus, he placed a biological association in space and time, recognizing it and associated species that were often found together in discernible patterns. Over time, the definition became much more complex, to where it included arrangements of plant species and even animal species which came to be associated with certain plant species in certain climates. The reasons for these criteria were simple: many plant assemblages were limited in distribution by conditions of soil and climate; many of the plants found together were found together in varying geographical locations; and many of the insect and animal species seemed to be common to these assemblages.

It is thus clear how such pragmatic definitions came to be further utilized in the later development of "succession" theories, in which such assemblages became relegated to temporary positions in time in a given geographical location, particularly after environmental perturbations (fire, earthquakes, human effects like deforestation, etc.) When early ecologists noticed that the patterns of succession in similar conditions of soil and climate could be predicted, it soon gave way to the essentialist notions of "stability" and "equilibrium." Even when they recognized the danger of espousing such ideas, and called it "loose equilibrium" and other not-so-stringent names, it all resulted in the same

net effect. Early ecologists largely seemed to think that natural systems existed in some sort of stable "balance", and that even though it might shift this way and that, the shifts all existed around some point of equilibrium.

This is, importantly, somewhat analogous to the Lotka-Volterra equations, in which the numbers of both predator and prey oscillate around each other. When predator numbers go down, prey numbers go up, all things being equal; eventually, predator numbers will rise due to the abundant prey, reach a certain level at which they cannot be sustained, and then crash - and then the cycle begins anew. Granted, the equations are never *in equilibrium*, but the whole relationship is in a sort of loose equilibrium, and so ecologists thought that perhaps this is how ecosystems functioned.

There are two obvious candidates for why such an explanatory mechanism arose: a metaphysical predetermination toward a view of nature, which is not necessarily due to a similar metaphysical notion of an omnipotent, omniscient creator who knows what is best for His creation; or, it may be a result of an epistemological prejudice of sorts towards a world in which there exists such equilibrium, such stability. Simply put, such a world would be easier to understand for many people brought up in a western worldview, rather than a natural world of perpetual upheaval and entropy (after all, living systems are all counterexamples to the Second Law of Thermodynamics, insofar as they remain "living".) However, regardless of the explanation for such conceptualizations of essentialism in ecology, such prejudices continue to prevail in both the public sphere and the sphere of theoretical ecology. In defending such a notion of equilibrium, an ecologist may posit "How else are we to explain how an ecosystem - particularly a geographically-large community like the northern boreal forest in North America - can seemingly stay the same for such a long period of time, at least several thousands of years?" This is a difficult question to refute for an ecologist, although there are two main options: 1) What criteria can be stipulated so that all ecologists may make the same observation?; and 2) What about other ecosystems, for example prairies and perturbation sites, where different species may proliferate from year-to-year or decade-to-decade? Further, if a site were to have a number of consecutive perturbation episodes, when might we decide that perturbation is the norm for that site?

In asking such questions, it becomes evident that one solution might be for ecologists to try to reach a consensus in stipulating such definitions and descriptions. That may be acceptable for an interim time period, but in some circumstances it may not be acceptable at all. For example, the now-infamous case of the rapidly disappearing northern spotted owl of the Pacific Northwest. Some alternative explanations of its population decrease may be normal fluctuations; loss of habitat, which is apparently old-growth canopy cover; disease, of which we are witnessing the aftereffects; normal extinction of a previously-dwindling population over time, which may or may not have been accelerated by human activities; increased predation and/or mortality rate; decreased prey availability or prey mortality, particularly if subpopulations had become overly specialized predators; genetic drift in subpopulations, and/or increased negative effects of a small gene pool; etc.

In positing such possible explanations, it becomes clear that a mere consensus of ecologists will likely not help to arrive at the better or best conclusions. For instance, there may be discrepancies between theoretical and practicing ecologists over both methods used and data interpretation, and even within each division. Furthermore, much of this debate transpired within the public eye, and there were additional political forces to account for as well. In such a milieu of opinion, can there ever be a right or wrong answer? Probably not. One positive effect of this largely public debate was to place the question of *values* in ecological methodology in the spotlight, literally *forcing* ecologists to acknowledge the role of values both in their science and in their subsequent evaluation of data, methods, evidence, and existing theory. Therefore, it is to the topic of values in ecological science to which I will now turn.

## VALUES IN ECOLOGY

There are several different types of values in any science, and there are additional values which are specific to ecology. Understanding the role of values in science, from the choice of a design to the more difficult choice of deciding on the acceptability of data, frequently represent very difficult decisions for both experimental and theoretical

scientists. Following a discussion of these values, and an elucidation of the importance of these values, I will present some arguments in favor of the use of models in ecology, and particularly a form of model called case studies. In lieu of these arguments, I will then proceed to present some ways in which case studies may be used to evaluate the effects of GEO introductions into the environment, in Chapter Four.

The apparent dichotomy of fact and value was a bastion of sorts for the logical positivists, and was one of the reasons for the downfall of positivist influence in philosophy of science, as I have already discussed (see also Rosenberg 1985; Shrader-Frechette and McCoy 1993). However, as scientists are all human beings and accordingly fallible, many decisions have to be made in the course of an experiment, from its conception and design to the evaluation of its data and the conclusions that are drawn from the data. The positivists wished to eschew any sort of partiality or opinion in the guise of "values", and instead emphasized the gathering of empirical data and a strict subscription to the methodology of hypothesis-deduction, also known as the Hypothetico-Deductive (H-D) model. Clearly, these are excellent goals, but in the several steps that must be taken in the course of an experiment many choices are made, data is evaluated, a hypothesis is likely generated or falsified, and eventually theories are created which must be compared to older theories. Each one of the steps I just described requires making an evaluative decision, and even further beneath the surface lie more sacrosanct values like *predictive ability* and *falsifiability*. The closer one looks at scientific methodology, the closer one finds that the method of model creation and analogy is heavily used -- and each of these scientific tools require evaluative assumptions, as well. This is particularly true for any scientific field attempting to quantify "unobservables", which are by definition less empirically-sound then data obtained by direct observation.

Unfortunately, much of this philosophically-fascinating topic is far beyond the scope of this thesis, and I intend to cover the topic of values in science (and particularly, in ecology) in a very brief fashion, barely touching on many important issues. However, the issues that I do reveal are of tremendous importance to ecology, both as a science and as a tool for creating scientifically- and ethically-sound environmental policy.

There are seven main values in science that I consider to be the most influential, and most frequently mentioned: predictive ability; falsifiability; simplicity; parsimony; empirically sound (and therefore testable) data; heuristic power; and theories that approach a lawlike status. This latter value is part of the discussion of the autonomy/provincialism debate discussed at the beginning of this section, and accordingly I will not address it further. Similarly, I discussed Karl Popper's emphasis on falsifiability at the outset of this chapter, along with the emphasis on empirical data (which is both "available" in the public domain, as well as replicable.) Simplicity is a complicated value, which has *prima facie* value due to the principle of Ockham's razor : if something can be explained equally well in simple terms or in complex terms, the simple explanation is much to be preferred. However, there is another side to simplicity, which makes its inherent value dubious. Many models in ecology are aimed at merely gaining more understanding of complex phenomena, and so exceedingly simple models are created and systematically refuted in the process of obtaining a greater understanding of such complex phenomena. An oft-witnessed result of this use of models is that the data can be interpreted incorrectly, or that too much information can be *read into* the data, or that invalid conclusions are drawn as a result of the data and/or the scientists' own conclusions from the model. I will return to a discussion of models in ecology in the following section.

Heuristic value is by nature instrumental, and I suppose it is unnecessary to defend heuristic value for the simple reason that anything that leads to greater understanding in science, and particularly in a young science like ecology, is valuable. Parsimony, on the other hand, is a far more conservative value in science, and is addressed more to theory-building and theory-evaluation. If a theory has been replicated numerous times under a wide variety of conditions, and a single instance of falsification is then recorded, to immediately discard the old theory would be akin to "throwing out the baby with the bath water." Ironically, if Popperian doctrine were carried out to the letter, than such an immediate refutation might be justified, as Popperian falsification required only one instance, as compared to many efforts at corroboration. Much of this

discussion can be easily reduced to pedantic epistemological considerations about what constitutes knowledge in science, which is far beyond the scope of this thesis. For good or for bad, ecologists are *expected* to make actual contributions to public, environmental policy on the basis of their knowledge base in the scientific discipline of ecology - and this is the issue I shall return to shortly, in my discussion of ecological models. And, of course, ecologists may differ in their perceptions of responsibility in the public policy sphere, even though they agree on the criteria for evaluating ecological theories.

Finally, there is the extremely problematic arena of predictive value, which is difficult for all of the life sciences, but especially difficult for ecology due to its innate complexity. This is an issue that I touched on in the autonomy/provincialism debate, and one which I will return to momentarily. It is important to appreciate at the outset, however, that it is entirely possible that *no theory in ecology may ever possess absolute predictive value*. When asked to assess the potential ecological damage of a human action, e.g. polluting a body of water, an ecologist may be able to ascertain a certain percentage range of mortality for fish or macroinvertebrates, based on existing information. However, there may be additional mitigating factors for or against the survival of those aquatic organisms, which were unknown at the time. The resulting public outcry due to 100% fishkill might then be aimed at the ecologist, who ironically could not possibly have known that there was also an algae bloom occurring upstream. Such examples of synergistic environmental stimuli are widespread in ecology, and are frequently beyond the range of human abilities to identify at any given time. This example of an ecologist's predictive <u>inability</u> is not intended to claim that ecology is predictively useless, but merely that ecological events often have multifactorial causes which often defy human foresight. Such cautious messages from ecologists have been ubiquitous in the arena of biotechnology, and I will return to this issue in Chapter 4.

It is important to note that predictive ability in ecology is hard to come by, and that when it is possible it is usually due to a preponderance of available data for evaluation. In most instances, there is an alarming paucity of data available for the ecologist, and particularly data obtained over a long period of time. Again, predictive ability (and the lack thereof) is at the heart of this thesis, and I will return to this topic

several times in the remainder of this paper; but for now it is sufficient to note that although predictive ability may be easy to come by in the well-established theories of the physical sciences, that is simply not the case in ecology.

Some values in the sciences are used to fill the gap that is always present between hypotheses and evidence, and this is one of the reasons that the scientific method requires repeated attempts at corroboration and replication of the results of a given experiment. However, Shrader-Frechette and McCoy describe three acute problems for the H-D model in ecology:

> (1) The lawlike status of ecological hypotheses is often questionable;
> (2) It is difficult to construct uncontroversial null models to test hypotheses;
> (3) Cognitive or methodological value judgments in ecology often determine the relationship between evidence and theory. (1993,81)

Such methodological values often take the form of evaluative assumptions. A common example of an evaluative assumption is a mainstay of models in bioenergetics, in which it is assumed in every model that all individual organisms wish to maximize their energy intake and minimize their energy use through their behavior, so as to be a net energy consumer. Associated assumptions are that the energy quantification methods are sound; that organisms are even aware of an energy budget, and that such awareness may lead to changes in their behavior; and that organisms have the time and other available resources to place some concern on their individual energy budgets. Unfortunately, these assumptions and their attendant weaknesses are often implicit within the energetics model -- that is, they are undeclared. Such assumptions also *further* assume that they do in fact replicate nature, which may not be true all of the time or even any of the time. I will return to a discussion of the instrumental use of models as "conceptual tools" in the following pages.

There is another fashion in which ecologists may go too far in the use of evaluative assumptions, and the example of conservation biology is important for two reasons. The obvious reason is that:

A) conservation biology has definite normative values which are an explicit part of its domain, including

(1) species diversity is good;
(2) The more species that remain, on a global scale, the better (i.e., any extinction, human-caused or otherwise, is bad); and
(3) Humans have no right to cause extinctions as a result of their actions;

B) much of this thesis is dedicated to analyzing why it is philosophically problematic to ask ecologists to make normative declarations *on the basis of their science*, while conservation biologists are declaring that it is not only acceptable to ask that of ecologists, but that ecologists should take an active role because it is their *responsibility* (see the discussion of the related Noss-Kangas controversy in Shrader-Frechette and McCoy 1993, Ch. 4 & 7).

The second and more implicit reason is that conservation biology has actively extended island biogeographic theory to mainland ecosystems, for theoretical purposes and recently for practical purposes - the design of biological preserves intended to stave off extinctions, loss of important habitat, and loss of wildlands or wilderness areas. Shrader-Frechette and McCoy astutely point out that this is a questionable analogy, and possibly one that is incapable of empirical confirmation as to its validity. For example, it is assumed that the species-area relationships for endangered populations resembles that of island populations, and this is a scientifically-dangerous assumption. However, given point B in the above paragraph, if a conservation biologist has been assigned the task of saving an endangered population, such questionable assumptions are of small importance if that population is gone in 10 years, while ecologists were striving to gather scientifically-sound data and evaluate theories. Again, I am hinting at the difficulty in combining the duties of a scientist in an imperfect discipline with the public expectations of that scientist, and the result is a philosophical quandary.

In the earlier section on "Ecological Knowledge", I discussed the difficulties associated with much of the key terminology in ecology. Given that the terms cannot, *prima facie*, have an "absolute" definition, they require some form of consensus among ecologists regarding shared use, along with a declaration by experimental scientists to the effect of "this is how I intend to use and define the ecological community (or ecosystem, or plant association, etc.) I am studying." One reason for such caution is that it can help to

avoid tautological definitions - e.g., "of course a niche is defined by its occupants!" - and also because the way that ecological processes are defined helps to shape the confirmations that result. If an experiment is defined ambiguously, and is subject to numerous alternative explanations, then the empirical data that is generated will be similarly ambiguous and incapable of confirmation. (Although it is important to note that an improperly designed experiment does not necessarily lead to inaccurate data, as the processes of data collection and organization are a different aspect of the research design.) A similar issue is that many scientists do not seem to make a fair attempt at nullifying their null hypotheses, therefore rendering their null models and experimental conclusions inadequate. One reason is that there may be too many equally valid alternative hypotheses, most or all of which cannot be ruled out due to a relative paucity of data. If this happens, it may mean that the scientist has picked an insufficient starting point for the experimental investigation, and must now perform more basic experiments as the first step of a systematic reduction of "unknowns" and alternative hypotheses. However, the process by which scientists *appear* to create null models according to the H-D method and then intuitively support them is a thinly-veiled form of modeling in ecology, which is meeting with increasing acceptance amongst other members of the scientific community. It is to this topic which I will now address myself.

## MODELS IN ECOLOGY

First, it is important to distinguish between informal or verbal models, and the more scientifically rigorous mathematical models. One example of verbal models is the Philip Regal (1987) article suggesting different ways in which GEO introductions to the environment may be hypothetically predicted, discussed in Chapter Two, "Natural Selection and GEOs in the Environment." Mathematical models take one of two forms: statistical models which seek predictive value in probabilistic terms, or models which attribute mathematical values to certain processes or behaviors and establish formulas to predict what may happen. A classic example of a mathematical ecological model is the bioenergetic form, in which the behavior of an organism (or the direct reaction to a

stimulus) can be mathematically predicted using a number of stipulations, formulas, and of course assumptions.

One of the most important rules for the correct application of models as an investigative tool is to *honestly present all known assumptions at the outset*. In this manner, the data collected, the methods employed, and the conclusions drawn may be fairly analyzed by an impartial investigator. Why is that important? Because while the data and the methods may be excellent, a scientist may draw the wrong conclusions, or vice versa. Similarly, all of the information may be correct, but the conclusions may be misapplied due to an improper understanding of the assumptions that the investigator utilized. Granted, this last case is not the direct responsibility of the investigator, but it is helpful for the sake of all ecologists if such misunderstandings could be reduced where possible.

Unfortunately, some critical assumptions may turn out to be practically *intrinsic* to a given model, and one example was already given: applying island biogeographic theory, a heavily-investigated and widely-respected theory, to mainland examples of habitat "islands". The assumption that such extension works has not been adequately addressed, and one of the main reasons is *time*. Because of the often high rate of migration/immigration on coastal islands, the importance of time as a relevant factor could be minimized by MacArthur and Wilson, the original authors of island biogeographic theory. This is probably *not* the case for nature reserves, and although researchers have strived to estimate Minimum Viable Populations as well as Estimated Time to Extinction (MVP and ETE, respectively), ETE may have many more variables on a mainland habitat island than it would on a true island - although in practice the opposite may be the case. In any event, the application of island biogeography to conservation biology is now so far advanced that the question of its validity will probably never be adequately addressed, although that makes it no less of a philosophical problem.

Another interesting aspect of models, which I hinted at in the previous section, is that their methodology is nearly the opposite of the H-D method. Whereas the null model may ask "Given conditions X,Y,Z, will A occur?" and then "expect" that A will not occur

(the formulation of the "null hypothesis"), models seek to find that A will occur. Usually, such model-formation will begin with asking about X, Y, and Z as precursors of A separately; then, the investigator can examine to see if the conditions may counteract each other to prevent the occurrence of A. If not, then under what conditions similar to XYZ will A not occur?

Interestingly, such negative questions are a frequent goal of ecology, as it is essentially a science of limits. Examples range from examining the age- and population-density limits for individual organisms, to the notion of limiting nutrients for aquatic and terrestrial ecosystems. Further examples include tolerance limits for such environmental effects as heat, light, drought, slope and altitude, or toxicological limits such as maximum amounts of certain chemicals for ingestion or other exposure. To continue with a negative question, a scientist may inquire as to what effects an environmental perturbation may have on a given population, community, or even ecosystem; similarly, the ecologist may choose to determine how much disturbance a population or system can tolerate, which is another way of wording the same issue. Even more important, it is another way of wording the objective of this thesis, in the following manner: (1) Given that GEOs are almost certainly going to be introduced to the environment at some point in the future (granted, limited field introductions have already occurred, but nothing deemed to be especially risky), might they act as a perturbation to existing populations and/or communities? (2) If so, what are the possible effects *and* how might they be predicted and/or addressed?

These two questions are exactly what ecologists are now being asked, and they are also the same questions that scientists have been asking of themselves since the early 1980s (see Krimsky, 1982). The informal verbal models presented by Regal in his 1986 paper "Models of Genetically Engineered Organisms and their Ecological Impact" are an excellent introduction to some ways in which ecologists may choose to conceptualize the impacts of such novel organisms, but as scientists their research interests must delve much further and be more critical in nature (for a competing position see Brill, 1985). The conclusion hinted at by Regal and mentioned by many other reasonable ecologists

are that there is only one method that will be suitable for adequate results, a form of model called "case studies." It is on this topic that I will now focus my efforts.

## CASE STUDIES

In the previous sections, I have sought to establish a number of scientific "difficulties" in ecology which help to prevent it from attaining the status of a "hard", physical science with strong predictability and well-established ecological laws. Key terms in ecology are commonly misunderstood and used in more than one fashion - words like community, stability and equilibrium. Other equally important words are used carelessly, like "species" - though there is a shared understanding about this word among biologists, it continues to be a term which wears many different hats depending on its contextual usage. The uniqueness and constant change of communities and their resident populations typically only allow for general mathematical models (like the Lotka-Volterra equations) that "do not express a general, empirical law, but rather a more-or-less accurate fact about a particular situation"(Shrader-Frechette and McCoy 1993,116). The frequent change in community structures also allows for historical differences, so that the spatiotemporal community dimensions remain critical.

Given the admittedly historical contingency of community ecology, it may then be fair to ask whether it is even reasonable to expect it to meet the traditional scientific expectations of predictability and strong explanatory value. It is clear that the method of case studies, utilizing investigations of a singular spatiotemporal community which may or may not be applicable to other such communities, has strong explanatory value in ecology, particularly where very little knowledge of the community relations exists already. Further, given this lack of knowledge of ecological relations, it is also clear that the "covering-law model" of explanation becomes a difficult standard to achieve. The covering-law model, like all models based in part on deductive methodology, require a knowledge of *all relevant initial conditions* in order to make the deductions valid. If there are any "unknowns", historical or otherwise, it is clear that a scientist cannot effectively rule out some *exceptions* to that deductibility. As I just explained, all communities have

at least some historical contingency, the degree of which is usually unknown but which is sufficient to rule out full deductibility from original premises. Furthermore, ecologists frequently lack a full understanding of the levels of organization in a community or ecosystem, which casts further doubt on the status of their knowledge. The case study method attempts to minimize the problem of contingency the same way that any model does - through the use of assumptions. Case studies, more so than most models, attempt to make the assumptions explicit at the outset, and then subsequently minimize the use of assumptions, to make the case study more relevant.

These are all reasons why case studies and the statistical form of the covering-law model are so heavily favored by ecologists. Although statistical confirmation introduces a degree of fuzziness between induction and deduction, it has many more benefits which outweigh such doubt. The random occurrence of many important ecological events, the aforementioned difficulty of exact replication of relevant initial conditions, and the importance of disturbances and perturbations are all arguments in favor of statistical confirmation in ecological research.

Statistical confirmation is just one aspect of ecological case studies, albeit an important one. Many case studies simply attempt to develop predictive models based on what is known, and try to discover under what conditions the scientists' predictions may or may not occur. Naturally, an ecologist may fail to account for certain critical initial conditions, and this is one reason to endorse peer review and objective discussion of the results of case studies (a well-known devil's advocate for conservation biology case studies is Florida State University's Daniel Simberloff.) In their 1993 text Method in Ecology, Shrader-Frechette and McCoy note that their strategy is to "provide a new account of rationality and explanation in ecology....based on the method of case studies"(107). Although I do not make such an inclusive claim, I do however share their goal of endorsing case studies. More importantly, I share their vision of the public responsibility of ecologists to help shape environmental policy through informed discussion of their research. For these reasons, I will continue with my outline of the methodology of case studies, and then evaluate some curent case studies involving GEO introductions in the next chapter.

One of the key advantages of case studies is that they utilize a so-called "bottom-up" methodology. Rather than starting with a general ecological theory and attempting to apply it to one or two taxa in a particular situation, a case study typically will utilize the available natural history data for those one or two taxa, and then move in a broadly inductive manner toward making predictions about the interactions of those taxa and their environment. Case studies utilize a quasi-experimental methodology, which falls in the spectrum between "observational" and "classical-experimental" methodology. A true "classic experiment" includes the following four components: manipulation, a control, replicated observations and randomization. Typically, case studies lack at least one of these four components, and in ecology the randomization aspect is nearly always impossible.

The so-called quasi-experimental technique is also important because it cannot control for infinite alternative hypotheses (a benefit of randomization), and therefore must attempt to control for one or two alternative hypotheses. This, naturally, involves a choice on the part of the experimenter, and there is unfortunately no proven algorithm for making that choice. Therefore, the key heuristic value of the case study may depend on the manipulations, and the ability to perform (partially) replicated observations. Naturally, due to the spatio*temporal* nature of the case studies, such replications are difficult, if not impossible. However, it is of course the complex interactions of community ecology that make accurate case studies most difficult, and there are five key difficulties, according to Shrader-Frechette and McCoy :

> (1) uncertainty regarding subject and target systems; (2) unknowable boundary conditions; (3) unknown bias in the results; (4) the nature of the underlying phenomena; and (5) poor data. (1993,122)

It is important to note that these five problems make it impossible to perform classic experimentation, as too much manipulation may no longer replicate natural conditions, and may also make it difficult to stipulate a valid null hypothesis. For this latter reason, many researchers really do not try to specify a true null hypothesis, but rather propose a possible hypothesis and then analyze the possibilities for limiting the viability of

alternative hypotheses. This is basically ecological brainstorming, but is extremely important because it plays the same role as specifying the assumptions in a typical model, as I discussed earlier.

Because of these benefits and limitations, case studies possess four key aspects. They are *particularistic*, as they apply to a single specific process or phenomenon; they are *heuristic*, in that they are a stepping-stone to more knowledge, if not hypothesis confirmation; case studies are explicitly *inductive*, reflecting their dependence on natural history information; and they are typically *holistic*, as they are applied to real-life situations within the actual context in which they occur (see Shrader-Frechette and McCoy 1993, and references within).

Two key goals of case studies are (1) attempting to determine a causal explanation for a certain phenomenon through informal, practical reasoning, and (2) evaluate alternative explanations for that phenomenon, to determine whether and how they might be applied to other situations. All case studies have a primary goal of discovering an explanation for one particular set of circumstances, and a secondary goal of being able to extrapolate that information and apply it to similar circumstances. Shrader-Frechette and McCoy (1993) list five components of all case studies:

1. The research design of the case study;
2. The characteristics of the investigator;
3. The types of evidence accepted;
4. The analysis of the evidence;
5. The evaluation of the case study. (125)

The research design, essentially a blueprint for the case study, examines both the collection and organization of the data as well as a plan for how to evaluate the findings. As I mentioned earlier, the case study does not seek to disprove a null hypothesis, but rather it seeks to verify what the investigator already believes may be true according to existing information. Therefore, a case study may not be involved in the collection of information as much as it is involved in finding new ways to assemble and evaluate that information. There are five key components to the research design, which are frequently shared with the H-D method:

1. The questions to be investigated;
2. The actual hypotheses;
3. The units of analysis;
4. The logic linking data to hypotheses;
5. The criteria for interpreting the findings. (Shrader-Frechette and McCoy, 1993,125; and references within.)

It is important to note that many of these aspects of the research design may also be directly related to the known data. In the examination of genetically engineered plant species in the next chapter, for instance, it is not possible to know whether interactions between the GEO and wild relatives may result in hybrid vigor (successful hybrids), outbreeding depression (decreased hybrid fitness), hybrid fertility, or even no known effects, due to the absolute lack of natural history information on genetically engineered populations in the wild (see also Rissler and Mellon 1996). Furthermore, much of this data and subsequent hypothesis formation may be heavily dependent on the spatiotemporal scale that is used, which may be an asset or a liability for the investigator (e.g., a 25 year time scale may present a number of difficulties.) Rissler and Mellon note that in any event, based on current information, whether hybridization will result in vigor or outbreeding depression cannot usually be predicted (1996,141). Given such difficulties, it may not be possible to actively formulate the desired questions or even hypotheses, other than to simply design the case study to (1) observe which effect occurs, and (2) perhaps manipulate some of the sample pools to further test their fitness. It is extremely important for the researcher to formulate the research design carefully, taking into account these and other difficulties.

Clearly, another problematic area for case studies is (4) above, the logic linking data to hypotheses. What kind of logic might be acceptable? The uniqueness of a case study is frequently enough in itself to prevent the use of the H-D method for hypothesis confirmation. In some instances, a "pattern" method of inference may be effective. This is based on statistical methods in which patterns of data are searched for, and often a conclusion of "no effects" may be reached. However, such inferences are subject to the same doubts and fallabity as statistics, especially questions like "Is there enough data?" or "Was the data collected over a long enough period?" For the question of hybrid vigor

that I raised in the previous paragraph, a related concern might be that the first generations suffered decreased hybrid vigor, but for reasons that may or may not be relevant the hybrid fitness might increase in subsequent generations.

This concern with the acceptable logic also carries over to (5), the criteria for evaluation. Ideally, this should be stipulated at the outset where possible, for the same reasons that scientists need to identify their relevant assumptions in the formation of models at the outset. How much might annual, periodic, seasonal or other forms of variability affect the findings? Without controls or prior natural history data, such a question is difficult, and it becomes yet another form of methodological value judgment. Since such value judgments proliferate in science, this realization is not a problem *per se*, but it does need to be identified and addressed by the investigator, and preferably at the outset of the investigation. Some of the criteria issues can also be resolved by the use of what Shrader-Frechette and McCoy (1993) term tacit knowledge, the use of which is common in all sciences. This is a result of the unformalizable training of scientists, and takes the form of answers to questions like "What reagent might be best to use for the next test?" or "Where should we set the live traps for the next experiment?"

This leads to a consideration of some of the problems that are associated with case studies, which are by no means unique to case studies. There is a definite possibility for investigator bias, just as there is in all experiments, but perhaps more so since in this case the investigator is openly seeking to confirm a predicted outcome. One approach to eliminating investigator bias is to promote a process of peer review, and at the same time to openly ackonwledge such bias; the ongoing debates between Reed Noss and Daniel Simberloff in the field of conservation biology, alluded to earlier, are a classic example of such acknowledgement. In fact, Reed Noss has gone so far as to advocate a position proclaiming conservation biology to be a "normative science", discussion of which is unfortunately far beyond the scope of this thesis. As I mentioned earlier, investigators also need to state the "rules" and assumptions inherent in their case study, which may help to assuage concerns of investigator bias as well as spell out the methodological value judgments up front.

Another problem of the case study method is that it is difficult to generalize from a single case, or a single instance. This problem holds true for all areas of science, and for all experiments. This is also known as the "problem of induction", and the degree to which it is a problem for case studies will vary according to the situation. For instance, if a field researcher is merely trying to establish some very basic knowledge about certain characteristics of a species in the field, a case study with some degree of control can probably establish that information quite reliably. However, if the researcher wishes to establish answers to "why" questions regarding those same characteristics, that may be another matter entirely. This is a difficult area for ecology and any other science that wishes to utilize case studies, as much of that research may be utilized for pragmatic purposes - perhaps even without the knowledge of the researcher. Of course, this is a particular concern for ecologists, and it will be an area of concern which I will return to in the next chapter.

The final two concerns involve claims of "circularity" and "non-testability" against case studies. Some scientists or philosophers might consider data derived from case studies to be circular, due to the fact that the interpretations are presupposed. This is not a strong claim, nor is it one that is unique to case studies. Clearly, a scientist utilizing the H-D method can construct a null hypothesis that is really nothing more than a "straw man", and then easily refute it (as he/she originally expected.) This problem may be worsened by a failure to adequately examine alternative explanations for one's results, and I have attempted to emphasize that consideration of alternative explanations is highly stressed by proponents of case studies; as I just mentioned above, this is one reason why it is difficult to extrapolate from a single case study, particularly when seeking answers to "why" questions. Finally, case studies are really no more or less testable than any other scientific "method". Systematic testing is often possible, by varying one or more characteristics at a time, and by attempting to keep all other variables the same (e.g. season, time of day, population size, sex ratio, etc.). However, case studies also fall victim to the very same problems that the H-D method has, particularly in field research - simply put, a scientist may never know whether a key variable has been overlooked, and which also has key explanatory value for that particular research endeavor.

## SUMMARY

The recent advances in the philosophy of ecology have been offshoots of similar debates in the philosophy of science during the latter half of this century. With the announcement of the Heisenberg Uncertainty Principle and the discovery of radioactive phenomena, accounts of the necessity of direct causality in scientific explanation were heavily tempered. Similarly, the positivist contention that metaphysical principles introduced cognitive meaningless to science had to be dropped, as this was exposed to be a metaphysical belief in itself. Ironically, at approximately the same time advances in molecular biology and organic chemistry led to a renewal of the provincialism/autonomy debate in the philosophy of biology, although the doctrines of vitalism and finalism were no longer part of the autonomist position. Instead, great evolutionary biologists like Ernst Mayr were utilizing adaptationist reasoning to advocate a separate realm for the philosophy of biology, opposed to the mechanistic, reductionistic views of the provincialists. In adopting this autonomist view, I illustrated why the young science of ecology suffers from the same epistemological difficulties as biology, and why this has severe repercussions for ecologists. In the public sphere, ecologists are also expected to participate in the environmental decision making process, and their epistemic dilemmas have to be subsumed by the demands of their public role. To this end, I am following Shrader-Frechette and McCoy (1994) by advocating the use of case studies as an alternative to the H-D method, particularly as an aid for environmental planning. In the next chapter, I will attempt to employ the case study method in evaluating the release of various forms of genetically engineered oilseed rape (*Brassica napus*), and then evaluate the efficacy of this method.

## CHAPTER 4: HOW TO ASSESS THE POTENTIAL IMPACTS OF GEO RELEASES?

### INTRODUCTION

The process of scientifically assessing the risks inherent in environmental releases of GEOs poses numerous philosophical and methodological difficulties. The philosophical problems are chiefly *epistemological* (e.g., What counts as "knowledge" for the relevant scientific experiments that have been conducted?); *metaphysical* (e.g., What is the place of humans in the natural world?); and the philosophical aspect which incorporates both of the above aspects, the *ethical* aspect. One's metaphysical worldview necessarily influences one's ethical worldview, and for some belief-systems, the release of GEOs into the environment would have little or no ethical relevance, until and unless such releases might affect humans. For obvious reasons, examining the interaction of environmental GEO releases and metaphysical worldviews is far beyond the scope of this thesis, although it does remain ethically relevant. I will return to the ethical importance of GEOs in the environment at the end of this chapter.

I have already touched on some epistemological considerations that are latent in the philosophy of science, and I have attempted to place my focus upon the life sciences, particularly ecology. My conclusion in the preceding chapter was that ecological case studies may allow for the best possible determination of the ecological effects of large-scale GEO releases, especially given the political pressures from the biotechnology companies who have invested considerable time and effort to produce the GEOs. This conclusion is reinforced by numerous scientists who have also called for evaluations of GEO releases on a case-by-case basis. One of the difficulties with performing case studies is the frequent paucity of relevant data for "ecological baseline information", and that lack of relevant data will not change until a substantial amount of research has been performed.

In this chapter I will attempt to establish a single, detailed model of a case study for evaluating GEO releases, including the assembly of much "baseline information". In

so doing, I will point to the shortcomings of the method, the difficulty in meeting public expectations for ecological "expertise" in providing and analyzing relevant data for GEO releases, and then evaluate the case study methodology with regard to the conventional scientific method.

In the following pages, I will develop a case study for oilseed rape, and demonstrate some of the difficulties that face ecologists and environmental planners when faced with evaluating transgenic microbial populations in the environment, and plants that are engineered for virus-resistance. These are all areas of acute environmental concern, and the resources of ecologists are strained simply by the daunting nature of the task that confronts them. As such, I will attempt to illustrate the benefits and weaknesses of the case-study method in ecology, and hopefully illuminate some particular areas of epistemological and (to a limited degree) ethical concern.

## Genetic Engineering of Crop Plants

The demands on global agricultural production continue to rise every year, along with world's human population. These demands are presented in Table 4-1, below, along with some conventional responses to these demands, and some relevant examples of biotechnology solutions to these demands. Interestingly, agricultural scientists have thus far been able to utilize biotechnology to help solve all but one of these shortcomings, and even that may have a future solution from biotechnology.

Since the earliest days of genetic engineering, and the first production of transgenic plants in 1982, scientists have acknowledged the possibility for ecological risks (Seidler and Levin 1994). Almost immediately, these risks were met with derisive responses based entirely on conjecture, such as the "genetically-crippled" model (see Regal 1986, and Brill 1985). This model claims that, for example, a crop engineered for pest -resistance simply cannot become a weed, due to the large metabolic costs of that pest-resistance gene. Such conjecture may well be borne out in laboratory tests and field trials, but it remains difficult to design an experiment that will eliminate all possible

explanations *except* metabolic costs, if that plant should fail to survive in natural conditions[2].

**TABLE 4-1. Some Responses to Continuing Demands from Agriculture.**

| Agricultural Demands | Conventional Responses | Ex. of GEO Responses |
|---|---|---|
| Increased yield | Conventional breeding<br>Increased chemical use<br>Increased use of marginal land | Herbicide-resistance<br>Pesticide-resistance<br>Incr. oil production in OSR<br>Flavr-Savr tomatoes |
| Increased automation, from planting to processing | More and better mechanical inventions | (none yet) |
| Increased nutritional value | Conventional breeding | Incr. oil production in OSR |
| Decreased predation (and loss of yield) by plant pests and pathogens | use of *Bt* sprays<br>alternating rows of diff. crops<br>Increased use of chemicals | Pest- and pathogen-resistance<br>Pesticide- and *Bt*-resistance |
| Decreased chemical use (including herbicides, pesticides, and fertilizers) | Increased use of *Bt* sprays<br>Organic farming | Incorporation of *Bt*-toxin<br>Herbicide-resistance<br>Pesticide-resistance |
| Increased yield from increasingly marginal land | Increased chemical use<br>Conventional breeding | Crop plants engineered for various stress-tolerances |

[2] I should note that a weed is simply an unwanted plant, and that by this definition, there is an inherent value judgment relative *only* to humans; i.e., there are no plants that have weediness relative to a natural ecosystem. Similarly, for the purposes of this paper I am defining "natural ecosystems" as those which are unaided (and largely unaffected -- that is, not tilled, watered, weeded, or otherwise intensively managed *for* human use) by humans, and which may include the boundaries of farmland.

Furthermore, there are far more complex issues surrounding the introduction of genetically engineered crop plants. For instance, can the crop plant hybridize with related species, possibly contributing to a rise in invasive weeds that have incorporated the engineered trait? P.J. Dale (1994) has listed no less than 21 relevant concerns for evaluating this possibility, which are listed in Table 4-2. Bartsch, Sukopp and Sukopp (1993) list nine relevant factors for interspecific crop-wild hybrids, found in Table 4-3. I will closely examine the hybridization issue in my OSR case study.

The incorporation of pesticide-resistant traits in genetically engineered crop plants presents another difficult issue, for scientists and philosophers alike. This will likely result in very strong selection pressures for that trait in the targeted pest species, and the (potentially rapid) evolution of resistance to that pesticide in both targeted and non-targeted pest species, including fungi and insects (Gould 1991). Such evolution has already been amply demonstrated (references in Gould *et al* 1992.) One particularly important insecticide is the soil bacterium *Bacillus thuringiensis* (Bt), which aggressively attacks the midgut of Lepidopteran insects.

The Bt-toxin has been widely used by organic farmers for decades as an alternative to chemical insecticides, which (1) frequently target a broad range of insects, (2) can be noxious and long-lasting in the environment, and (3) are frequently toxic to many more organisms as the result of bioaccumulation (e.g., DDT). However, there is increasing evidence that insects can also evolve resistance to Bt, which makes any indiscriminate use of the Bt-toxin a reason for concern (Gould *et al* 1992; Van Rie *et al* 1990).

The gene for Bt-toxin has now been introduced into a number of crop plants, including cotton, tobacco, tomato, soybean and canola (Stewart *et al* 1996; Gould *et al* 1992; Van Rie *et al* 1990). Similarly, a variety of insect species have now developed resistance to Bt, although there is some evidence that the Bt-resistance will disappear upon removal of the selection pressure (the Bt-toxin.)

Possible mechanisms for ecological disruptions due to transgenic Bt-toxin plants have been examined by Jepson, Croft and Pratt (1994). This is yet another aspect of

potential ecological risks from transgenic crop plants, although very indirect, and is equally difficult to assess. I will return to their findings shortly.

I have now touched upon several aspects of ecological risks associated with transgenic crops, and I will now proceed to outline a broad list of such concerns, followed by a model case study of transgenic OSR, and the relevant philosophical difficulties that are attendant to such organisms.

**Table 4-2. Factors determining the likelihood of intraspecific crop-wild hybrids**

| **The production of viable hybrid seeds** |
|---|
| 1. Compatibility of the two parental genomes (mitotic and genetic stability)<br>2. Ability of the endosperm to support hybrid embryo development<br>3. Direction of the cross: one parent may support embryo and seed development better than the other<br>4. Number and viability of hybrid seeds<br>**Establishment of hybrid plants from seeds in soil** |
| 5. Seed dormancy<br>6. Vigor of the hybrid plant<br>7. Direction of the cross: maternal effects influencing seedling vigor<br>8. Nature of the habitat: wild, semi-wild, or agricultural<br>9. Nature of competition from other plants<br>10. Influence of pests, diseases and animal predators<br>**Ability of the hybrid to propagate vegetatively and sexually** |
| 11. Method of vegetative propagation<br>12. Persistence of vegetative propagules in agricultural habitats<br>13. Dissemination of vegetative propagules<br>14. Invasiveness of vegetative propagules in natural habitats<br>15. Sexual breeding system: cross compatible, self-compatible, ability to cross to either parental species<br>16. Male and female fertility: meiotic stability and chromosome pairing<br>17. Seed number and viability<br>18. Seed dormancy<br>19. Nature of habitat: wild, semi-wild, or agricultural<br>20. Nature of competition from other plants<br>21. Influence of pests, disease, and animal predators |

from: Dale, P.J. 1994. "The impact of hybrids between genetically modified crop plants and their related species: general considerations." Molecular Ecology 3:31-36(32).

**Table 4-3. Factors determining the likelihood of interspecific crop-wild hybrids.**

| |
|---|
| 1. Extent of pollen production |
| 2. 2. Degree of inter- and intraspecific pollination in both populations |
| 3. The ways and means of pollen transport. |
| 4. The amount of transported pollen. |
| 5. The properties of insects transmitting the pollen (in the case of insect pollination). |
| 6. The spatial distribution among individuals of the two populations. |
| 7. The phenological differences of the two populations. |
| 8. The density of the individuals of the two populations. |
| 9. The compatibility of the two genomes. |

from: Bartsch, D.; Sukopp, H.; and U. Sukopp. 1993. "Introduction of plants with special regard to cultigens running wild." in *Transgenic Organisms*. Wohrmann, K. and J. Tomiuk, eds. Birkhauser Verlag Basel, Switzerland.

In February 1994, the journal Molecular Ecology (1994, vol. 3, no. 1) published a special issue entitled "The Ecological Implications of Transgenic Plant Releases", following a 1992 symposium in Maryland. A glance at some article titles indicates a portion of the spectrum of concern:

- Hybridization, including spatio-temporal persistence;
- Introgression of hybrids into wild populations;
- Increased weediness of crop-wild hybrids;
- Biodiversity and the impact on ecological communities;
- Potential effects on soil fauna;
- Implications for biogeochemical cycles;
- Potential models and test systems to determine such effects.

Unfortunately, this is only a small portion of the possible ecological effects, and yet it occupied almost 90 pages of the one of the finest scientific journals. Before proceeding to create a "model" case study of transgenic oilseed rape, I will first examine these philosophical and ecological issues more closely.

Philosophical Concerns:

The primary philosophical concerns are twofold: What counts as scientific knowledge? How can we design experiments to determine the answers to these open-ended questions of risk? In Chapter 3 I attempted to answer the first question, and concluded that case studies would be the most applicable form of scientific validation. The answer to the second question is far more complicated.

First, Regal notes that we must ask if such experiments are actually "scientific tests of realistic ecological concerns"(1994,11). This requires extensive consideration of several factors, listed in Table 4-4.

**Table 4-4. Aspects of transgenic plant introductions relevant to ecological risk assessment**

| |
|---|
| 1. The type of genetic modification under consideration. |
| 2. The actual crop plants to be modified. |
| 3. The target organisms, if any, for the trait (e.g. Bt-toxin and insects). |
| 4. The current normal range and density of the crop plant, including international use. |
| 5. The potential extended range of the crop plant (e.g., drought- or frost-tolerant plants). |
| 6. The known wild (or weedy) relatives of the crop plant, and its ability (if known) to hybridize with them. |
| 7. The known (existing) range and density of related species. |
| 8. Any available information on seed dormancy for the transgenic and nontransgenic crop plant, and its potentially hybridizable related species. |
| 9. The potential impacts on seed dormancy from transgenic traits (e.g. increased oil production in canola may lead to increased seed dormacy and viability). |
| 10. What is the flowering phenology (time and amount of pollen production, which also has to coincide with receptivity *for* the pollen) for the transgenic plant and its related species? |
| 11. Will the transgenic traits potentially affect the flowering phenology, and if so, how? |
| 12. How are all of the above factors affected by weather, seasons, or any combination thereof? |

Second, it must be determined whether laboratory (i.e., greenhouse) conditions or field plots of a few to a hundred acres can adequately replicate the potential conditions in which the ecological risks, if any, may be incurred. Is this even an empirical question? One may argue that the questions will be answered, given enough time, and that it will then be theoretically possible to determine how and why such results obtained in a particular scenario. It took ecologists many years to connect the thin eggshells of raptors with the bioaccumulation of DDT, and it is widely acknowledged that it was probably *not* a foreseeable consequence of using DDT. However, gene flow between (1) corn and teosinte (various species of the *Zea* genus); (2) various squash (*Cucurbita*) species, and (3) oilseed rape and wild mustard (*B. napus* and *Sinapis arvensis*, respectively) has been well-documented (Doebley 1990; Wilson 1990; Kerlan, Chevre and Eber 1993). But it is not well understood why the same does not hold true in other species, with the exception of breeding male sterility into numerous cereal crops, to prevent normal fertilization and germination in natural (i.e., non-agricultural) environments.

Given that there is a limited pool of existing natural history information to give answers to the "why" questions, ecologists and botanists are left with purely empirical information upon which to base their recommendations. Again, without knowing the "why" answers, it is very feasible that GEO introductions determined to be "safe" based on laboratory and field trial data could have devastating ecological effects, although it is highly unlikely. It is equally important to emphasize that although biotechnology continues to advance by leaps and bounds, botanists are only beginning to develop the molecular tools to help them understand how many plant-environment interactions are controlled -- the likely keys to answering the "why" questions.

In fact, Bartsch, Sukopp and Sukopp (1993) note that the first eight of the nine factors in Table 4-3 are:

> Attributes [which] are mainly influenced by ecological factors and can only be quantitatively studied in natural conditions at a relatively high expenditure.... [and]only the analysis of all [9] parameters gives a reliable estimate of the probability of a successful hybridization event among individuals of two plant populations.(142)

These are all specific aspects of some issues in the philosophy of science that I discussed in Chapter 3. In the life sciences, unlike the physical sciences, prediction is simply not a mirror image of confirmation. It is simply not possible, or even realistic, to be capable of predicting (and later replicating) the exact conditions that will occur in the natural environment; perhaps more importantly, it is not possible to predict the *ecologically-relevant* conditions that will occur in one, or more, of the agricultural or semi-wild environments that will receive transgenic plants.

One method of delineating ecologically relevant conditions that ecologists have employed is the study of biological invasions, a means of evaluating that ephemeral characteristic known as "invasiveness", which is often justifiably conflated with "weediness". Ecologists have long sought to define what allows some 10% of biological invaders to succeed (and occasionally wreak havoc), while the other 90% of invaders frequently disappear unnoticed (Williamson 1994). Most attempts have been largely unsuccessful in determining the key factors involved, other than the obvious ones like "lack of competitors", "plenty of available resources", or that it is simply due to the invader being a "cosmopolitan species", capable of surviving in diverse habitats. I will hereafter be using the term "invasive" in a somewhat casual manner, for a plant population that was at one time not present in a given area and is now present in substantial numbers, and may even have pestilential qualities.

There are three categories of plants used by humans: wild plants, obligate cultigens (possessing attributes derived from the cumulative effects of conventional breeding), and facultative cultigens, "wild plants which are cultivated either in their natural habitats or transferred to regions where they did not originally exist" (Bartsch, Sukopp and Sukopp, 1993,139, and references within). These three categories have obvious implications for the consideration of the ecological impact of genetically engineered hybrids, and particularly their potentially invasive qualities. Transgenic facultative cultigens may hybridize and escape cultivation via the direct escape of hybrid progeny, or by reversion to the wild-type form (with its weedy qualities), or by introgression of the transgene into the existing wild population.

Clearly, for obligate cultigens the escape of a transgene through crop-wild hybridization faces much more severe barriers than that of facultative cultigens. One such barrier is a *genetic* barrier, such as different numbers of chromosomes, which might limit the amount and direction of gene flow. Other barriers might be morphological, such as flowering phenology or male-sterility. However, for many plants that are obligate cultigens, there are also closely related species that are weeds (e.g. sorghum and johnsongrass), and this fact seems to obscure the line between the three types of plants used by humans. The use of plants also changes over time, which is also of ecological concern. *Bromus secalinus*, today a cereal crop weed, "was used by humans from neolithic to modern times"(Bartsch, Sukopp and Sukopp, 1993,143).

Ellstrand and Hoffman (1990) note that there are congeneric wild relatives for eight out of ten major California vegetable crops, which are presented in Table 4-5, below. Similarly, 1439 interspecific and intergeneric hybrids have evolved from about 2000 wild vascular plants of the British flora, according to Bartsch, Sukopp and Sukopp (1993, and references within). Because this latter figure does not include a measure of time but does emphasize an evolutionary element, it is important to consider that such evolution may occur only over a great length of time, although just how much time is necessary for such extensive hybridization remains empirically indeterminate. Furthermore, only 975 of the hybrids were observed in the British Isles, with the rest in Central Europe; such migration will continue to be enhanced, of course, by humans acting as transportation vectors. Indeed, 17 out of 18 of the "World's Worst Weeds" are also cultivated, which serves to emphasize that the use of plants is also globally relative (Holm *et al.*, 1977). Ellstrand and Hoffman (1990) discuss numerous tropical and subtropical weedy relatives of tomato (*Lycopersicum esculentum*; 3 total), tobacco (*Nicotiana tabacum*; 5 total), and potato (*Solanum tuberosum* ssp. *tuberosum*; 8 total) -- all of which are likely candidates for early release of genetically modified variants.

**Table 4-5. Wild relatives of 10 major California vegetable crops.**

| Vegetable crop | Wild relatives |
|---|---|
| 1. Asparagus | Same species |
| 2. Broccoli | 9 congeneric species |
| 3. Carrot | Same species |
| 4. Cauliflower | 9 congeneric species |
| 5. Celery | Same species |
| 6. Sweet corn (*Zea mays*) | None |
| 7. Lettuce | 6 congeneric species |
| 8. Onion | 37 congeneric species |
| 9. Potato | 21 congeneric species |
| 10. Tomato | None |

from: Ellstrand, N.C., and C.A. Hoffman. 1990. "Hybridization as an Avenue of Escape for Engineered Genes." *Bioscience* 40(6), 439.

In the consideration of invasive plants, time and space are two of the important ecological variables that require consideration. Time is important because it takes time for an invader to become established, and also because the utility of plants changes over time. Predicting the success of an invasive species is also difficult, as some invaders may require the absence of competitors to succeed, while other invaders will succeed as long as their resource needs are met; both of these conditions may change for any given invasive species, given the effects of time and space. Interestingly, there are no good hard-and-fast rules for predicting the success of invaders (Williamson 1994), and this is an excellent reason to support the autonomy of ecology from a philosophical point of view, as I argued in Chapter 3. I will return to that issue later in this chapter. I will now illustrate a case study model for transgenic oilseed rape.

## A CASE STUDY OF TRANSGENIC OILSEED RAPE

There are eight central aspects that are common to all case studies, according to Shrader-Frechette and McCoy (1993,201):

1) The background information;

2) The questions to be investigated;

3) The hypotheses;

4) The types of evidence;

5) The analysis of the evidence;

6) The criteria for interpreting the findings;

7) The conclusions reached;

8) An evaluation of the case study as a whole.

In applying these eight factors to transgenic oilseed rape (OSR, or *Brassica napus*), I will be exceptionally brief, as my chief intent is to demonstrate how this process is carried out. In Table 4-6, I have included several ways in which OSR has been genetically modified, and the references in Table 4-6 are just a few of the many authors who offer excellent background information on this plant. In addition, the natural history of OSR is well-studied, so I will include only the relevant information for steps 2-8, where possible.

*Brassica napus* is a cultivated plant from which canola oil is derived. It is a pereniial plant capable of self-pollinating, or "selfing". As a transgenic crop, it has been modified in many ways, both useful and purely experimental, as seen in Table 4-6.

**Table 4-6. Some genetic modifications of oilseed rape, *Brassica napus***

| **Trait** | **Reference** |
|---|---|
| Incorporation of the *Bt*-toxin insecticide | Stewart *et al*, 1997 |
| Seed oil variation (high laurate/high stearate) | Linder and Schmitt, 1995 |
| Antibiotic (kanamycin) resistance | Crawley *et al*, 1993 |
| Herbicide resistance - glufosinate | Chevre *et al* 1997 |
| - glyphosat | Frello, *et al* 1995 |

Some of the questions to be investigated, both now and in the future, are as follows:

(1) Will genetically engineered OSR plants pose special ecological risks?

a) Will transgenic OSR plants escape and form weedy feral populations?

b) Will transgenic OSR plants hybridize with weedy relatives?

- If so, will those hybrids pass the transgene on to their progeny?
- If so, will those hybrids have a selective advantage over existing plant populations?

(2) Will OSR plants transgenic for insecticides pose special ecological risks?

The next step in the case study is formation of the hypotheses. For this case study, there are four hypotheses:

A. Transgenic plants pose no special risk to the environment. (I am assuming this to be an active hypothesis, as field trials are continuing and some GEOs have been approved for human use and consumption.)

B. Transgenic oilseed rape will not form feral populations that are of any more ecological significance than nontransgenic OSR.

C. There will not be any ecologically significant hybridization between transgenic OSR and weedy relatives, such that there are selective advantages introduced into the weed population or the crop-wild hybrid population.

D. There will be no ecological effects, broadly speaking, from GEOs. Examples might include insects rapidly evolving resistance to an otherwise beneficial trait, e.g. insecticides.

Again, proceeding according to the method outlined by Shrader-Frechette and McCoy (1993), there are inferences, based on certain types of evidence, which are used to afirm the hypotheses of the case study.[3] These inferences and their respective evidence

[3] It should be noted that this is a form of induction, which was criticized in Chapter 3. However, (1) it was also noted that induction is utilized often in the everyday workings of scientists, chiefly for its excellent pragmatic value; and (2) other methodologies were also heavily criticized in Chapter 3, and yet their

are problematic for evaluating the potential effects of GEOs, particularly due to the novel features of many transgenic organisms, but are vital to the case study method. The main inferences for transgenic OSR are:

INF-1. Scientists can distinguish transgenic OSR from nontransgenic OSR, hybrids, and weedy relatives (the *Brassicacae*).

INF-2 Scientists possess the necessary data on life history, resource requirements, and predators of OSR and its relatives to make predictions about the ecological success of feral transgenic OSR and/or hybrids expressing the transgenic trait.

INF-3 Scientists currently possess enough data on the ecological relations of OSR and its predators to determine that there will not be any increase in the evolution of resistance from insecticidal traits possessed by transgenic OSR.

Analysis of the Evidence

As it is my intention to merely model the case study approach through the use of an example, my analysis of the evidence for each of the inferences described above will be brief.

Unfortunately, much of the evidence for INF-1 is anecdotal, albeit from scientists having long experience with oilseed rape cultivation. However, its life-history traits are well-established in the literature, as are its habitat requirements (which are extremely varied). The *Brassicacae* as a whole are very hardy and adaptable plants, and so OSR is not only capable of hybridization with as many as 17 species (Scheffler and Dale, 1994), but it is also capable of surviving in diverse habitats. It is also possible to use "genetic markers" to identify transgenic OSR. These may be molecular, e.g. the RAPD (randomly amplified polymorphic DNA) markers that were used by Mikkelsen, Jensen and

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influence in science has continued, unabated (e.g. the H-D method). So, it would seem that induction has crept in by the back door, although it was duly noted that it is applied as a tool of the case study method.

Jorgensen (1996), or phenotypic traits such as antibiotic resistance, used by Crawley *et al* (1993). Unfortunately, depending on the method of modifying the genome and whether more than one gene is used (for more than one trait), after hybridization events and subsequent introgression, the expression of some traits may disappear. It is also theoretically possible to distinguish such plants by the presence of a transgene that confers a fitness advantage in a harsh environment, if it is determined that the OSR could not survive in that environment without the transgene.

The analysis of evidence for INF-2 is much more complicated and controversial. Crawley *et al* (1993) claimed that after a three year, controlled experiment with transgenic OSR, the transgenic plants performed much worse than either nontransgenic OSR or charlock (*Sinapsis arvensis*, a weedy relative). This noteworthy experiment examined dormancy, germination, plant survival, and fecundity, including seed burial at two different depths, retrieval of seeds and seedlings after 12 and 24 months, the use of 12 different habitats, and the seed burial and retrieval was repeated four times in the 3 year period. This is a classic example of a controlled experiment, and had seemingly conclusive results: >60% of the *S. arvensis* seeds survived, in all habitats; only 2% of the nontransgenic OSR seeds survived; and <2% of the OSR modified for herbicide-resistance survived. Still, the authors drew only modest conclusions:

(1) The low seed survival of the transgenic OSR was not *necessarily* due to genetic engineering, as alternate explanations are available.

(2) Interspecific competition was apparently the main determinant of $\lambda$, the finite rate of increase of each population. The main support for this conclusion was that OSR was successful in disturbed landscapes, as were its competitors -- but the disturbed sites had much less vegetation than the other sites, making for reduced interspecific competition.

(3) Although the transgenic lines were less successful, "there were no substantial [metabolic] costs involved in expressing a kanamycin- or glufosinate-tolerant phenotype"(Crawley *et al*, 1993,622).

(4) There were no known selective pressures for the transgenic lines in this experiment.

The first conclusion is noteworthy, in that a common conjectural response to the potential risks of genetically engineered plants is that "most crop plants are so dependent on human nurturing as to be unable to survive on their own"(Williamson, 1994,76). Williamson goes on to state that such conjecture is simply *wrong*, and that "what cultivated plants often cannot do well is to disperse or to reproduce {e.g., many garden flowers]"(*ibid.*).

It is the issue of potential hybridization which has received perhaps the most attention. In their literature review on OSR hybridization experiments, Sheffler and Dale (1994) note that *Brassica napus* produced interspecific hybrids from 17 species by hand poliination, of which 12 had no progeny, and 8 had no progeny after backcrossing. Furthermore, second generation ($F_2$) *B. napus* could openly pollinate and hybridize with its relatives *B. rapa* and *B. juncea*, and could hybridize with *B. adpressa* and *B. raphanistrum*, but only if there was a male-sterile, female *B. napus* parent. When reviewing such data it is important to ask (1) were enough samples tested to make the results statistically significant, and (2) are the exact determining factors known which control the conditions for hyridization? If so, how might those factors change in the future, and will it be directly or indirectly as a result of human intervention? Both of these answers are still negative, and the data becomes much less conclusive, following more along the pattern of natural history data. Indeed, the exact conditions which limit or further such hybridization events (both in the field and in the greenhouse) remain unknown, so it is wise to be cautious when considering how much confidence may be had from existing scientific sources.

In a hybridization study between OSR and wild radish (*Raphanus raphanistrum*) in France, Chevre *et al* (1997) found that if OSR heterozygous for an herbicide transgene was the female parent, first generation ($F_1$) hybrids had poor initial female fertility, but in the $F_2$-$F_4$ generations both female fertility and seed germination virtually mimiced the

weedy radish.[4] Because of heterozygosity in the transgenic parent, the $F_1$ plants displayed Mendelian segregation for the trait (a 1:1 ratio), but that the gene transmission decreased dramatically in subsequent generations. However, in the $F_4$ plants that displayed the trait, they also displayed both the chrmosome number (an indicator of genomic stability) and morphology of the wild radish - making those plants, essentially, a potentially troublesome weed. Finally, this experiment was designed to assess the likelihood of spontaneous gene flow under field conditions, for two plants with similar flowering phenology. Clearly the hybridization resulted in significant gene flow, but it is noteworthy that the experiment did *not* occur under the influence of a particular selective pressure - *viz.*, application of the herbicide for which the OSR was resistant. In addition, the authors were not clear whether there was or was not any vegetative competition for resources; the term "field conditions" can be used for either a cultivated plot not currently being cultivated, the margins of cultivated land, or a plot in a field somewhere, which may or may not have been cleared. In my discussion of explanatory fertility as a criterion for interpretation and evaluation of case studies, I will describe some of the complexity that is involved in the interpretation of ecological research, particularly when one seeks to apply those findings to matters of environmental policy.

In a similar experiment with the same transgenic trait (glufosinate-tolerance), Mikkelsen, Andersen and Jorgensen (1996) produced transgenic hybrids of *B. napus* and the weedy relative *B. campestris* "spontaneously" (meaning unclear), and then grew the transgenic hybrids together with the weed in an experimental plot. The $F_1$ plants were fertile (an average of 450 seeds per plant), and the first back-crossed generation ($BC_1$) had a significant proportion (44/865 were analyzed, but the basis for choosing them is unstated) that had *B. campestris* morphology, including 20 chromosomes and >90% pollen fertility. Of these, four were chosen to cross with genuine *B. campestris* individuals, and $BC_2$ had the following *B. campestris* traits: "pronounced seed

[4] In the study by Frello *et al* (1995), the authors emphasized that they utilized the direction of cross (i.e., crop-wild vs. wild-crop) that they found to be most successful; for them it was a male, transgenic *B. napus* and a female weedy relative, *B. juncea*. This is probably an appropriate strategy at this early stage of ecological experimentation and confirmation, as it will maximize the ability to predict worst-case scenarios, viz. increased hybridization events.

dormancy", 20 chromosomes, a similar external morphology -- and 42% of the 416 $BC_2$ plants were glufosinate-tolerant! In addition, the authors found two glufosinate-tolerant plants with 20 chromosomes, high pollen fertility, and *B. campestris* morphology that had overwintered and survived among other plants at the experimental site. This is a very important study, as it both (1) demonstrates significant hybridization followed by introgression (with subsequent integration of the transgene into a population of viable individuals), and (2) occurred without any selective pressure for the transgene under consideration. This second point is important, as most traits for "resistance" or "tolerance" do have metabolic costs, namely the greater resource acquisition needed to produce the substance under consideration -- in many cases, 1 or more chemicals. In addition, this experiment also lacks some of the vital ecological data (e.g. competition for resources; type of experimental plot; etc.) that were also lacking in the study by Chevre *et al* (1997).

The most powerful voice in this debate over the ecological significance of GEOs may be that of Peter Kareiva. In a recent article, Kareiva, Parker and Pascual (1996) performed a bootstrap analysis of the data in the landmark transgenic-ecology study by Crawley *et al* (1993), and reached dramatically different conclusions.

After performing a variety of statistical analyses, it was found that the magnitude of error resulting from the analysis of less than three years of data could be as much as 100%. It was concluded from that result that for OSR, *the number of years was more important than the number of sites!* Furthermore, the analysis by Kareiva, Parker and Pascual noted that:

> If this example portends a general trend, then experimental assessments of GEO risks will *require several years of data* [original emphasis], with shortcuts to speed up the process coming at high costs in terms of predictive power. This is because year-to-year variation in plant success can be staggeringly large -- a phenomenon well-known to any gardener.(1996,1673)

The second point addressed by Kareiva, Parker and Pascual (1996, and references therein) is the large discrepancy to be found in conventional analyses of historical weed establishment and persistence data. They determined that (1) a weed's invasion rate over

the first decade after introduction is not a very good predictor of its final invasion extent; and (2) the extent of initial invasions is similarly not a good predictor of the ultimate extent of a weed's presence. The first of these two points is very much contrary to the conventional wisdom, but it was reached after the analysis of nearly 90 years of data in the American Northwest. Likewise, the second point was founded upon the same evidence, and was mathematically analyzed, but there is also a very reasonable explanation. Weed introduction typically occurs in an ebb-and-flow population distribution pattern; the weed often does not establish after the initial introduction, but rather may not succeed until after several opportunities, when the environmental conditions (including human land use) may have become more favorable. Most importantly, the authors point out that short-term studies may not be very conclusive about the invasive properties of weeds, and the same may well be true for the evaluation of transgenic plant releases with similar potential for weediness.

The final inference has received a great deal of recent attention, and perhaps deservedly so. Andow (1994) emphasized that resource acquisition patterns should be a focal point for evaluating transgenic plant releases, and plants engineered for resistance to primary consumers (predators - namely insects) may significantly alter these patterns in a community. He illustrated how a single disease on a single plant species (chestnut blight on the American Chestnut tree) altered the structure of the entire eastern deciduous forest ecosystem. Therefore, conversely, a gene for a trait such as disease- or pest-resistance could potentially affect the entire structure of a plant community.

In an excellent example of classic experimentation, Stewart *et al* (1997) examined OSR plants transgenic for the *Bt*-toxin (an ICP, or insecticidal crystal protein), and compared their success against Bt-susceptible specialist and generalist herbivores, in both naturally-vegetated and cultivated plots. The authors considered not only the difference in defoliation compared to the nontransgenic controls, but also overwinter survivorship and reproductive success. The conclusions that the authors reached were as follows:

1. "Herbivory by insects was an effective selection agent in favour of transgenic insecticidal plants"(776);
2. The insecticidal rapeseed "is highly resistant to [certain] ubiquitous susceptible defoliating lepidopterans"(777);
3. "A single episode of herbivory by diamondback moth decreased the overwinter survivorship of nontransgenic rapeseed"(777);
4. Extensive defoliation correlated with low seed production in nontransgenic OSR;
5. There was "low overwinter survivorship of rapeseed in the uncultivated plots"(777), suggesting that the OSR is a poor competitor (and therefore likely to be noninvasive) in undisturbed habitats;
6. They note that in areas in Georgia where rapeseed cultivation has only been practiced for approximately 10 years, there are prominent roadside populations which were presumably nonexistent prior to cultivation (demonstrating that OSR is a potential invader in the proper habitat conditions, which are likely to be near cultivation sites);
7. "Given the above data and scenarios, and the complex taxonomic relationship in the Brassicaceae, a gene conferring insect resistance will likely become fixed in weedy mustards in ecological time. Insect-resistant wild mustard is certainly not a desirable organism in either agricultural or natural ecosystems"(778).

These are all strongly-worded and definitive conclusions, which may or may not be justified on the basis of a single experiment, in this instance. R.J. Abbott (1994) has raised the issue of insect-resistant transgenic crops also, including the possible effects on the nontarget organisms (e.g. the soil macrofauna). He claims that for transgene escape

> the main danger will occur when genes that confer improved adaptation to the natural environment, for example, insect and disease resistance, become incorporated by design into ecologically competent crops or by accident into their wild relatives.(1994:281)

In a reply to Abbott's letter, Thacker (1994) notes that for transgenic properties, as for chemical control of pests, no advantages "will be realized if the control system itself

breaks down because the target species becomes resistant to the transgenic product"(281). Even further, he asserts that

> ...constant toxin expression in transgenic plants is like pushing the accelerator pedal down on resistance development, [and] then the usefel lifetime of toxic products that are encoded in transgenic plants becomes sevrely reduced. In fact, if you wanted to demonstrate resistance development, I cannot think of a better way to do it than to produce a plant in which the toxin is continually expressed. (ibid.)

Such strong wording is admittedly uncommon from scientists, but this is a common theme among their peers. There is accumulating evidence for the establishment of resistance to conventional pesticides and *Bt* alike, although the latter poses a more complex problem: it is one of the few pesticides in common use by organic farmers. This added ethical element will be addressed later in the chapter. The more optimistic agricultural researchers emphasize that the use of insecticidal plants must be very selective (e.g., by planting alternating rows of nontransgenic plants, or clusters of both types in the same cultivation), alternate in the years it is applied, or perhaps genetically engineered in such a way that the trait is only expressed (a) in certain parts of the plant which are targeted by the insect, or (b) at appropriate times in the target insect's life cycle, where it can have the most effect without being a constant selective pressure. This aspects of transgenic plants are very difficult to address, and perhaps form the greatest hurdle for both advocates of GEO introductions and those who wish to accurately assess the relevant risks alike (see, e.g. Alstad and Andow, 1995).

## CRITERIA FOR INTERPRETATION OF FINDINGS

This is perhaps the most difficult aspect of the case study, although I have hinted at some problematic areas while analyzing the available evidence. Again following Shrader-Frechette and McCoy (1993), there are four main criteria: internal consistency; external consistency; explanatory fertility; and predictive power. Because I have already covered these topics in some depth in the previous chapter, I will not go into great detail at this time.

Internal consistency simply requires that the inferences and evidence used to evaluate the hypotheses must be consistent with each other. For example, I cannot claim that on the basis of Crawley *et al* (1993) that it is acceptable to introduce OSR transgenic for herbicide-resistance into the environment, and subsequently claim that OSR transgenic for insecticide-resistance is not acceptable for environmental introductions, and then conclude that "by and large, GEO introductions pose no special risk to the environment". That would be horribly inconsistent, and would really not present any grounds upon which to raise any conclusions whatsoever.

External consistency is an evaluation of all of the evidence used to support or argue against the hypotheses; are the background information surveys and controlled experiments and statistical evaluations all consistent with each other? Are similar methodologies being utilized by the authors? How can I know that? Some amount of faith must be placed in the peer-review process in order to judge *any* of this data as having external consistency, to assure proper methodologies and reporting procedures. I do not feel that this is much of a problem for most areas of science, although ecology can be problematic in its own right. for example, in the Crawley *et al* (1993) experiment, how does anyone know just how much the "12 different habitats" actually differed? Did they differ in ways that mattered, regarding such characteristics as resource availability, presence or absence of interspecific competitors, presence or absence of predators, etc.? Or is it perhaps better to simply alternate one variable at a time, to achieve a better sense of adequate "controls" for the experiment? These are all difficult methodological questions, and I will return to them shortly.

Similar to the issues presented above, explanatory fertility is an amorphous quality which ecology is always chasing after, or so it would seem. Frederick Clements' early theory of community ecology, in which terrestrial ecosystems were structured around a stable association of plant forms which served to define that community, continues to have great explanatory fertility today, even though most theoretical ecologists have abandoned the notion as too determinate and prefer to lean towards emphasizing the forces of entropy as determinants of community structure. Indeed, some might argue that Clementsian communities are more the norm than the exception, and

that most exceptions also happen to occur in either severe environments (deserts and the arctic) or in areas of common disturbance regimes (like prairies), which would merely require a looser notion of "community". Understanding the concept may also be facilitated by reference to Newtonian physics, which continues to function well for most applications of physics, although it has been pushed aside by modern quantum mechanic theory.

All of the data that I have presented and evaluated thus far has considerable explanatory fertility, except in one crucial aspect: Do we know what limits or allows a given event to occur? For example, why is it that sometimes hand pollination works for hybridization experiments, and sometimes it does not work at all? Why is it that some hybrid progeny are viable, and some are not? Will that always be the case? These questions are enough evidence to realize that at this point in time, no, ecology does not have as much explanatory fertility as environmental planners might wish for it. However, many of the other life sciences suffer from the same shortcomings (including human genetics), and this was a primary reason that I wished to support the autonomy of biology in Chapter 3. Unfortunately, much of these indeterminate questions are critical to evaluating these GEO risks, and I will address that next.

Predictive power is a shortcoming of all of the life sciences, as I discussed in Chapter 3. From seemingly trivial examples of a doctor who cannot profess to a patient how severe her reaction might be to a recently acquired viral disease, to great and complex issues like that of global warming. I have discussed at length in my analysis of the evidence many of the variables involved in these ecological studies, and the fact that the results of the studies may also change from one year to the next. This point was addressed by several authors, and many scientists clearly intend to perform multiyear studies to determine what some of the controlling variables might be for GEOs like transgenic, herbicide-resistant OSR. I continue to maintain that such predictive power is largely foreign to many areas of ecology, and that not until many studies are both performed and then repeated under varying conditions, with similar results, will I feel that the predictive power afforded by the methodological approaches of community ecology are conclusive enough for the process of risk assessment. For that reason, I will later

describe and assess the tiered approach to risk assessment recommended by Rissler and Mellon (1996), and compare it to the case-study method for equivalent results.

Finally, it is possible after reviewing all of the above criteria to draw some conclusions about the relevant hypotheses. I will address each hypothesis in turn.

Hypothesis A, that transgenic plants pose no special risk to the environment, is somewhat of a rhetorical statement. For one, field trials have occurred at an accelerated rate for several years, and some genetically-engineered products have already been made available for consumers(see e.g. Balder 1997; Ronald 1997). As such, there is an inherent sense in which the USFDA and APHIS have already decided that the transgenics are pretty much OK for people. However, if transgenics are bad for the environment,they are likely to be bad for people, too, and so in a very real sense this is not a rhetorical statement. Clearly, many ecologists consider transgenic plants and other organisms to pose acute and unique environmental risks (Abbott 1994), and for that reason alone the experiments will continue. I feel that overall the case study demonstrates that this hypothesis is false.

The second hypothesis, that transgenic OSR will not form feral populations of any greater significance than nontransgenic OSR, is also decidedly false. However, I must first hedge my remark by noting that (1) there is a great deal of evidence that demonstrates poor overwinter survivorship for transgenic OSR, particularly in the presence of interspecific competition; (2) the nontransgenic OSR sometimes fares much better in the same regard (possibly due to the associated metabolic costs of the transgene); and (3) without at least a certain proportion of plants to constitute that population, if the transgene is heterozygous it will probably nearly disappear due to normal Mendelian segregation. But, these three points without exception revolve around the absence of critical selective pressures, or at least selective pressures that are considered critical based on the available evidence. This is yet another unknown that can be added to the negatives in the "predictive power" collection. Finally, this point is borne out by the available evidence for insecticidal transgenic OSR, and it is a point for which many of the

scientists felt very strongly (Stewart *et al* 1997; Kareiva, Parker and Pascual, 1996; Abbott 1994; Thacker 1994).

Again, the available evidence for the third hypothesis, "no ecologically-significant hybridization between trangenic OSR and its weedy relatives..." has been demonstrated to be decidedly false. Hybridization is common and frequent in natural conditions, and has been shown to result in fertile progeny which then demonstrate weedy characteristics. The importance of this aspect of the transgenic OSR plants will again rest on the nature of the transgene, as well as various life history traits and particularities of the conditions in which the transgenic OSR is used.

The fourth hypothesis is also somewhat of a rhetorical statement. Experience has dictated that nearly all human actions on the landscape have some sort of environmental effect, and certainly the actions that have the effect of either increasing, decreasing or adding a new selective pressure will have some sort of ecological effects. The problem is, some effects are much more insidious than others. For example, if a transgenic OSR plant with the *Bt*-toxin is somehow prevented from hybridizing - they are made completely sterile, perhaps - then they cannot hybridize and augment the selective pressures in favor of a weedy relative, but they can still have enormous selective pressures for insects to become resistant to *Bt*. Certainly it is possible to reduce the magnitude of those effects, as discussed in Gould *et al* (1992) and Shelton *et al* (1993), but the status of such selective pressures is no less an ecological effect just because humans are trying to prevent it from having an effect. Another example of an insidious ecological effects might include stress-tolerant plants that are cultivated in previously inaccessible environments, which would therefore displace the existing plant community, regardless of the potential for hybridization. The potential ecological effects of transgenic organisms released into the environment are limited only by the scientists' ability to express a trait in an organism, and by the extent to which that organism is released into the environment.

Shrader-Frechette and McCoy note that "one of the best ways of evaluating a case-study conclusion is to analyze the objections likely to be made to it and therefore to determine if the objections can be answered in a reasonable manner"(1993,234). This is

an important ideal, especially in a case study such as my transgenic OSR model, in which all likely hypotheses are at least partially refuted. In their 1993 book *Method and Ecology*, Shrader-Frechette and McCoy develop a very extensive case study of the nearly extinct Florida panther (*Felis concolor coryi*), and evaluate the case study according to conservation biology theory. Their conclusion is highly uncertain (as are most attempts to save endangered species), and it is an area of concern for them. It is enough of a concern that they address some political aspects, namely the potential repercussions of failing to take the steps that are seemingly necessary, even though there is very little evidence that those steps will be successful (e.g., using conservation corridors between habitat preserves to help increase the size of their range - an admittedly costly measure). Following this methodological criticism, the remaining objections to their conclusions are more properly addressed in the public policy arena.

I have attempted to show that for each inference in my case study, there are two distinct possibilities for interpreting the data (it is ecologically safe or unsafe), and in some instances there may even be a third (no action yet, and do more studies). The methodological/ epistemological questions continue to remain open issues, and they always will, perhaps. For example, the data and respective conclusions of Crawley *et al* (1993) were very well-received, and the article has been cited in nearly every other source on transgenic OSR since 1993. However, Kareiva and his colleagues (1996) were able to arrive at dramatically different conclusions from the same data, and three years later, no less! I prefer to think that the community of professional ecologists, along with their peer review processes and their intellignet scrutiny of each other's work, are probably much more likely to sort out the methodological difficulties of the numerous transgenic plant experiments in a better fashion than any philosopher is likely to do via prescriptive means. They have achieved great epistemological successes thus far, and ecology as a discipline is still barely 100 years old.

The second problem that I have with framing a prescriptive recommendation regarding the case study's conclusions is that a certain proportion of such decisionmaking properly belongs in the public arena. Following Rollin (1995), I am inclined to recommend that such policy battles should be waged on a local front as much as possible;

if farmers in the Deep South wish to use transgenic organisms and farmers in Kansas and Nebraska do not, then perhaps that is the best solution -- to use them locally only. In cases where that GEO may have far-reaching implications, the regulation is properly left in the hands of the USFDA and APHIS. However, the degree to which such localized decisionmaking can and will be implemented remains to be seen.

## TRANSGENIC VIRUS-RESISTANT PLANTS: A CASE FOR SPECIAL CONCERN

The level of understanding of viral evolution and viral host specificity in plants has increased dramatically in recent years, but there is still a great deal to be learned about both. Rissler and Mellon mention four specific areas of concern regarding the ecological risks posed by transgenic virus-resistant plants: (1) the potential production of new strains of a particular target virus; (2) the genetic engineering techniques may allow the virus to infect new hosts (an increase in its host range); (3) the techniques may exacerbate existing viral diseases, through various mechanisms; and (4) the potential for movement into wild/weedy populations of crop relatives (1996, 60, and references therein). As I have addressed the fourth concern at length with regard to other genetic engineering techniques, I will only address it in passing as it relates to the issue of virus resistance. The other variables that relate to the ecological risks of virus-resistance are the mode of transmission of the virus, e.g. seed-transmissable or insect-transmissable, and the potential for recombination between the host plant and nontarget viruses.

Most plants genetically engineered for virus resistance utilize a gene that produces the coat protein of the virus, which offers protection from the virus by an unexplained mechanism. This is known as coat protein-mediated protection, and is similar to another unexplained naturally-occurring mechanism for virus protection, "cross protection", in which a plant is "inoculated" to severe strains of a virus following infection by a mild strain of the virus. However, coat protein-mediated protection is not immune from the danger of new viral strains arising via recombination between the nucleic acids of the coat protein gene and the nucleic acids of related viruses. Such recombination may result in new biological characteristics, including a new or expanded host range, and there have

been documented occurrences in Cauliflower Mosaic Virus (CaMV) in transgenic tobacco plants (*Nicotiana bigelovii*), resulting in an increased host range for the CaMV (Schoelz and Wintermantel 1993). Greene and Allison (1994) also demonstrated that a noninfectious mutant strain of cowpea chlorotic mottle virus (CCMV) became infectious after recombination with a plant that was transgenic for a CCMV coat-protein gene, which may present a more serious ecological risk, given the evolutionary implications foir such host-pathogen interactions, which I will discuss momentarily.

A related risk of virus-resistant transgenic plants is from *transcapsidation*, the inclusion of the genome for virus Y by the coat protein of virus X. This is generally a short-lived phenomenon, but it still allows for a potential temporary expansion of the viral host range. This phenomenon is known to occur in nature, albeit rarely, and it may fairly be asked whether the phenomenon is truly any different for genetically modified plants. For transcapsidation to increase the host range of a virus, there must be an insect vector, and the coat protein (and no other virus particles) must be sufficient for virus transmission into the new host (Palukaitis 1991). Given these requirements, it may seem to be even more rare in agriculture than in a natural setting, but it is also important to consider that transgenic plantings are likely to occur on a large scale (perhaps even millions of acres), and insects are highly efficient viral vectors. This particular aspect of transgenic virus-resistant plants will probably be more of an economic issue than an ecological concern, although there remains one more way in which transcapsidation presents a risk.

Given that 1/5 of all known phytopathogenic plant viruses are seed transmissable, it is possible that viruses may either become seed transmissable or their seed transmissability may be enhanced (e.g., cucumber mosaic virus, or CMV, is variable in its degree of seed transmissability.) This is also mainly an economic issue, although it can become more of an ecological concern if it occurs repeatedly during a single growing season (Rissler and Mellon, 1996). Most importantly, the risks inherent in transcapsidation are *empirical* -- they can be assessed via rigorous testing in the laboratory, followed by manipulation of the relevant variables in greenhouse and/or field conditions. This should also be noted cautiously, as researchers must first determine the

extent to which transcapsidation of the transgenic plants occurs, and then attempt to determine the controlling factors for the event, if any.

A second genetic engineering technique for virus resistance utilizes the small pieces of RNA associated with certain virus strains, known as satellite RNA, or simply satRNA. The satRNA is not associated with the viral genome, yet requires the presence of the virus to replicate; its presence in transgenic plants inhibits the replication of the genome of the target virus, thus preventing infection of the host plant. The three major risks associated with the use of satRNA in plants are :

> 1) the satRNA may be benign in the transgenic plant and virulent in a different plant species;
> 2) the difference between benign and harmful satellite RNAs may involve a change in only one or a few nucleotides; and
> 3) sat RNA has a high mutation rate. (Rissler and Mellon 1996, 62, and references therein)

The dangers of mutation and viral synergism are discussed as the largest risk factors for plants engineered with satRNA, and researchers currently do not know much about either (Palukaitis 1991). As such, it is impossible to clearly state any predictions about the presence of such transgenics in the environment, although it remains a largely empirical issue. Theoretically, at least, researchers will be able to systematically experiment with satRNA-transgenic plants in a variety of environments, and eventually determine the extent to which the satRNAs mutate, and what factors affect increased virulence in the target virus or its mutated forms.

The evolutionary implications for virus-resistant plants are vast and complicated. As intracellular obligate pathogens (they require the use of the host's molecular machinery to replicate themselves), the evolutionary relationship is very complex. Plant defense mechanisms utilize receptors coded by *R*, or resistance genes, to recognize the presence of the avirulence, or *avr*, genes of the pathogen. This is a direct gene-for-gene complementarity mechanism, and it requires that the pathogenic virus periodically loses the particular form of the *avr* gene now recognized by the plant's *R* gene, by mutation (see Staskawicz, *et al* 1995). Such gene-for-gene complementarity does not bode well for

the potential success of transgenic plants, especially given the rapid evolutionary capability of viruses. For instance, a consequence of the gene-for-gene complementarity in natural systems is a diversity of *R* genes "within different individuals of a host species and a corresponding diversity of avirulence genes in different pathogen races"(Staskawicz *et al* 1995, 665).

Given the known existence of gene-for-gene systems on plant-pathogen relationships, it is exceedingly difficult to assess the ecological risks of transgenic virus-resistance plants by examining the potential for evolutionary change. Simms (1996) discusses several attempts at modeling the cycling of the genes and their traits. When virulence and resistance are costly[5], the "simple models predict cycling of host and pathogen allele frequencies around a locally unstable equilibrium point"(138, and references therein). She then notes that if the models are modified to more closely approximate reality, for instance by "treating the host as diploid with several alleles at a single resistance locus and incorporating multiple alleles at a haploid pathogen virulence locus...[the models] are likely to produce patterns resembling chaos"(ibid.). However, by modeling resistance independent of virulence, "the tradeoffs between costs and benefits can produce stabilizing selection for an intermediate optimal value of resistance"(ibid.).

This is an interesting conclusion, as the modified models would also assist in predicting a maximum rate of viral evolution as a response to a high level of resistance in the host plants -- particularly in the event of large-scale plantings of such transgenic crops. Accordingly, agronomists, plant ecologists and virologists may then be able to estimate the *durability* of resistance alleles (the time it takes pathogens to evolve virulence to the new resistance allele) prior to release in the environment, and a form of integrated pest management may then be utilized to help slow the evolution of virulence by the viral populations. However, because the biochemical costs of resistance are not well known, and may differ for a given plant from region to region, this is a very difficult evolutionary relationship to assess. It is in fact an empirical determination, but again, it is

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[5] Simms describes fitness costs, or tradeoffs, as "the fitness decrement experienced by an individual possessing a trait, either virulence or resistance, as measured in an environment where it is not needed....[and]some tradeoffs can arise from the costs of maintaining relevant biochemical pathways or allocating resources to resistance or virulence" (1996, 137-138).

dependent on a number of variables, each of which may vary on a regional basis, and scientists are a long way from predicting the results of such complicated ecological relations.

Although a great deal is now known about the relationships between plants and their viral pathogens, and despite the fact that transgenic plants for viral resistance are even now being produced, much of the work is still focused on how such relationships may change in a natural setting. The risks of viral transgenic plants in the environment is somewhat unique compared to other transgenic plant concerns, but that is mainly due to a lack of knowledge about microbial ecology and viral evolution. It is possible that viral evolution will never have a predictive element for ecologists, and that it will be strictly numerical in nature -- i.e., expressed as a strict probability, with no estimation of environmental influences. As such, there is a great need for caution in this sphere, although many more questions will be answered as experimental results become available.

Perhaps more than any other issue I have discussed, viruses would seem to offer great hope for a provincialist view of ecology, and yet it seems that whenever there are a number of variables (as is inherent in any living system), there does not seem to be much reason to hold such a reductionistic view. The interaction of viruses and plants serves to demonstrate that these ecological relations are just as complex and devoid of quantifiable predictive value as any other ecological relations that have been examined thus far.

## A METHOD OF RISK ASSESSMENT FOR GENETICALLY ENGINEERED CROPS

Rissler and Mellon (1996) conclude their text with an outline of a suggested methodology for performing risk assessments of genetically engineered crops, and before I briefly examine the issue of genetically engineered microbes, I will discuss some problems with their methodology.

For risk assessment, Rissler and Mellon note that "the fundamental question...is whether the presence of a transgene alters recipient plants in ways that make them a new or worse weed compared with nontransgenics"(1996,72). There are two problems with

this conceptualization. First, transgenic plants may be considered properly unique, to a degree that such comparison may be rendered meaningless. Some possible examples of "properly unique" genetically modified plants might include the following considerations:

- Might a conventionally-bred or naturally-occurring plant alter virus transmissability to the same extent that a plant engineered to produce a viral coat protein might?
- Might certain examples of horizontal gene transfer in plants ever occur naturally or spontaneously, especially in cases of protoplast bombardment?
- Could conventionally-bred or naturally-occurring plants ever conceivably evolve the ability to produce an insecticidal toxin, like *Bt*-endotoxin? (Considering the biochemical costs and type of substance involved, it is highly unlikely that such a trait could both mutate and not reduce the fitness of its producer, particularly in the early life stages.)

It is important to also consider that in Chapter Two I examined a number of reasons for excluding verbal models that claimed that GEOs were *not* any different than other organisms, and those reasons continue to hold true at this juncture.

Second, given the past examples of ecological disasters that resulted from the intentional release of foreign species into an ecosystem (e.g. gypsy moths; kudzu vine; European rabbits in Australia; goats on Santa Catalina island in California; starlings throughout North America; etc.), it may fairly be asked whether it is even reasonable to take a chance on the introduction of GEOs into the environment -- particularly those with wild/weedy relatives *and* fitness-enhancing traits. I will return to these two issues at the conclusion of this chapter, but before discussing risk assessment, it is important to bear in mind these two possibilities.

A strictly ethical aspect of risk assessment in the Rissler and Mellon system is: What to do if a given transgenic plant reaches the third tier, and is found to be unacceptable? Further, on the basis of existing data, can *known risks* be deemed *a priori* unacceptable, due to a conservative determination of risk by the individuals responsible for such decisions? The latter is a feasible choice, given a number of socially valid reasons that I will further develop after explaining this risk assessment system.

Rissler and Mellon (1996) review two aspects of genetically engineered crop plants for risk: weediness and the potential for gene flow. These are somewhat related, but for the sake of simplicity I will consider them separately as well, and start with "weediness".

The first question asked, the Tier 1 level, is as follows: Is the crop plant weedy, or does it have weedy relatives? Here, again, weediness is a trait defined simply as "undesirable to humans", and more practically, as an invasive species. If the answer is negative, as e.g. soybeans and corn (*Zea mays*) in the U.S., then abbreviated Tier 2 experiments are probably sufficient. However, if the answer is potentially affirmative (e.g. canola), then so-called "standard" population replacement experiments are required for that GEO. Sources of information at the Tier 1 level include literature searches, examination of weed lists from local cooperative extension offices, existing agronomic and botanical surveys (ideally not limited to national borders, but extending throughout a crop's ecological range), and consultation of experts. Over time, as information accumulates, this level of examination may be eliminated for some species (especially male-sterile species), with the possible exception of plants engineered for virus resistance, which will likely remain problematic.

The Tier 2 level consists of population replacement experiments, in which transgenic plants are compared to nontransgenics of the same species. There are two main variables: net replacement rate (R), the number of seeds produced by plants which were produced from a known number of seeds; and seed bank persistence, measured by half-life, which is the length of time required for a given seed bank to lose half of its viable seeds. If R=0, then the population is not replacing its original numbers, and may be expected to become extinct unless the trend is reversed; if R=1, it is replacing its numbers exactly; and if R>1, then the population is expanding, and is "successful". Similarly, if a seed bank has a long half-life, then perhaps the conditions have not been conducive, and more research is needed on the abiotic factors that stimulate production of seedlings.

There are two main advantages to the Tier 2 methodology: both aspects measure a "culmination of an array of interdependent events: seed germination, seedling survival, vegetative growth, adult survival, reproduction, and others - all influenced by the

environment"(Rissler and Mellon 1996, 80); and the experiments can easily be designed to be statistically valid and amenable to statistical analysis. However, there are a number of potential disadvantages, as well.

Without examining the individual life stage data, it is possible to both overlook critical variables, and therefore underestimate the risk posed by a given plant; this is exacerbated by the general lack of botanical and ecological data for weedy plant species, which would extend to a crop plant with weedy relatives. The selection of sites and time intervals for collection of data may also be problematic, especially if there is a lack of natural history data and a lack of information regarding what some of the critical variables may be. A related possibility is that there may not be an adequate representation of potential sites for that crop plant upon becoming weedy, e.g. field sites vs. field margins and nonfield sites. Another intrinsic difficulty is the difficulty in evaluating a number of engineered traits within a given species. As more data accumulates, the widely-hypothesized "genetically-crippled" model -- based on too many biochemical costs for the individual -- may be seen to be quite erroneous, particularly if all other conditions are acceptable. In addition, there may be synergism between the traits, such that data independently derived on a per-trait basis may be invalid for such a multitrait plant. Finally, in direct comparisons of transgenic vs. nontransgenic populations of the same species, what conclusion follows if the transgenic plants outcompete the nontransgenics only some of the time? There is no clear answer to this dilemma, unless (1) there is a critical variable involved at one site that had a direct, known correlation to an increase or decrease in fitness, or (2) more experiments are performed to determine the reason, or (3) it is considered a high-risk situation, and the researchers move to Tier 3.

If, after extensive field experimentation at the Tier 2 level (which has the advantage of potentially coinciding with the standard battery of field tests currently required by EPA and APHIS) it is determined that there remains a high level of risk, the experimenters have the choice of reconsidering commercialization or performing further experiments. Rather than explain the detailed methodology of testing at this level, I will merely note that many of the articles that I used for my canola case study were individually representative of the methods that would be used to examine all aspects of a

high-risk GEO crop introduction. At this level of risk, it becomes imperative to examine all the potential avenues for ecological disruption, such that in the best-case scenario those instances will be avoided at all costs in practice, while at the worst-case scenario the researchers would be literally forced to reconsider commercialization, if it was not disallowed outright. In considering the severe nature of the Tier 3 conclusions, it is also important to consider the reexamination of Crawley *et al* (1993) by Kareiva, Parker and Pascual (1996) -- in which it was shown that time was the most important variable for the initial experiment, whereas in the assessment of invasive weed species, time was *not* a critical variable, in part because many of the weedy species reviewed in the 1996 paper required several attempts before establishing themselves. Epistemologically, then, the conclusions may not warrant a definitive decision on the risk of the plant; but realistically, it is a pretty good method for eliminating a large number of variables, and at best *reducing* the risk of an ecologically-problematic genetically engineered crop species.

The second aspect of risk assessment for transgenic crop plants is the risk of gene flow to other populations. As with the determination of weedy relatives, there is a general lack of information on the potential for hybridization between crop plants and wild species, and the first step (Tier 1) would be to organize and assess the existing data. Rissler and Mellon (1996) suggest 6 important questions:

1. Is the crop sexual?
2. If so, does the crop have sexually-compatible wild relatives in its ecological range?
3. If so, do the breeding systems allow for gene flow between populations?
4. Does the flowering phenology overlap, and by how much?
5. Do the crop plants and wild relatives share the same means of pollination?
6. Do the crops and relatives naturally cross-pollinate and have fertile, viable seeds in field conditions?

As demonstrated in the oilseed rape case study, even for a relatively cosmopolitan crop such information is largely unknown, though quantifiable. In the assessment of gene flow, there will likely be no "abbreviated" set of population experiments in the near

future, for any crops that have positive answers to two or more of the above six questions. However, the population replacement experiments are certain to face some difficulties for hybrid transgenic plants, including: a frequently statistically-invalid sample size; a sample pool of viable hybrids that exhibits great deviation from year-to-year or even season-to-season; wide variation in seed viability and fertilization capability; and even fluctuation in the extent to which a hybrid exhibits an engineered trait, if at all.

The assessment of gene flow between populations as a potential risk is a scientifically difficult one, and one that I will not examine in any more detail since that difficulty was already demonstrated in my OSR case study. Again, I think it may suffice to say that all of this methodology suffers severe epistemological shortcomings, some of which will be improved upon over time; but it nonetheless allows scientists to both establish baseline ecological data for future use, and also provides some degree of predictive capability for ecologists who are expected to provide a degree of expertise to a public seeking to arrive at a decision on a problem of real import.

Before concluding, I will first devote some time to a discussion of the potential ecological risks that are unique to transgenic microorganisms, which pose similarly unique dangers to the environment and potentially to human health, as well.

## SPECIAL CONCERNS OF GENETICALLY ENGINEERED MICROORGANISMS

The extensive presence of naturally-occurring microbial populations in the environment pose unique problems for evaluating the release of genetically engineered microbes, or GEMs. These problems include normal genetic transfer among those populations, difficulty in identifying the ocurrence and extent of genetic transfer, random dispersal patterns (e.g. via wind or water), and the phenotypic qualities of the microorganisms themselves. For instance, Roszak and Colwell (1987) found that certain soil bacteria populations would go into dormant states in which they remained *viable*, but were not found in normal culture methods. In my brief discussion of the ecological impact of GEMs, I will begin by describing the methods of gene transfer in the environment, some evidence for gene transfer and its known or suspected means, and

some laboratory difficulties in assessing genetic transfer. I will then describe the use of microcosms, and their attendant advantages and disadvantages. Although my discussion will mainly entail agricultural applications, I will also briefly examine some aquatic applications of microbial ecology, as this is both an area of extensive biotechnology use and an area of important ecological concern.

Conjugation, as I described in Chapter 1, is the exchange of genetic material between a bacterium and another organism through plasmids, small bodies of extrachromosomal DNA. The recipient organism is usually another bacterium, but in the case of *Agrobacterium tumefaciens* (the source of crown-gall disease), there is a direct transfer of either Ri or Ti plasmid DNA into the plant tissue, which then becomes part of the plant's genetic material. This technique has been exploited by agricultural engineers to get the desired DNA into the plant, while silencing the effects of the *Agrobacterium* tumor-inducing DNA. Comeaux, *et al* (1990) cite numerous references for conjugal transfer in nature, including :

*In planta* occurrences:

- the transfer of oncogenicity in two *Agrobacterium* species;
- the transfer of antiobiotic resistance (a) from *E. coli* to various strains of *Pseudomonas syringae*, and (b) among different strains of the phytopathogen *Erwinia chrysanthemi*;

(The *in planta* occurrences were at a greater rate than *in vitro* experiments, which in itself is noteworthy, as it relates to the epistemological value of extrapolating from the laboratory to the environment.)

In aquatic systems:

- Extensive conjugal transfer occurred between *E. coli, Proteus mirablis, Salmonella enteriditis, and Shigella sonnei* in sterile sewage; this was less than the *in vitro* results, however.

Conjugation also occurs extensively in soil systems, and I will return to this topic shortly. Transduction is also widely present in natural systems, which is the transfer of

genetic material by bacteriophages. This is a common mechanism for the spread of antibiotic resistance, along with conjugation, and Bale, Hinton and Beringer (1992) note that is very common in *E. coli* and *Salmonella enterica*. These authors also emphasize research that has documented (1) the presence of extracellular DNA both in sediment and attached to sand grains (free from the effects of nuclease enzymes) which were involved in gene transfer (via transformation in aquatic habitats; and (2) that *Salmonella* has evolved resistance to antibiotics from plasmids -- which is also a response to a selection pressure.

Other authors note that at least for agricultural microbial communities, the GEM considerations must include a consideration of host-pathogen interactions (Van den Eede and Van Montagu, 1992). For example, in the consideration of the now-famous "ice-minus" *Pseudomonas syringae* variants, there are three key factors:

> 1. The extent of the selection pressures in a given habitat, with regard to the engineered trait (increased frost resistance);
> 2. The "extent to which competition for limiting resources affects intraspecific and interspecific fluctuations";
> 3. The interactions between the phytopathogenic bacteria and other microbes, particularly if the trait only affects a region of the plant (in this case, the leaves). (1992,28)

There are three main options for monitoring the survival and persistence of GEMs in the environment: Unusual phenotype tracking, which utilizes genetic markers; The use of nucleic acid probes, like PCR, which is very time- and labor-expensive; and the use of immunological detection, e.g. antibody presence, which is also not a viable option for agricultural applications. Phenotype tracking can be difficult, mainly due to the lack of gene expression which may occur in a natural setting (e.g., if it is induced by poor climatic conditions or nutrient deficiencies). As mentioned earlier, the microbes may become dormant, and then become impossible to monitor by normal culture methods. Finally, these issues are all compounded by the difficulty in determining the viability of transgenic microbial populations. Much more so than plants, microbes are expected to be hindered by the metabolic costs of a foreign gene that codes for the production of a given substance (e.g. the *Bt*-toxin), but this will be very difficult to establish in field studies. It

is more important to avoid a false-negative conclusion, due to the increased risk of such a conclusion (Van Den Eede and Van Montagu, 1992).

In the January 1998 issue of Scientific American, R.V. Miller describes his recent research documenting horizontal genetic transfer through the transduction of bacteria in the slimy layers of rocks on the bottom of a lake., the epilithon. He also cites other research indicating conjugation in the epilithon of a lake in Great Britain, and the fact that the microcosm results differed greatly from the laboratory results. For example, conjugation occurred at temperatures as low as six degrees Celsius in the river, which was far too low for conjugation to occur in the laboratory (Miller 1998). Similarly, Miller notes that evidence is accruing for some abiotic factors which control the availability of free DNA for transformation in aquatic environments; such factors can be as simple as diurnal fluctuations (1998). Finally, in what is perhaps the most important conclusion in the article, Miller notes that :

> [it was]originally thought [that] transduction would not be an important means of gene exchange in the environment, because it requires viruses and bacteria - both of which were thought to be present in low concentrations - to interact. But my co-workers and I have recently found bacteriophages in very high concentrations (often 100 billion virus particles per *milliliter* [my emphasis] in fresh and marine waters. These observations have caused a reevaluation of the frequency of interactions, including transduction, that occur between bacteriophages and their hosts. (1998,71)

Aquatic ecosystems are important for several reasons. For one, they provide much of the drinking water for the human population, and it is not clear to what degree the current methods of surface water treatment will act as an impediment to the proliferation of GEMs. Similarly, aquatic ecosystems are essential recreational areas for human use, which may also add to the exposure of potentially harmful GEMs. Finally, aquatic ecosystems are the receptacles for nearly all of the world's sewage effluent, which often has a very high concentration of pathogenic organisms in it, for obvious reasons. In addition, biotechnology is increasing by leaps and bounds in both the wastewater industry as well as the pollution clean-up industry, as more and more metal- and toxin-resistance genes are being isolated and engineered into recipient organisms. These are all reasons

for concern, and provide a good rationale for increasing the accuracy and predictive potential of research aimed at assessing the risks of GEMs in the environment.

I have already hinted at some of the difficulties that are attendant to GEMS, and the above quote from Miller (1998) also enumerates a problem: the continued existence of latent assumptions that are based on theory alone. The current perspective on evaluating genetically engineered microorganisms involves the initial isolation and incorporation of the trait, viability studies of the newly engineered organism, laboratory studies of the GEM, microcosm studies of the GEM, and then field trials.

Microcosm studies have long been supported by the U.S. Environmental Protection Agency, and are defined as "an intact, minimally-disturbed piece of an ecosystem brought into the laboratory for study...that behaves ecologically like its counterpart in the actual field"(National Research Council, 1989). Interestingly, they were originally used to evaluate the environmental impacts of pesticides, for which some authors have strived to point out the disanalogy to GEMs (e.g. Comeaux *et al*, 1990). One weakness of the microcosm technique is that they have not yet been standardized, so it remains questionable whether or not microcosm results can be considered comparable or repeatable with regard to later microcosm studies.

Krimsky *et al* claim that the purpose of microcosm studies is "to add a level of ecological reality to laboratory experience while maintaining many of the advantages of the controlled environment"(1995,594). However, there is still no way to guarantee that the *critical* or ecologically-relevant variables are present or controlled in the microcosm study, and so there will always be deviation from the "ecological reality". Further questions must be asked of each microcosm: Have the "edge effects" from the high surface-to-area ratios been minimized? Is it the appropriate size and shape? What is the best design and use for it? How long should the microcosm experiment be performed?

For GEMs, there may be more particular problems: How to test for vector-mediated escape or exchange of genes? How to eliminate the inherently static nature of the microcosm, so as to introduce environmental factors like diurnal fluctuations? How to address the difficulties presented when bacteria are existing in a viable, but dormant (i.e.,

non-culturable) condition? (This is even a problem for molecular-based detection, as in such a condition the bacteria cannot even be sufficiently concentrated.) Finally, since the microcosm results cannot be "calibrated" to field conditions, how can we insure that they do in fact represent what occurs in natural systems? This last issue is crucial, and Krimsky *et al* (1995) feel that an agreeable consensus may be reached by a forum of experts; furthermore, they recommend precalibration of the microcosm whereever possible, according to such parameters (for a soil system) as "respiration, nutrient cycling, and primary productivity, and/or structural attributes [e.g., community composition]" (1995,596).

Some examples of microcosms include soil samples that are generally 15 cm diameter X 60 cm deep, a number which is gaining acceptance as a sort of *de facto* consensus. In running the experiment, sterile field-application soil will be used for a control, a standard mixture of soil may be used for another control, and an intact soil core will be used for the experimental plot. Other variations may include "feeding" one or more of the soil samples with a specific culture aimed at promoting or furthering growth of one or more microbial populations, which would of course reduce the degree to which the microcosm is relevant to actual field conditions.

Microcosms used in aquatic studies range from using autoclaved source water as a control, to placing chambers fitted with water-diffusion membranes to allow flow-through directly into the source water. As mentioned earlier, some aquatic microcosm studies have resulted in surprising results, in which the results were nearly opposite what the researchers had predicted.

Clearly, the ability to assess potential ecological risks from GEMs suffers from many of the same methodological and epistemological shortcomings as genetically engineered plants. However, some of these are unique to GEMs, and accordingly there is more dependence on the extrapolation of laboratory data to the field than there is for plants, although some of this is mediated by the increasing use of microcosms in microbial investigations. This is an area of tremendous importance for humans, particularly those GEMS that are used in aquatic environments and agricultural settings,

and it is one that will likely receive increasing attention in the years to come, as biotechnology products continue to arrive at the door of the marketplace.

## SUMMARY

After determining that case studies were the best option for assessing GEO releases into the environment, I proceeded to describe a representative example of a case study, for oilseed rape (*Brassica napus*). This process included:

1) Assembly of ecological baseline information (e.g. what sexually-compatible relatives does OSR have? How do their naturally histories compare? What is the likelihood of crop-wild hybrid formation, and subsequent introgression of the new trait into weedy populations, as in Tables 4-2 to 4-4? etc.);

2) A detailed breakdown of the eight relevant issues for all case studies;

3) The current types of genetic modifications of OSR, as in Table 4-6;

4) A highly detailed analysis of the evidence, both for and against, the four hypotheses that I stipulated for the case study -- including an examination of the "Criteria for Interpretation of Findings".

Then, I briefly examined some reasons why transgenic virus-resistant plants pose a special concern for ecologists - particularly due to the lack of natural history data for many viruses, and because of their demonstrated capacity for extremely high mutation rates.

To make the OSR case study more relevant in the public decisionmaking domain, I then analyzed the tiered approach to risk assessment for genetically modified crops presented by Rissler and Mellon (1996). Their detailed scheme has two problem areas, one methodological and one ethical:

(1) Is it possible that perhaps GEOs should be considered properly unique, such that modeling their ecological behavior as analogous to that of related species is inherently problematic at worst, and simply an artifact of human understanding at best?

(2) Such potentially troublesome human activities in the environment may also be due special consideration, given some past examples of invasive species problems -- and what

are we to do in the event that a transgenic crop is found to be a high-risk variety, despite its potential economic benefits? This will not be an easy solution, and in fact Rissler and Mellon (1996) were content to merely indicate that "commercialization should be reconsidered" in such cases. Given that a large investment of both capital and research funds will have gone into the research and development of that GEO, it is likely that industry leaders will find it acceptable to merely "reconsider commercialization".

Finally, I briefly addressed the potentially critical issue of genetically engineered microorganisms, and their release into the environment. The two central concerns with GEMs are (1) their ease of transmission through such vectors as wind and water, and (2) the fact that horizontal gene flow does in fact occur spontaneously in microbial populations. This indicates that desirable traits may very well find their way into undesirable microbes, and that the beneficial traits like antibiotic resistance are likely to both find their way into nontarget microbial populations and increase the evolution of resistance to those beneficial traits. The last concern with GEMs is that of public health, which may be a particular concern for aquatic microorganisms.

## POTENTIAL PROBLEMS IN THE ANALYSIS

In the previous pages, I have made some very general assumptions which may not be obvious to the reader, and which require some attention.

First, it may be asked why I used the example that I chose for the case study – oilseed rape. In arguing for a risk assessment approach based on the *product* of genetic engineering and not the *process* itself, in Chapter 2, I left an important point implicit. I feel that for purposes of ecological risk assessment, species that have long been domesticated are not likely to pose a sudden ecological risk (i.e., as a feral organism) on the basis of a small genetic change. However, species that have wild relatives that are capable of interbreeding with their domestic relatives are far more likely to pose such a danger, as the newly introduced genetic material may well find its way into the "wild-type" genome. Hence, oilseed rape was chosen because (1) it has numerous wild relatives, (2) it is being widely used for genetic engineering, and (3) it has a very large

area of geographical distribution. The last item is important because even if a particular form of genetic manipulation is outlawed in one country, the recombinant plants may still arrive in that country and pose the exact danger that had been foreseen. In addition, oilseed rape has been heavily studied in both laboratory and natural environments, and a large natural history database is being developed for this plant. For that reason alone, it is somewhat "ideal" as an example of how a case study may be performed.

Some species are more problematic, and will have to be addressed on their individual merits. For example, if horses or pigs are engineered to have some new trait that may have a particular selective value (perhaps resistance to a disease that is harsh on their wild relatives), it will have to be considered whether it may be possible for such GEOs to mate with feral relatives. This is an admittedly farfetched example, but one that needs to be addressed, nonetheless.

Fish species pose a particular problem, in that it is somewhat difficult to predict whether a new trait may have selective value in a wild environment. Fish engineered to have extra growth hormone, for example, have obvious economic value because they can grow to market size in a shorter time. But, will such rapid growth be an asset or a handicap in a natural environment, if that trait were passed on to wild relatives and then maintained in the population? This is an empirical issue, and can therefore be addressed as a scientific question. However, the ability to extrapolate ecological information from one habitat or ecosystem to another is a more problematic issue, and that will have to be addressed when experiments are designed to assess these issues. Genetically engineered fish are beyond the scope of this thesis, but the interaction of gene pools in aquatic environments is an important contemporary issue.

A second point that may be raised is why emphasize plants, when genetic manipulation is becoming so commonplace. There are several reasons for choosing plants:

(1) Agricultural products are a potentially worldwide market, and so the use of genetically engineered plants has the potential for worldwide impact, across a variety of ecosystems;

(2) There are phylogenetically-related plant species of agricultural import all over the world, raising the possibility of spreading novel traits to a higher level;
(3) Because agriculture is such an economically important area, genetic manipulation of agricultural plants is likely to continue far into the future – the process will continue to receive funding, and the products will continue to be welcomed by farmers;
(4) The bulk of biotechnology research is already being done on agricultural plants.

These are all powerful and important motivations for having the proper framework in place for the evaluation of ecological impacts of agricultural GEOs. I have sought throughout this thesis to take both a theoretical and a pragmatic approach, and for that reason alone I think it is important to emphasize the potential ecological impacts of genetically modified plant species. Furthermore, in Chapter 3 I discussed the importance of natural history data for establishing a case study procedure, and I feel that as experimentation continues with agricultural plants, it is important to have a proper scheme in place for the evaluation of ecological risks.

Third, I have offered a brief discussion of genetically engineered microorganisms (GEMs) in this chapter for similarly pragmatic reasons. The risk that is presented by the so-called "pathogen paradigm" – that genetic manipulations may increase the pathogenicity of GEMs – is very real indeed. I will now briefly examine this threat, and illustrate an approach for evaluation of this risk.

## THE PATHOGEN PARADIGM

One commonly expressed fear regarding GEMs is the possibility that new, supervirulent pathogens may result from the "genetic tinkering". Further, with the worldwide increase in infectious emerging diseases like hantavirus pulmonary syndrome and the Ebola virus, this threat seems even more vivid. To elaborate on this potential problem, I will first describe the variables involved in evaluating the potential for new viral diseases. I will then attempt to illustrate how viruses may be used as a model to

examine the potential for new bacteriological pathogens, as a result of genetic engineering.

Frank Ryan, in his recent book *Virus X*, notes that there are six main requirements for viruses to be able to attack new hosts:

1. The virus must be able to establish itself in the new host.
2. A "route of trafficking" must be present for the virus to get to the new host.
3. The virus must be able to penetrate surface defenses (e.g. skin and mucous membranes).
4. The virus must be able to protect itself from attacks by the host's immune system.
5. Once into the new host's body, the virus must then discover a cell in the new species similar enough to that of the original host cell to enable it to replicate itself (Ryan notes that replication is usually highly evolved in viruses.)
6. Finally, the virus must be able to infiltrate the "genomic machinery" of the new host cell to finalize its replication. (1997, 312)

Furthermore, there are evolutionary "tradeoffs" that are assumed to be involved in the process of adapting to a new host. Initially, more aggressive viruses are assumed to have a selective advantage, but the advantage will exhibit a marked decrease over time – at least in theory. Ryan notes that some mathematical models have demonstrated that natural selection would favor aggression equally with decreased aggression, over time (1997, 310). This is an interesting example of the problems encountered in ecological and evolutionary modeling, because the conjectural approach and the mathematical approach are both severely limited in their application to the natural world. Unfortunately, attempting to study viral evolution in a laboratory setting has different liabilities, and I have examined these problems in detail in Chapter 3.

Viruses also have certain advantages for establishment in new hosts, and Ryan described three main advantages:

1. Rapidly evolving viruses will have obvious advantages, and for an example we may consider HIV.

2. The manner of transmission, or "avenue of contagion", may help or hinder viruses in their attempts to become established in new hosts.
3. Finally, increased contact with both old and new hosts increase the likelihood of coevolution with a new host. [6] (Ryan 1997, 314)

Many of the theories associated with increased virulence by viruses are based on conjecture, i.e., verbal models. Furthermore, these verbal models are impossible to prove or disprove, until some counterexamples arise either in nature or in the laboratory. With that in mind, it seems clear that any risk assessment based on these conjectural models are likely to be incorrect or merely unfalsifiable, with certain caveats.

I mentioned in Chapter 2 that for the assessment of novel food allergens, even the U.S. FDA has noted that there is no possible anticipatory structure for that determination. As such, it is only possible to be very careful about the host and donor organisms, and note that any risks associated with the host and donor are likely to be present in the GEO. Similarly, it would not be wise to utilize a known pathogen for genetic engineering, except in circumstances where the virulence locus is known and can be eliminated (e.g., avirulent *Staphylococcus* strains are commonly used in college microbiology laboratory experiments.) However, even with avirulent strains, there remains the potential for mutation into a virulent strain – and not necessarily the same form of virulent strain that was previously known. The regulatory structure has been in place for many years to prevent such random occurrences, and it is reasonable to assume that any risks will be addressed in a cautious manner.

Clearly, the risks from bacteriological pathogens are considerably different than those from viruses, although it would be arbitrary to claim that one or the other is a more severe risk. Many of the aforementioned virulence factors for viruses hold true for bacterial pathogens, with two main differences. The bacterium does not need to infiltrate the genome of the host cell, nor does it need to find an exact type of cell in which to reside.

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[6] It is important to note that all viruses coevolve with their hosts, in the sense that viruses share the host's genetic machinery and therefore are intimately involved in the evolutionary process.

However, regarding the last statement, the bacterium does need to find a suitable environment in which to reside. Of course, for bacteria groups like the Enterobacteria, any warm-blooded organism will be fine, and the virulence risks for such bacteria are increased accordingly.

Fortunately, bacteria are also much better understood than viruses, and this makes the project of risk assessment much easier for those scientists. However, the problem of transmission (i.e., from host-to-host) is qualitatively different for bacteria, as well. Because many bacteria are motile, the risks of transmission are considerably higher – particularly for spore-forming bacteria. In addition, colony-forming bacteria pose a different problem, as they are much more difficult to infiltrate and destroy via antibiotics and UV radiation.

Clearly, the method of risk assessment that continues to be most appropriate for all GEMs is the same as that of food allergens: carefully consider both the donor and host organisms, and evaluate accordingly. If, for example, a mycobacterium is being used (a group which includes the tuberculosis and leprosy pathogens), then extremely cautious measures are called for in all stages of the experiment. However, if a strain of *E. coli* that has long been benign will be utilized, somewhat lesser precautions may be merited. Fortunately, such measures have long been in place in the biotechnology field, and these good work practices are likely to continue in coming years.

Finally, any time that there is a discussion of risk assessment procedures there are a number of problems that arise. I have repeatedly referred to the "social and professional consensus" that surrounds the professional field of risk assessment, and I hold to the position that there is such a consensus. However, there are always disagreements in this arena, and a full discussion of these discrepancies is far beyond the scope of this thesis. However, a few comments are certainly warranted.

In every particular scientific field, there are "standards" for what constitutes an acceptable methodology and acceptable results. In psychology, there is usually a "random error" of either 1% or 5% for a study to be considered "statistically acceptable". This is known as the "p-value", and is usually expressed in decimal form: 0.01 or 0.05.

In ecology, there is typically a great variation in these values, and the standard depends on the type of study being done, and especially on the extent to which variables are manipulated and controls are in place. If there are too many variables, than there are likely to be too many possible alternative explanations for the data (including stochasticity), and the p-value will accordingly be higher, as well. If the experiment is rigidly controlled and there are few variables, than it is not considered to be a study that effectively replicates the "real world", and so has less explanatory value. In the former example, the p-value or expected randomness may be as much as 15%; in the latter, as little as 1% or 5%.

Is there a single, proper manner of conducting ecological risk assessments? Of course not. The bias may be dependent on a particular researcher, who wishes to have the lowest possible p-values, period. Other researchers may wish to have experiments that more accurately portray the "real world", and so they may include so many variables that the p-value is far too high to be considered "statistically-valid".

It is my opinion that the current method, involving systematically proceeding from small-scale lab experiments to small-scale field experiments to large-scale, precommercialization testing is an excellent procedure. Certainly, there is always going to be some potential for problems, but science is a human endeavor and mistakes are going to occur. It is important to have regulatory oversight at the last two stages (both of which are field experiments), to insure that there is academic honesty in the procedure. Finally, as I have noted numerous times in this thesis, ecology is simply <u>not</u> a predictive science. Predictions can be made, some of which may be accurate, but the predictions will always be based on inference. Why is inference problematic? Because there is no way to know all of the data before reaching a conclusion, and some of the "unknowns" may be crucial problems. Statistically, this is unlikely, but there is a definite tradeoff between probability of occurrence and the potential severity of occurrence. Unfortunately, further discussion of this tradeoff is far beyond the scope of this thesis.

Science, clearly, will always be plagued by uncertainty. It is how we, as humans and scientists, address this uncertainty which will help to determine the outcome of our genetic manipulation of other life forms. There is no other way.

# CONCLUSION

Soon after the identification of the DNA double-helix structure, biochemists were hard at work trying to insert the genes of one organism into the genome of another. Perhaps this is just one more example of the human inclination toward tinkering, and humans are a product of evolution, and evolution is itself the "great tinkerer", in the words of Francois Jacob (1977). As such, there is probably nothing inherently wrong with such tinkering, and there is truly a great deal of good which may eventually come of genetic engineering: increased agricultural production with a possible decrease in chemical investment (especially herbicides and insecticides), as well as the potential for human gene therapy to treat any number of diseases, including some chromosomal abnormalities and debilitating metabolic storage disorders.

However, there are definite grounds for due consideration when releasing GEOs into the environment. The change in responses to selective pressures, in addition to the potential for gene escape into undesirable populations, and the potential for negative effects on existing ecosystems are all reason for concern with regard to GEOs in the environment. Ethical discussion of these introductions is necessarily difficult, as there are two potential views which may conflict: A decidedly anthropocentric outlook versus an ecocentric outlook.

For evidence, one needs only to observe the current dialogue regarding genetically engineered plant releases, in which the relevant question is: Does the plant have any wild or *weedy* relatives in the target ecosystem? Though "wild" may not necessarily be a normative term, the term "weedy" is decidedly normative, and is centered on human uses and concerns. If a plant is pestilential in any way to humans, it is termed a weed, whereas for an ecologist a weed is generally synonymous with an invasive plant species ("invasive" refers only to the status quo of the ecosystem, either at that time or historically -- it is not a normative term, although it may take on normative meaning). Such a conflation of meaning for a given term is both curious and troublesome, because it is representative of the difficult nature of the GEO debate, both methodologically and epistemologically.

For the ecologist interested in GEO releases, there are two main difficulties relating to the design of experiments to assess the role of GEOs in the environment. The first is methodological, relating to the best way to design an experiment to discern information about the GEO of interest and/or its related species. The second difficulty is epistemological - namely, what does the ecologist *know* after finishing an experiment? Can the ecologist exclude all reasonable alternative hypotheses relating to the original investigative question? Frequently, in ecology, that is simply not the case, and ecology is not the only science that suffers this shortfall.

All of the life sciences suffer from a similar shortcoming - how to exclude as many variables as possible from an experimental design, and still maintain a high degree of explanatory fertility. It may be argued that this is particularly true for field studies in plant ecology, as witnessed in the earlier quote from Kareiva, Parker and Pascual (1996) that "year-to-year variation in plant success can be staggeringly large" - and without necessarily even knowing why. Because of the tremendous degree of variation field studies are subjected to, and because of the complexities of living systems, it is clear that biology is different from the physical sciences in very important ways -- viz., causation. While one may logically state that an action B caused an action (or re-action) C, such causality is frequently absent or simply untenable in the life sciences.

For this reason, I argued in favor of a position of autonomy for biology, as contrasted with a vision of it as a sort of "province" of the physical sciences, as some reductionists would have it. I feel that it is fundamentally different in ways that really matter, and that that is one of the reasons for a frequent lack of predictive ability, and sometimes even of explanatory fertility, in the life sciences. A provincialist account would perceive biology as a manifestation of the electrochemical reactions that occur in the course of maintaining the survival of living beings; implicit in such a view is the notion, akin to faith, that someday molecular biologists will in face be able to assign a definitive *causative agent* for everything that occurs. I find this view to be both unsupportable and contrary to the evidence, given the difficulties in providing explanations for much of what occurs in the living, natural world (despite the continued gains in molecular biology and organic chemistry).

Part of the autonomist account is a need for alternative methods of explanation, especially given that the Popperian account of falsificationism fails on many grounds in biology and ecology (mainly the rich supply of plausible alternative hypotheses). A great deal of ecological science rests on induction, perhaps of necessity, and given that fact I proposed the method of case studies as the optimal route to warranted justification for ecologists. After all, much of ecology depends on models, and many of those models have collapsed in recent years - a fine example would be that of equilibrium notions in ecology, particularly as applied to community succession.

One advantage of case studies is that the hypotheses and assumptions are presented in explicit fashion, so that a case study can easily be reworked and reevaluated if some amount of information is found to be incorrect. Obviously, case studies cannot be used across the board, but as their name implies, they were not meant to be, either. A second advantage of case studies in this particular application is that nearly all researchers have proposed that GEO introductions be considered on a *case-by-case* basis, simply because there are too many unknowns. In addition, case studies in combination with classical experimentation allow ecologists to study both interactions between similar species and the influence of selective pressures on a given population - another area that is not easily translated from one GEO case to another.

The selective pressures acting upon GEOs are perhaps the most critical area for concern, when attempts are made to evaluate the release of GEOs into the environment. However, the philosophical debate over the proper level at which to evaluate selection pressures is far from over, and is a key issue for ecologists. There are three possible levels at which natural selection may operate : at the level of genes, at the level of individuals, or at the level of groups of individuals. Because genetically engineered organisms are receiving special consideration due to the incorporation of a single foreign gene into their genome, it would seem plausible that perhaps selection does indeed act at the genic level. On the other hand, the selection of individuals is what is observed in field experiments, and survivorship is measured as the number of surviving individuals; further, if those surviving individuals fail to produce fertile offspring, they are still being selected against,

as they will not pass their genes on to their offspring. It is for this reason that I have elected to follow the argument in favor of Ernst Mayr's "unity of the genotype", despite the fact that GEOs are being evaluated on the ability of the single engineered trait to provide a fitness advantage for them in the environment.

The final area of concern for GEO releases is the ethical sphere. If, after performing years of expensive ecological studies, it is found that a genetically engineered plant does in fact pose a potential ecological risk, what are we as a society going to decide regarding its fate? I have mentioned that Rissler and Mellon (1996) have simply argued for a "reconsideration of commercialization" of that crop, and I do not feel that is enough. The further issue of the scale of ecological risk is one that will be decided by the community of scientists and risk managers for both the government and private industry, and so I will focus only on some facts which may influence the decision about GEOs that are a potential risk.

In 1973 the U.S. Congress passed the Endangered Species Act (ESA), which aimed to provide measures to ensure the protection of endangered species in the U.S.A. This Act was the culmination of a gradual shift toward an appreciation of wild nature, an appreciation grounded in aesthetic, ecological and utilitarian terms. Furthermore, the passing of the ESA signaled a dramatic shift in views regarding the human-nature relationship, one that reflected a view of nature that was valued in and of itself, and not strictly according to human wants and needs. In the Act itself, Congress stipulated that endangered species had such value, and mandated that efforts be taken to protect and maintain such populations as remained of those species. Those "efforts" included protection for the species and its *habitat*, and with such a strong affirmation of the value of nature was afforded considerable attention (especially when combined with the Clean Water Act and National Environmental Policy Act, both of which were passed in 1970).

The passage of significant environmental legislation is clearly sufficient to demonstrate that in the United States, there is a definitive community consensus in which we place value on the environment, outside of that utilitarian value that humans always place on nature. I think that it is also clear that along with our ethical "obligation" (in an

admittedly weak sense of that word) to take active measures to protect nature, there is also an equally strong "obligation" to take measures such that we do not knowingly harm the environment. This is a way of describing the legal term "negligence", and it is directly analogous to the legal sphere. A person is held more accountable for his actions if he knowingly causes harm to another individual or that individual's property, as compared to causing such harm inadvertently. Similarly, I feel that there is a greater sense of wrong associated with continuing to do actions that we know to either cause harm to the environment, or that at least have the potential to cause harm to that environment.

Earlier, I established that there are numerous examples of disastrous intentional species introductions to the U.S. and elsewhere, and it is probably safe to assume that the number of unintentional introductions that have had adverse effects it quite high as well. Given the reality of this potentiality, it seems uncontroversial that any potentially adverse ecological consequence as a result of GEO releases to the environment should be considered unacceptable. Just as it is necessary to protect the habitat of an endangered species to protect the species itself, if we are to maintain the integrity of the natural environment we must as a society consider it to be imperative not to take unnecessary ecological risks. Genetic engineering is a wonderful technology, and it has the capacity to help feed the growing human population, produce new and better drugs and antibiotics, and perhaps even help to reclaim metal-contaminated environments; but it also has the potential to cause a great deal of ecological harm, and many of the potential avenues for such ecological damage have been described in this thesis. Perhaps just as we as a nation decided that it was worthwhile to forgo some economic benefits to protect endangered species, ecologists as a society can help to convince us that some GEOs will present undue ecological risks and their introductions should justifiably be thwarted, or at least reconsidered.

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