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Cytotaxonomy and Interrelationships of Pacific Basin *Salvelinus*

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INTRODUCTION

Salmonid fishes of the genus *Salvelinus* form an interesting group in terms of the systematic problems they present and those species living in the drainage area of the North Pacific basin are certainly no exception. Important questions concerning the relationships between chars on the western and eastern sides of the Pacific have not been answered by morphological studies. Also, the taxonomic status of nominal forms of the *Salvelinus leucomaenis* group occupying the Japanes Islands remains a controversial problem.

In this paper we have applied cytogenetic information on the chromosome morphology and placement of the nucleolar organizer regions (NORs) to the following questions: 1) Can the chars of the Japanese island be separated taxonomically on the basis of the chromosome morphology and NOR characters ? 2) Is the bull char, *Salvelinus confluentus*, more closely related to the other North American *Salvelinus* or are its relationships, instead, with the western Pacific forms ? 3) How similar, karyologically, are the southern subspecies of *Salvelinus malma* on each side of the Pacific basin ? and 4) How reliable are cytogenetic characters used in this study for hypothesizing relationships among the taxa of Salvelinus ?

Earlier work by Cavender (1984) demonstrated that information on karyotypes could easily separate Salvelinus confluentus from Salvelinus malma. The two species had long been confused, taxonomically, over a broad area of distributional overlap on the Pacific slope of North America (Cavender, 1978). Viktorovsky (1975a, 1975b) showed that the diploid chromosome numbers differed in at least three of the Salvelinus taxa living in the area of the Kamchatka and Sakhalin peninsulas. Uyeda and Ojima (1983a), using the C-banding technique were able to find characters that would separate the Hokkaido Dolly Varden from the Miyabe char. They also found differences in populations of Salvelinus "leucomaenis" from Japan (Uyeda and Ojima, 1983b). However, in their work Uyeda and Ojima made no attempt to distinguish between S. leucomaenis leucomaenis and S. leucomaenis pluvius as we have done.

The recent development in silver staining technique for marking the NORs in metaphase (Howell and Black, 1980) has enabled investigators to utilize N-banding information along with their conventionally stained karyotypes. C-banding technique as applied by Uyeda and Ojima (1983b) to S. leucomaenis and by Phillips and Ihssen (1985) and Phillips et al. (1988) with respect to S. fontinalis, S. namaycush and S. alpinus offers additional characters for cytotaxonomic analysis. In reviewing our own results along with the results of other workers we have attempted to test the congruence of hypotheses of Salvelinus interspecific relationships based on cytogenetic and morphological characters.

The *Salvelinus* taxa we have investigated are named as follows: amemasu char (*Salvelinus leucomaenis leucomaenis*), iwana char (*Salvelinus leucomaenis pluvius*), gogi char (*Salvelinus leucomaenis imbrius*), the southern subspecies of the Dolly Varden char in the western Pacific (*Salvelinus malma krascheninnikovi*, see Berg 1948, p. 307), the southern subspecies of the Dolly Varden char in the eastern Pacific (*Salvelinus malma lordi*), the Miyabe char (*Salvelinus malma miyabei*) from Lake Shikaribetsu and the bull char (*Salvelinus confluentus*).

For convenience we have used subspecific names to identify the *leucomaenis*-group of chars (amemasu, iwana and gogi) without any preconclusions intended on their taxonomic status. Behnke (1980) suggested the name *S. malma lordi* be used for the eastern Pacific subspecies. Chereshnev (1978) and Behnke (1984) last discussed the use of the name *S. malma krascheninnikovi* for the southern form of the western Pacific Dolly Varden.

MATERIALS AND METHODS

In the preparation of metaphase chromosome slides the technique of Kliegerman and Bloom (1977) was followed without modification. Whenever possible, giemsa, silver and double stained preparations were made for each species studied. For silver staining, the one-step method of

Howell and Black (1980) was used. Specimens were injected in the abdominal cavity with 50 μg/g body weight colchicine. After 3 to 6 hours they were sacrificed. Gill epithelial tissue was employed as a source of mitotic cells in all taxa examined. Chromosomes were classified according to the method of Levan et al (1964). For NOR position terminology, Takai and Ojima (1986), Phillips and Zajicek (1986) were consulted. As far as could be determined, silver staining produced consistent and repeatable results with each taxon. The ability of the silver stain ($AgNO_3$) method to specifically label the active NOR regions in metaphase chromosomes was discussed in Howell (1977) and Schmid and Gutterbach (1988). This stain has the advantage of pinpointing the rDNA regions through their associated proteins without also banding the constitutive heterochromatin that may be at different locations on the chromosome.

The Salvelinus and salmonid taxa studied, the number of specimens examined and their place of origin or source are given in Table 1. The salmonid taxa Hucho perryi and Oncorhynchus masou were use for outgroup comparison with Salvelinus in a phylogenetic analysis of the genus as explained further in the discussion section.

Table. 1.

RESULTS

For each taxon investigated the karyotype formula is given along with a description of the gross morphology of the chromosomes including those with secondary constrictions and N-bands. A photo karyotype is presented for each taxon along with illustrations of the NOR chromosomes. Emphasis is placed on those chromosome features that are useful for phylogenetic analysis.

Hucho perryi (Fig. 1): The karyotype formula found for this species was 42 msm (metacentric-submetacentric) 4 ST (subtelocentric) and 16 T (telocentric) for a 2n (diploid) total of 62 with an NF (arm number) of 104. Of the 42 msm the smallest pair were distinctly submetacentric chromosomes. This was the same formula as that reported by Uyeda (1987) for *Hucho perryi* from the Sorachi River, Hokkaido. However, Uyeda did not publish a photokaryotype of this species nor investigate the NOR chromosomes.

Fig. 1
Fig. 2

As pointed out by Uyeda (1987) considerable chromosomal evolution in the form of Robertsonian rearrangements has occurred in the phyletic line leading to *Hucho perryi* from its original split with one that produced *Hucho hucho*.

Hensel and Holčik (1983) reviewed the chromosome data on *Hucho hucho hucho* and gave the karyotype formula as 24-32 msm and 52 to 58 acrocentrics, with a 2n number of 82. Dorofeeva as cited in Viktorovsky (1978) gave the karyotype formula for *Hucho hucho taimen* as 18 msm and 66 ST+T (acrocentrics) for a diploid number of 84 and arm number of 102. This formula is closer to that expected in the ancestral *Salvelinus* (16 msm, 68 St+T, 2n=84, NF=100) and other primitive salmonines. The highly derived character in the form of many biarmed chromosomes of the Hucho perryi karyotype was unexpected considering that this species is morphologically the most primitive huchen (Vladykov, 1963; Shaposhnikova, 1968). As a comparative example of a salmonine species pair exhibiting a large differences in chromosome number, Uyeda (1987) used Salmo trutta which has 80 chromosomes and Salmo salar which has 56-60 depending on the population examined. The significance of the Hucho perryi karyotype, however, is its strong convergent similarity to that found in the western trout group (Parasalmo) especially Salmo clarki bouveri, the yellowstone cutthroat which has 64 chromosomes composed of 42 msm, 4 subtelocentrics and 18 telocentrics (Gold et al., 1977) and Salmo gairdneri, the rainbow trout, which typically has 60 chromosomes (44 msm 2 ST and 14 acrocentrics, Thorgaard, 1983). This point underlines the fact the salmonines undergo the same kinds of changes in their gross chromosome morphology independently in different phyletic lines.

Silver staining for NORs, did show Hucho perryi had an unusual type of NOR chromosome. There is only one pair as believed to be the primitive condition for teleost fishes (Takai and Ojima, 1985) and the condition found in most salmonines (Phillips et al., 1986). However, the NORs are placed interstitially in the middle of the slightly shorter arms of two metacentric chromosomes. These metacentrics are the fifteenth longest pair of 21 msm pairs. This, NOR placement is clearly derived from a terminal position probably by means of a pericentric inversion or as the result of a tandem fusion as described by Takai and Ojima (1985). As far as known most salmonines have the NORs placed distally on the short arms of a small acrocentric pair as we found in Oncorhynchus masou. Hucho perryi stands apart from other species in the genus Hucho by its larger scales, lower vertebral numbers (10 lower) and by possessing teeth on the basibranchial as well as medially on the basihyal. All of these features are most likely primitive. The species stand apart from all other native salmonids of Japan by being a spring spawner (Kimura, 1966).

Oncorhynchus masou (Fig. 2): Masu salmon, from the Ichani River, eastern Hokkaido. We used Oncorhynchus masou as an outgroup taxon to compare against the Japanese Salvelinus taxa. Karyotype formula, 36 msm 22 ST (including 2 NOR chromosomes), 8 acrocentrics, 2n 66, NF 102.

Uyeda (1984) reported nearly the same formula (38 msm, 28 ST+T, 2n 66, NF 104) for Oncorhynchus masou from a pond of the National Research Institute of Aquaculture, Tochigi Pref., but classified one pair of subtelocentrics as possible submetacentrics. Yamazaki and Arai (1987) published a photokaryotype with 17 pairs of metacentric-submetacentric chromosomes, 24 subtelocentrics and 8 acrocentrics. They did not recognize the two small biarmed chromosomes found in our sample and also in that of Uyeda (1984).

The one pair of relatively small NORs occur on acrocentric chromosomes. They are either centromeric in position or telomeric on the very short arms of a subtelocentric pair. Uyeda (1987) obtained identical results and published a photograph showing a metaphase spread with a larger NOR on one chromosome than on the other. It is interesting to note that the biwa salmon, Oncorhynchus rhodurus, has an identical karyotype to that reported here for O. masou except for the NOR pair (Uyeda et al., 1985).

Phillips et al. (1986) reported Oncorhynchus masou from an unknown locality with one acrocentric nucleolar organizer chromosome pair using both $AgNO_3$ and CMA3 staining. The same NOR type was also found in O. kisutch and O. tshawytscha.

Viktorovsky (1978) published a photokaryotype of Oncorhynchus masou possibly from Kamchatka with 66 chromosomes but showing much different morphology of the smaller biarmed elements.

Fig. 3
Fig. 4

Salvelinus leucomaenis leucomaenis (Fig. 3): Amemasu, the Ichani River, Hokkaido. The karyotype formula for this subspecies was 16 msm and 68 ST+T, 2n 84, NF 100.

Uyeda and Ojima (1983) found the same karyotype in Salvelinus leucomaenis leucomaenis from the Sorachi River, Hokkaido. Most of the S. leucomaenis they reported on in this and other papers (Uyeda and Ojima, 1984; Uyeda,1984; Uyeda, 1987), however, were S. l. pluvius from different drainages of Honshu. Also finding the same karyotype of S. l. leucomaenis from Hokkaido were Abe and Muramoto (1974) and Arai (1984). Viktorovsky (1975b) reported S. l. leucomaenis from Sakhalin with a karyotype of 2n=84-86, NF=100.

The most striking features of the S. l. leucomaenis karyotype are

1) the low number of biarmed chromosome (8 pairs) which is found also in southern *S. malma, S. fontinalis* and *S. namaycush* and is likely the primitive number for the genus. 2) the characteristic large submetacentric pair with an arm ratio of 1:1.6-2:0 which can be easily identified in the karyotype and serves as a marker chromosome (Viktorovsky 1975b). 3) the obvious subtelocentric chromosome pair with well developed short arms (arm ratio 1:3.0-3.5). This is the chromosome pair that carries the NOR'S. In giemsa stained slides a somewhat smaller subtelocentric pair can sometimes be confused with the NOR chromosomes in metaphase plates with contracted elements. One of the NOR chromosomes often shows a secondary constriction in the short arms. 4) the acrocentric chromosomes grade evenly from the largest to the smallest. The largest acrocentric is about the same length as the shortest biarmed chromosome. The NOR subtelocentric pair ranks as either first or second in length among the acrocentrics. In reference to this size ranking, one can observe that in the exceptionally well illustrated photokaryotype of *Salvelinus leucomaenis leucomaenis* (Plate XVI, Fig. 183 in Arai 1984) the chromosome pair labeled ST (subtelocentric is too large to match the subtelocentric pair in question and is more likely the distinctive submetacentric pair of *S. l. leucomaenis* mentioned above. The NOR bearing subtelocentric pair is probably the smallest biarmed pair in the figure. What might have occurred in this cell was the staining of the NOR regions with giemsa so that they appear as normal arms of metacentric chromosomes.

Salvelinus leucomaenis imbrius, gogi char (Fig. 4): The karyotype formula determined for the gogi char from the Takatsu River drainage was 16 msm, 68 ST+T, 2n 84, NF 100.

As far as we could determine the karyotype was similar in all respects to the amemasu char, *S. l. leucomaenis*, with the possible exception of a size difference in the NOR-bearing chromosome. In the latter species the short arms that carry the NORs are larger giving the chromosome a slightly longer dimension. With silver staining there is a particularly noticeable difference in size of the NORs. In both the gogi and amemasu one chromosome of the NOR pair often shows secondary constrictions in its short arms (Fig. 10).

Fig. 5 Fig. 6

Salvelinus leucomaenis pluvius (Fig. 5): Iwana, from the Akimoto Hatchery, Miyazaki Pref., Kyushu; originating from the Momose River of the Jintsu River system, Toyama Pref., Honshu.

The karyotype formula found for the iwana was 16 msm and 68 ST+T, 2n 84, NF 100. Uyeda and Ojima (1983) and Uyeda (1984) reported the same karyotype for this subspecies. A large submetacentric (marker) pair of chromosomes was present in the karyotype as characteristic of other member of the *leucomaenis* group of chars as well as the western Pacific forms of *Salvelinus malma*. The NORs occur on the short arms of a single subtelocentric pair. Two other pairs of subtelocentrics are similar to the NOR pair but differ slightly in size. They can be easily confused with one another in the conventionally stained metaphase plate. The karyotype of *S. l. pluvius* closely resembles that of the amemasu, *S.*

l. leucomaenis differing only in the short arm development of the NOR-bearing subtelocentric chromosomes when giemsa stained metaphase preparations are compared. This difference reflects the larger sized NORs in *S. l. leucomaenis*. When silver stained preparations are compared *S. l. pluvius* always has much larger NORs on one chromosome than on the other. In *S. l. leucomaenis* this lopsided difference is not as apparent. Uyeda (1987) also illustrated this characteristic of the NORs in *S. l. pluvius* from the Tone and Miya Rivers of Honshu. He found a difference between these two populations in the length of the NOR chromosomes. The C-banding patterns investigated by Uyeda and Ojima (1983) also showed intraspecific polymorphism in *S. l. pluvius*.

Salvelinus confluentus, bull char (Fig. 6): the karyotype of this species was reported by Cavender (1984) as 24 msm, 56 ST+T, 2n 78, NF 102. Attention was called to two pairs of submetacentric chromosomes. The larger of the two pair was of the type found in *S. leucomaenis* and *S. malma* but the smaller 10th pair was as far as known unique to *Salvelinus*. Further work on *S. confluentus* by Phillips (this volume) has shown the smaller "submetacentrics" are NOR-bearing acrocentric chromosomes. The NOR-bearing regions sometimes accept the stain in aceto-orcein or giemsa preparations and appear as the short arms of a biarmed chromosome. The same or very similar condition occurs in *S. l. leucomaenis*. It is tempting to propose that the NOR chromosome in *S. confluentus* is a slightly more specialized version of the type seen in *S. l. leucomaenis*. The change having been a further enlargement of the

NORs. It follows that the karyotype formula for S. confluentus can be written 22 msm, 58 ST+T, 2n 78, NF 100 which is the same as that of Salvelinus kronocius from Lake Kronosk, Kamchatka Peninsula (Viktorovsky, 1975a). However, it may well be the smallest biarmed chromosome in the photokaryotype published by Viktorovsky (1975a) is actually the acrocentric NOR chromosome. One of the elements of the smallest biarmed pair in Viktorovsky's karyogram is definitely a submetacentric. If this were the case, S. kronocius would have 20 instead of 22 msm. Morphologically, S. kronocius does not agree with S. confluentus in a number of characters indicating it is not a sister species to the bull char. S. kronocius has fewer mandibular pores and branchiostegals, more gill rakers of the type found in S. malma and more pyloric caeca. The gill rakers are identical to S. malma malma in shape and the dermethmoid and ethmoidal fontanelles are also similar to S. malma.

Fig. 7
Fig. 8

Salvelinus malma kraschenninnikovi, Dolly Varden char of Hokkaido (Fig. 7): This may be the same taxon as the Soviet ichthyologists term the southern subspecies of S. malma in the western Pacific at Sakhalin (S. malma kraschenninnikovi Taranetz) - see Berg (1948). The nomenclature is confusing since there is also a southern form called S. malma curilus (Berg, 1948).

The karyotype formula determined for the Ichani River population was 16 msm, 66 ST+T, 2n 82, NF 98.

Other workers studying the chromosomes of Japanese Dolly Varden char found the same karyotype (Abe and Muramoto, 1974; Uyeda and Ojima, 1983; Uyeda, 1984). In the eastern Pacific, Cavender (1984) found 2n, 82 chromosomes with 16 msm, 66 ST+T, NF 98 for *Salvelinus malma* from the Soleduck River, Olympic Peninsula, Washington.

Our results from karyotyping the Hokkaido population showed the characteristic very long first acrocentric chromosome pair designated as a marker chromosome by Cavender (1984) in a paper presented at the 1981 Charr Symposium in Winnipeg, Canada. Cavender proposed that this chromosome resulted from a Robertsonian fusion of two acrocentric chromosome followed by a pericentric inversion reducing the arm number by two.

Uyeda and Ojima (1983) independently called attention to this chromosome and postulated it was the result of a single tandem fusion event which may be a more parsimonious explanation than the two event sequence.

This very long chromosome pair occurs in the northern Dolly Varden of the Kamchatka River basin (*Salvelinus malma malma*) judging from the photokaryotypes published by Viktorovsky (1978, 1975b). The northern Dolly Varden has a markedly different karyotype formula that of the southern form (18-20 msm, 56-60 ST+T, 2n 76-78, NF 96). The relationship between the two forms is shown by the common inheritance of the very long acrocentric. This chromosome may prove to be a powerful systematic tool for marking the *malma* group of chars, since it also appears in the karyotype of *S. malma* from the southern Sakhalin (labeled

S. malma curilus by Viktorovsky, 1978) in *S. malma schmidti* (Fig. 10, Viktorovsky, 1978) and in the resident mollusc eating char (*S. malma malma*) of Lake Azabache, Kamchatka River basin (Fig. 6, Vasilyev, 1975)

The Hokkaido *S. malma krascheninnikovi* also posseses a characteristic subtelocentric pair which was usually the only pair with discrete short arms among the median sized acrocentrics. The NORs were found at the centromeres of the second longest pair of acrocentrics. The NORs of this pair may or may not stain with giemsa. If they do the NOR pair could be confused with the median sized subtelocentric pair. It is interesting to note that this type of subtelocentric occurs in the *leucomaenis* group of chars but has not been found in eastern Pacific Dolly Varden.

Another important character of the Hokkaido *S. malma* karyotype was the large submetacentric pair found in the second to fourth pair-position similar to that in members of the *leucomaenis* group. The arm ratio of this chromosome varied from 1:1.6 to 1:2.4 depending on the amount of contraction. It could occasionally be classified as a subtelocentric as possibly happened in the karyotype of *S. leucomaenis* (Arai, 1984).

Salvelinus malma lordi, Dolly Varden char of the southern Alaska at Juneau (Fig. 8).

The karyotype formula for the Juneau population was 16 msm, 66 ST+T, 2n 82, NF 98.

This is the same formula found for the southern Dolly Varden from Washington (Cavender, 1984). In gross features, it is identical to the

Hokkaido *S. malma* but in detail the karyotype lacks the median sized subtelocentrics characteristic of the Hokkaido population and the NORs were found on a different chromosome pair. They occurred terminally on the short arms of the distinctive large submetacentric pair described above. The NORs themselves are very large unlike those in the Hokkaido form. Secondary constrictions marking the NOR sites were observed in giemsa stained preparations. The SC's were much easier to locate on one member of the NOR pair than on the other.

Externally the Hokkaido *S. malma* from the Ichani River resembles that illustrated by Inamura and Nakamura (1962) from the Rausu River, in the eastern Hokkaido.

Salvelinus malma miyabei, Miyabe char from Lake Shikaribetsu (Fig. 9): The karyotype formula determined for this form was 16 msm, 66 ST+T, 2n 82, NF 98.

The NOR-bearing chromosome appeared as large submetacentrics in all giemsa stained preparations. Uyeda and Ojima (1983) classified them as submetacentrics and distinguished *miyabei* from *malma* on the basis of arm number with *miyabei* having NF 100. It is clear from both our work and that of Uyeda and Ojima (1983) as well as Uyeda (1987), that the same chromosome pair is involved in both subspecies but change has occurred in *miyabei* with an increase in size of the NORs. What has most likely occurred is that protein associated with the NOR body has accepted stain giving the appearance of an extended short arm on the acrocentric chromosome. In this study, we have been able to determine the difference between Hokkaido *S. malma* and the Miyabe char is in the NOR chromosome pair. As reported by Kimura (1976) and Maekawa (1977), *S. malma miyabei* had an high gill raker count (23-27, average 25.5) for the 6 specimens karyotyped.

DISCUSSION

One of the objectives of this study was to develop some cytogenetic characters that could be used in a phylogenetic analysis of *Salvelinus*. Enough has been learned about the chromosomes and NORs of *Salvelinus* and salmonids in general to establish the primitive states for a number of chromosomal characters. The following states can be considered primitive for *Salvelinus*.

1) 2n, 84 chromosome number, 16 msm and 68 acrocentrics.

2) NF 100 chromosome arm number.

3) One pair of NOR-bearing chromosomes.

4) NORs occurring at the centromeric position on two homologous acrocentric chromosomes.

5) NORs relatively small in size as in *Salvelinus fontinalis*.

6) Biarmed chromosomes with arms equal or subequal in size most with an arm ratio less than 1:1.5.

7) Acrocentric chromosomes grade evenly in size from the largest to the smallest. The largest acrocentric approximately equal in size to

the smallest (8th) pair of biarmed chromosomes.

8) All acrocentrics more or less uniform in morphology. Short arms when present are very minute and difficult to reproduce in micro photographs. No outstanding subtelocentric marker chromosomes.

Chromosome number is the most utilized chromosomal character because it is the easiest to obtain. In Salvelinus, karotype evolution through Robertsonian fusion has produced a variety a derived states with 2n, 76-78 the lowest number reported (Viktorovsky, 1975b). Apparently the direction of change where known has always been toward reduction in chromosome number. This character is subject to parallel evolution in Salvelinus and has been found to be common place in other salmonid groups. At least two and possibly three phyletic lines have produced parallel changes, such as the lines leading to S. confluentus 2n, 78, S. m. malma 2n, 76 or 78 and possibly also in S. alpinus 2n, 78. As far as can be determined from the literature, change in arm number has occurred only once in Salvelinus. This was the result of a probable centric fusion of two acrocentric chromosomes followed by a pericentric inversion producing a change from 100 to 98 arms (Uyeda and Ojima, 1983; Cavender, 1984) thus giving rise to a very long acrocentric marker chromosome. Cavender (1984) hypothesized that S. alpinus also inherited this character since it has an arm number of 98. However, to date, no one has confirmed the existence of this same chromosome in S. alpinus. Morphological evidence presented by Glubokovsky and Chereshnev (1981) on chondrocranium structure provides characters that are incongruent with the above hypothesis of a close relationship between S. alpinus and S. malma. The arm number of 96 proposed by Viktorovsky (1975b) for northern Dolly Varden, S. m. malma, has not been convincingly demonstrated.

One pair of NOR chromosomes was found in Oncorhynchus, Parasalmo, Hucho as well as Salvelinus. Silver staining in other teleosts groups supports the conclusion that one pair is the primitive condition (Takai and Ojima, 1985; Schmid and Guttenbach, 1988). Also, centromeric NOR position on an acrocentric chromosome was probably the primitive teleostean condition (Takai and Ojima, 1985). Change in NOR position must have occurred quite frequently in the evolutionary process. Much intraspecific variation can exist within Salvelinus malma, for example, as NORs are now known to occur in four different positions. Thus, little higher taxon level phylogenetic information is available from NOR studies. With respect to NOR size, there does appear to be a trend toward an increase in size with the more derived populations or taxa showing this state. Characters stemming from the morphology of individual chromosomes have the potential of being extremely helpful in phylogenetic analysis. Marker chromosomes in the biarmed and acrocentric series are present within Salvelinus. However, without banding patterns to confirm homologies between taxa, the use of distinctive marker chromosomes carries considerable risk.

Takai and Ojima (1985) have speculated that the increase in NOR size is associated with a accumulation of rDNA (a derived character) which as a final step in NOR evolution before redistribution to new sites on other chromosomes occurs. This process produces the multiple NOR state in *Salvelinus* seen in *S. fontinalis* (Uyeda, 1987), *S. namaycush* (Phillips and Ihssen, 1985) and *S. alpinus* (Phillips et al., 1988).

Derived characters states used in our phylogenetic analysis are listed as follows:

1) Reduction in the chromosome number from the primitive 2n, 84.

2) Increase in biarmed chromosome numbers.

3) Reduction in arm number to 98 from the primitive NF, 100.

4) Change in NOR position from an original centromeric condition on an acrocentric chromosome pair.

5) Increase in NOR size at one site.

6) Increase in NOR size at both sites.

7) Change to multiple NOR sites.

8) Change in morphology of a biarmed chromosome through a shift in centromere position.

9) Development of long acrocentric chromosomes through centromere shifts.

Fig. 11
Fig. 12

10) Presence and increase in number of subtelocentric chromosomes.

The karyotype evolution in *Salvelinus* as shown in Fig. 11 is based on the above derived character states. As pointed out previously nearly all of these states are found elsewhere in salmonids and thus subject to parallel evolution. It is not certain which states are uniquely derived within *Salvelinus* but the chromosomes with distinctive morphology such as the very long acrocentric in S. malma may have evolved only once in Salvelinus. The same type of chromosome is found elsewhere in Salmo trutta and Salmo salar, however (Hartley and Horne, 1984).

Eight taxa have been reported to have a reduced number of chromosomes. These are S. alpinus, S. confluentus, S. m. malma, S. m. kraschenínnikovi, S. m. lordi, S. kronocius, S. albus and S. m. schmidti. At least four of the above taxa are known to share the very long acrocentric chromosome: S. m. malma, S. m. kraschenínnikovi, S. m. lordi, S. m. schmidti and possibly also S. alpinus.

Three taxa share a multiple NOR condition (NORs on more than one pair of chromosomes). These are S. namaycush, S. fontinalis and S. alpinus (Phillips et al., 1988). Four taxa share a similar type of NOR acrocentric chromosome with dark staining NOR associated material in the short arms: S. l. leucomaenis, S. confluentus, S. l. pluvius, S. l. imbrius. These taxa are known to have derived states of the NOR from the primitive small NORs in centromeric position on acrocentric chromosomes. S. confluentus and S. l. leucomaenis show the most enlargement of the NORs. S. m. lordi and S. alpinus have telomeric NORs on the short arms of two large, distinctive submetacentric chromosome. S. m. miyabei has enlarged telomeric or interstitial NORs on the short arms of a subtelocentric chromosome pair.

All Pacific taxa share a distinctive large submetacentric chromosome pair with an arm ratio of about 1:1.6-1:2.4: S. confluentus, S. m. malma, S. m. kraschenínnikovi, S. m. lordi, S. l. leucomaenis, S. l.

imbrius, *S. l. pluvius*, *S. kronocius*, *S. m. schmidti* and *S. albus*.

Most of the above taxa have subtelocentric chromosomes but *S. m. lordi* lacks the distinctive medium sized subtelocentric pair found in *S. m. kraschenninnikovi*.

~~Fig.~~ Table 2

In order to compare the cladogram generated by cytogenetic characters (Fig. 11) with one constructed from morphological information, we have taken a set of morphological characters from the literature, listed their derived states within *Salvelinus* (Table 2) and illustrated the cladogram they produced (Fig. 12). Polarity was determined by consulting the outgroup taxa *Hucho perryi* and *Salmo trutta*.

There is strong concordance between the two cladograms generated by the cytogenetic and morphological characters, respectively. One problem exists with the *Salvelinus alpinus* clade which requires character 3 (presence of large fontanelles in the ethmoid cartilage) to be reversed. This character was one of several responsible for the pairing of *S. alpinus* with a group composed of *S. leucomaenis* and *S. malma* and not with *malma* itself in the phenogram of Glubokovsky and Chereshnev (1981). Ethmoid fontanelles are absent for the most part in *S. namaycush*, *S. fontinalis* and *S. alpinus* while being developed in the other *Salvelinus* taxa. The latter condition is almost certainly derived for salmonids since it is only found in *Salvelinus*. The question was asked as to whether *S. alpinus* shares any other derived states with the North American taxa *S. namaycush* and *S. fontinalis*. The latter two do share a well developed toothed crest on the vomer often found developed in S. alpinus (Cavender, 1980). The polarity of this character could not be determined although Cavender (1980) believed it was primitive because of its early appearance in the fossil record of Salvelinus.

CONCLUSIONS

Results of our chromosome studies show the NORs have considerable intraspecific variation and in a evolutionary context change at a relatively rapid rate. Thus, they are difficult to use for phylogenetic reconstruction. Instead, they seem particularly suitable at helping with problems at the subspecific level. Each subspecific taxon investigated had a different form or position of the NOR in Salvelinus malma. The taxon not investigated, S. malma malma, the northern Dolly Varden, predictably will also show a difference in the NOR chromosome. NOR information should help with the so called "Char Problem" of Salvelinus alpinus.

Several characters in the chromosome morphology such as the distinctive submetacentric and subtelocentric chromosomes suggest that the malma and leucomaenis groups shared a common ancestry. This conclusion agrees with that based on gross anatomy.

The characteristics of the NOR chromosomes tend to group S. confluentus with S. leucomaenis. The karyotype of S. confluentus lacks the derived long acrocentric chromosome that unites all members of the malma group of chars.

S. l. leucomaenis, S. l. imbrius and S. l. pluvius posses nearly identical karyotypes but show slight differences in the NOR-bearing chromosomes as might be expected in taxa of the subspecific level. The shared characters detected between imbrius and pluvius suggests that these two might be put together under one taxon S. pluvius. S. l. leucomaenis stands out from the other two by it larger NORs and possibly more accumulated rDNA.

Hucho perryi has a highly derived karyotype (2n, 62 with 42 biarmed elements) unlike any known Salvelinus species and parallels that of western trout (Parasalmo) and Pacific salmon (Oncorhynchus). It has the NOR positioned interstitially on a medium-sized metacentric chromosome pair which is a different type than any reported thus far for the Salmonidae. Not only is the chromosome number of Hucho perryi similar to that found in the Pacific salmonines Parasalmo and Oncorhynchus but the arm number of 104 also agree with this group and is quite unlike the evolutionary trend toward a lower NF found in Salvelinus. Apparently the karyotype evolution in Hucho independently followed very similar pathways to that in Oncorhynchus and Parasalmo - a fact which suggests they may belong to sister groups. On the other hand, karyotype evolution in Salvelinus at least in one case shows a trend in arm reduction through centromere shift which parallels that found in Salmo. This fact also suggests that Salmo and Salvelinus might have shared

shared a common ancestry and are thus sister groups.

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Legends to Figures

Fig. 1. Karyotype of *Hucho perryi* from an aquarium of Faculty of Pharmaceutical Sciences, Hokkaido University. 2n 62, NF 104.

Fig. 2. Karyotype of *Oncorhynchus masou* from Ichani River, Hokkaido. 2n 66, NF 102.

Fig. 3. Karyotype of *Salvelinus leucomaenis leucomaenis*, from Ichani River, Hokkaido. 2n 84, NF 100.

Fig. 4. Karyotype of *S. l. imbrius* from Takatsu River system, Shimane Pref. 2n 84, NF 100.

Fig. 5. Karyotype of *S. l. pluvius* from Akimoto Hatchery, Miyazaki Pref. 2n 84, NF 100.

Fig. 6. Karyotype of *S. confluentus* from Flathead River drainage, Montana. 2n 78, NF 100.

Fig. 7. Karyotype of *S. malma krascheninnikovi*, Ichani River, Hokkaido. 2n 82, NF 98.

Fig. 8. Karyotype of *S. m. lordi* from Juneau, Alaska. 2n 82, NF 98.

Fig. 9. Karyotype of *S. m. miyabei* from Lake Shikaribetsu, Hokkaido. 2n 82, NF 98.

Fig. 10. Different type and location of NORs on chromosomes of salmonids studied. Type A & B, *Salvelinus malma krascheninnikovi*; Type C, *S. leucomaenis imbrius*, *S. l. pluvius* and *Oncorhynchus masou*; Type D, *S. l. leucomaenis*; Type E & F, *S. m. miyabei*; Type G, *S. m. lordi*; Type H, *Hucho perryi*.

Fig. 11. Hypothetical relationships of *Salvelinus* taxa based on the cytogenetic characters listed in the text.

Fig. 12 Hypothetical relationships of *Salvelinus* taxa based on the morphological characters listed in Table 2.

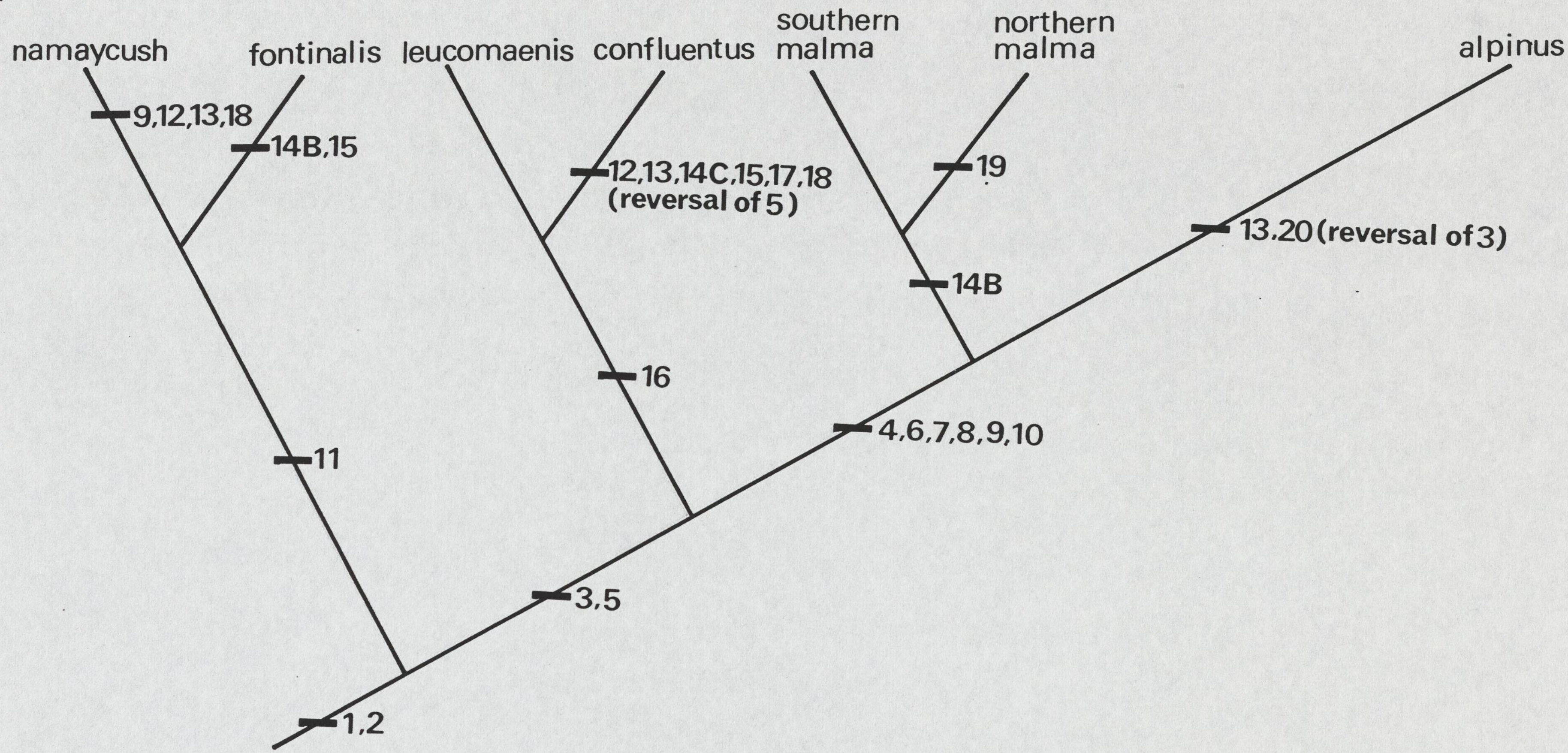
Table 1 Numbers and source of specimens used in this study for chromosome analysis.

Taxon	Locality	Numbers used
Hucho perryi	Hokkaido (Hokkaido Univ. laboratory)	13
Oncorhynchus masou	Ichani R., eastern Hokkaido	6
Salvelinus malma kraschenInnikovi	Ichani R., eastern Hokkaido	6
S. m. lordi	Second generation hatchery stock, Hokkaido, Univ., originating from Juneau, Alaska	3
S. m. miyabei	Lake Shikaribetsu, Hokkaido	6
S. leucomaenis leucomaenis	Ichani R., eastern Hokkaido	7
S. l. imbrius	Takatsu R., Shimane Pref., Honshu	13
S. l. pluvius	Akimoto Hatchery, Kyushu (planted from Toyama Pref., Honshu)	4
S. confluentus	Bear Creek tributary, Middle Fk Flathead R. Flathead Co., Montana	9

Table 2 List of morphological characters for <u>Salvelinus</u> taxa showing polarity states, character discussion is found in Cavender (1980) except where noted.

Character	Plesiomorphic State	Derived State	Reference
1. No. of infraorbitals	6	5	
2. Lateral line scale	overlapping	do not overlap	
3. Ethmoid cartilage	fontanelles absent	fontanelles present	Glubokovsky (1977)
4. Hyoid bar	elongate	short and deep	(this study)
5. Dermethmoid	elongate, narrow posterior extension	adult with wide posterior extension	
6. Gill raker teeth	marginal teeth present	marginal teeth absent	
7. Neurocranium dorsal profile	narrow	wide	(this study)
8. Branchiostegals	12-14 (one side)	10-11 (one side)	
9. Mandibular pores	7-9 (one side)	6 or 10 (one side)	
10. Mouth position	terminal	subterminal	
11. Pars jugularis	wide ossified bridge	narrow bridge	(this study)
12. Parasphenoid	with strong flexure	weak flexure	(this study)
13. Dermethmoid lateral process	present	reduced or absent	Glubokovsky (1977)
14. Maxilla	A (slightly curvature)	B (straight) C (strongly curved)	Glubokovsky & Chereshnev (1981)
15. Basibranchial	well toothed	teeth weak or absent	
16. Parr marks	appear on alevin	appear after swim up	Kimura (1974, 1976, 1977)
17. Neurocranium lateral profile	deep	depressed	
18. Sexual dimorphism	well developed	reduced	
19. Gill raker shape	tapered	tips strongly attenuated and curved	
20. Posterior margin of frontals	angled with midline	squared off	Glubokovsky & Chereshnev (1981)

Fig. 12



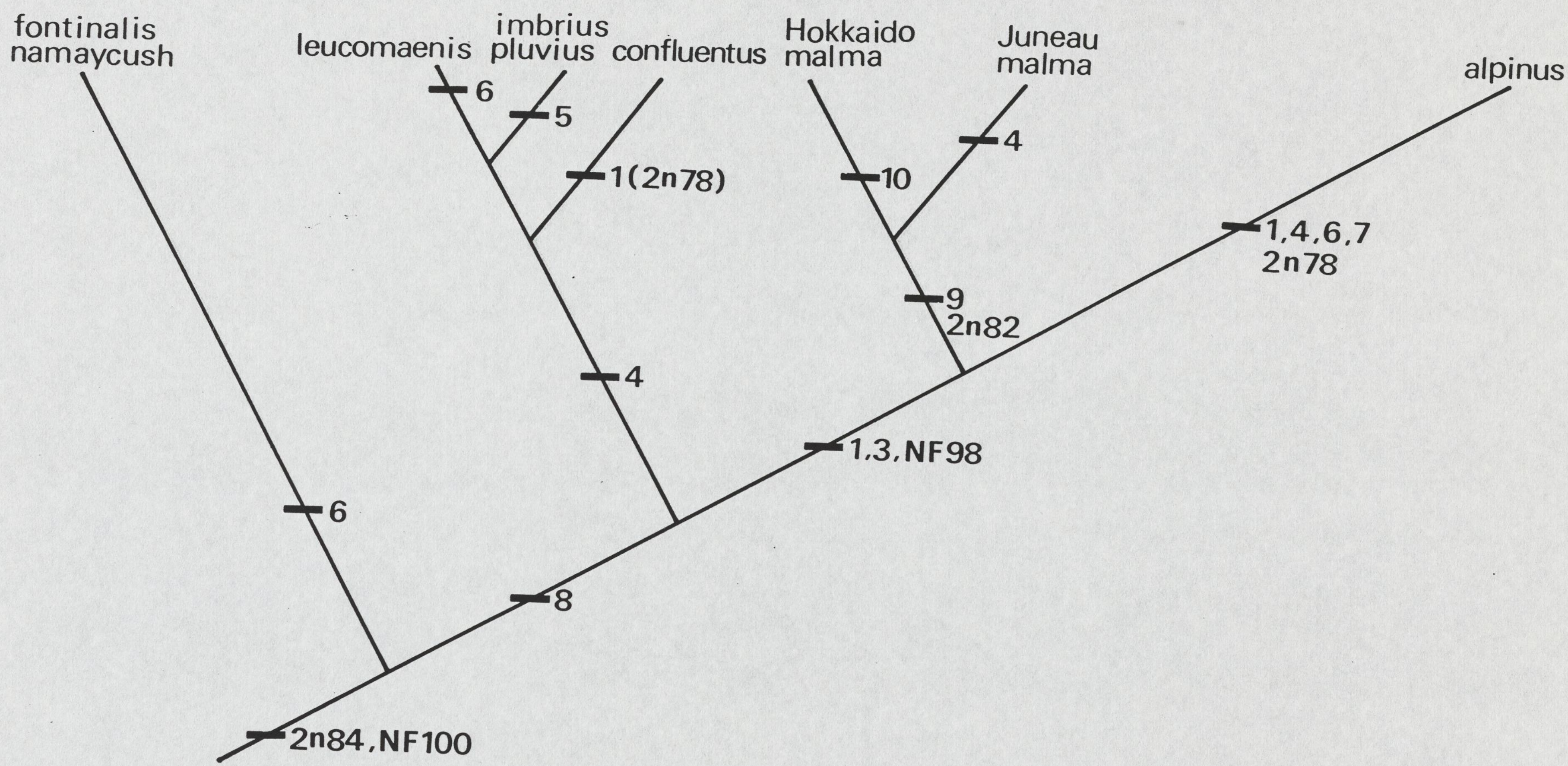
原図返却

横幅を頁いっぱいに縮少

Fig.12

Fig.12

Fig. 11

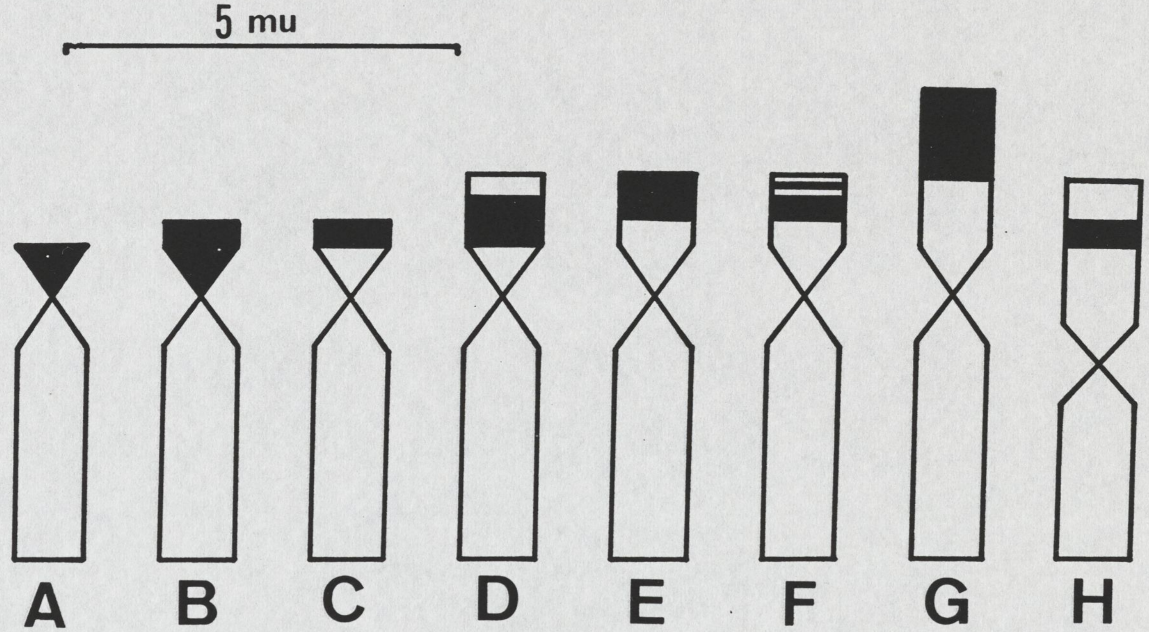


原図返却

横幅を頁いっぱいに縮小

Fig. 11

Fig. 10.



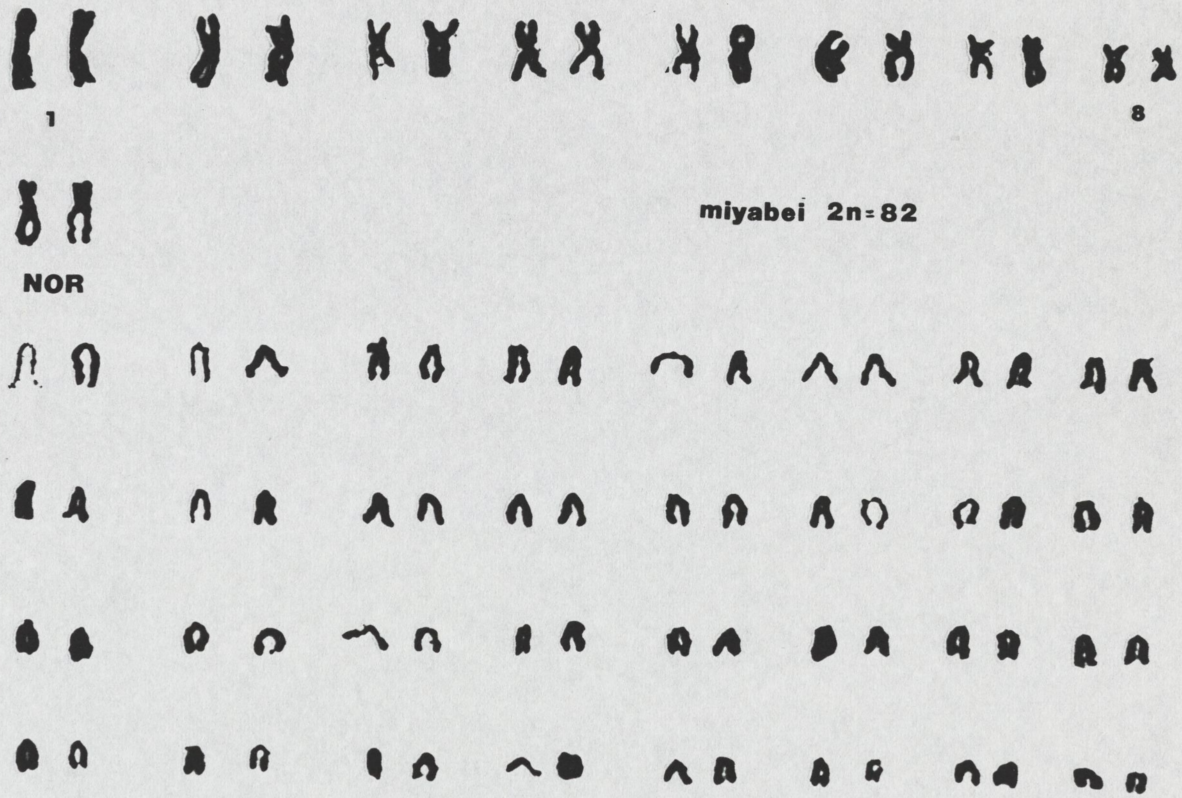
原図返却.

Fig.10

横幅を頁いっぱいに縮小

Fig.10

Fig. 9

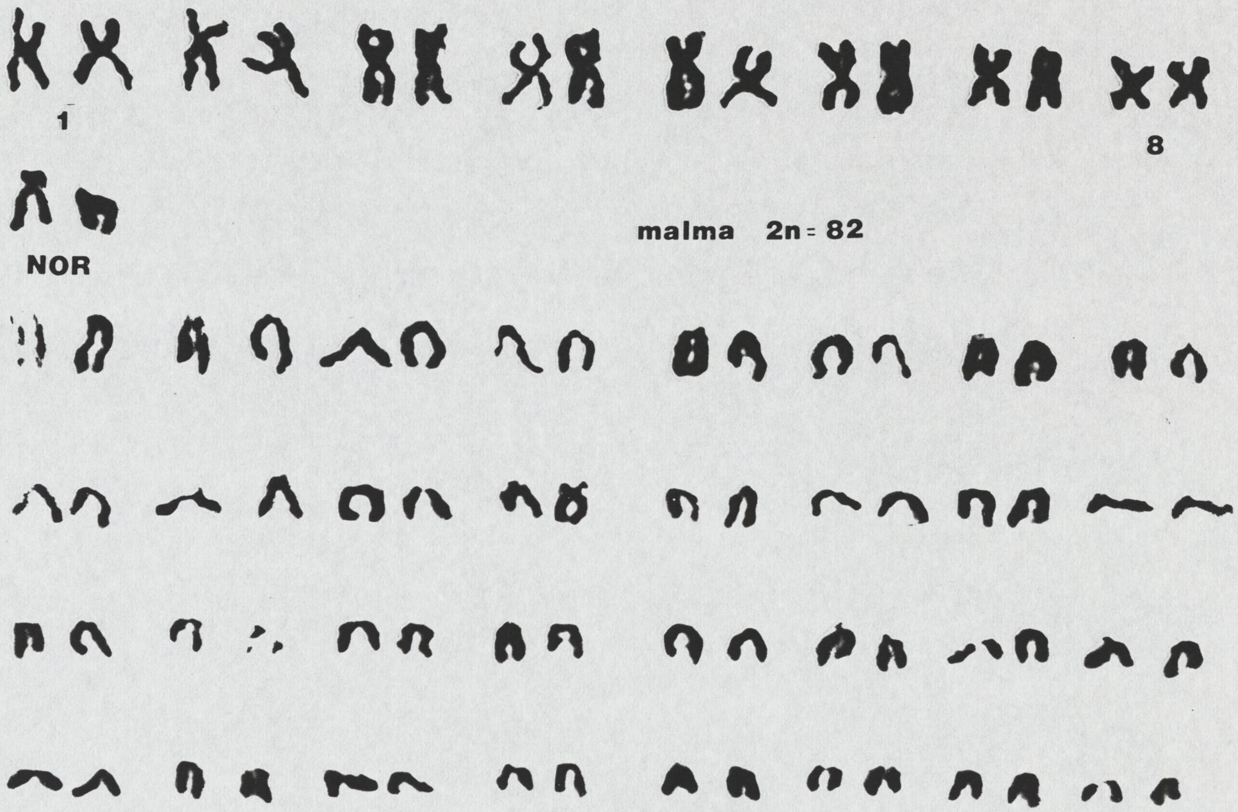


原図返却.

横幅を頁いっぱいに縮め

Fig.

Fig. 7.

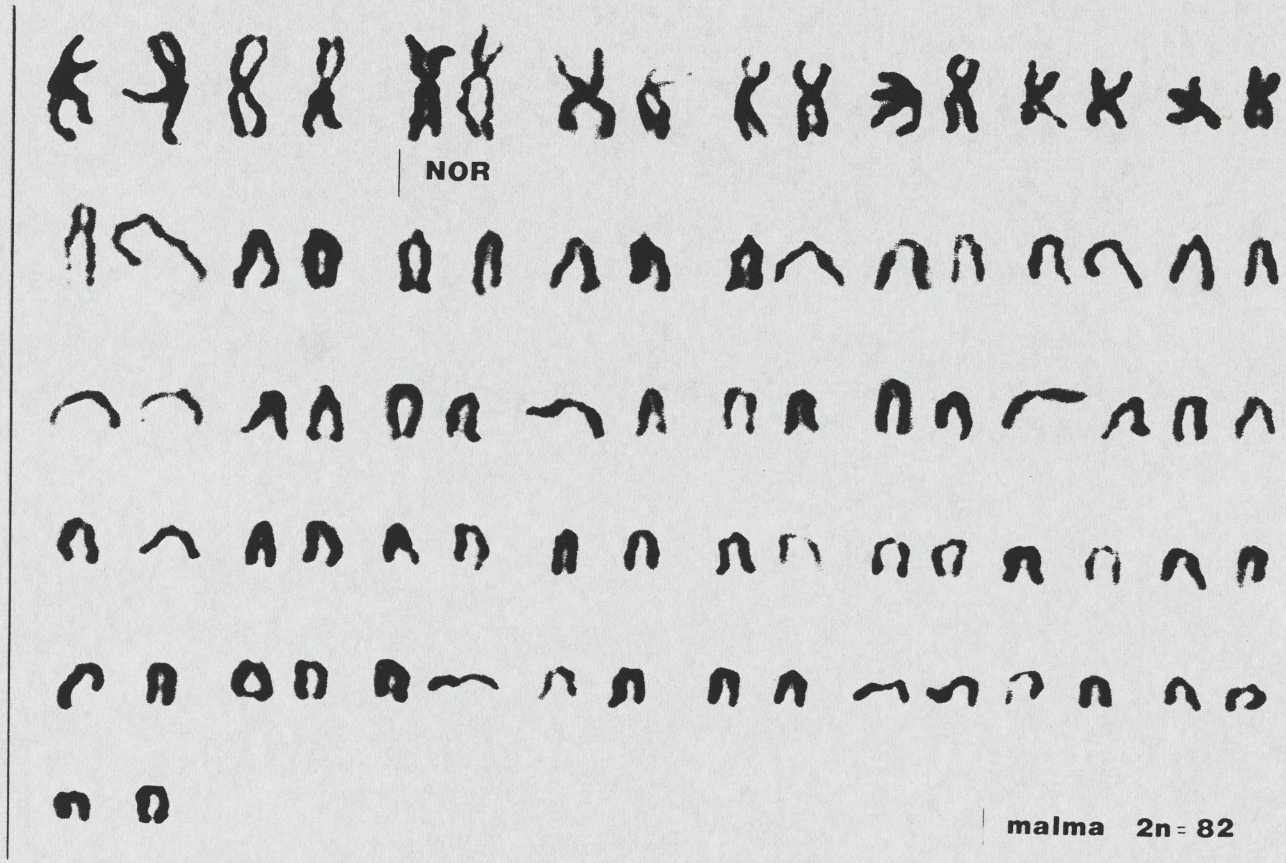


原図返却.

横幅を頁いっぱいに縮小
(Fig. 8 と組み合わせて 1頁を作って下さい)

Fig 7

Fig. 8.

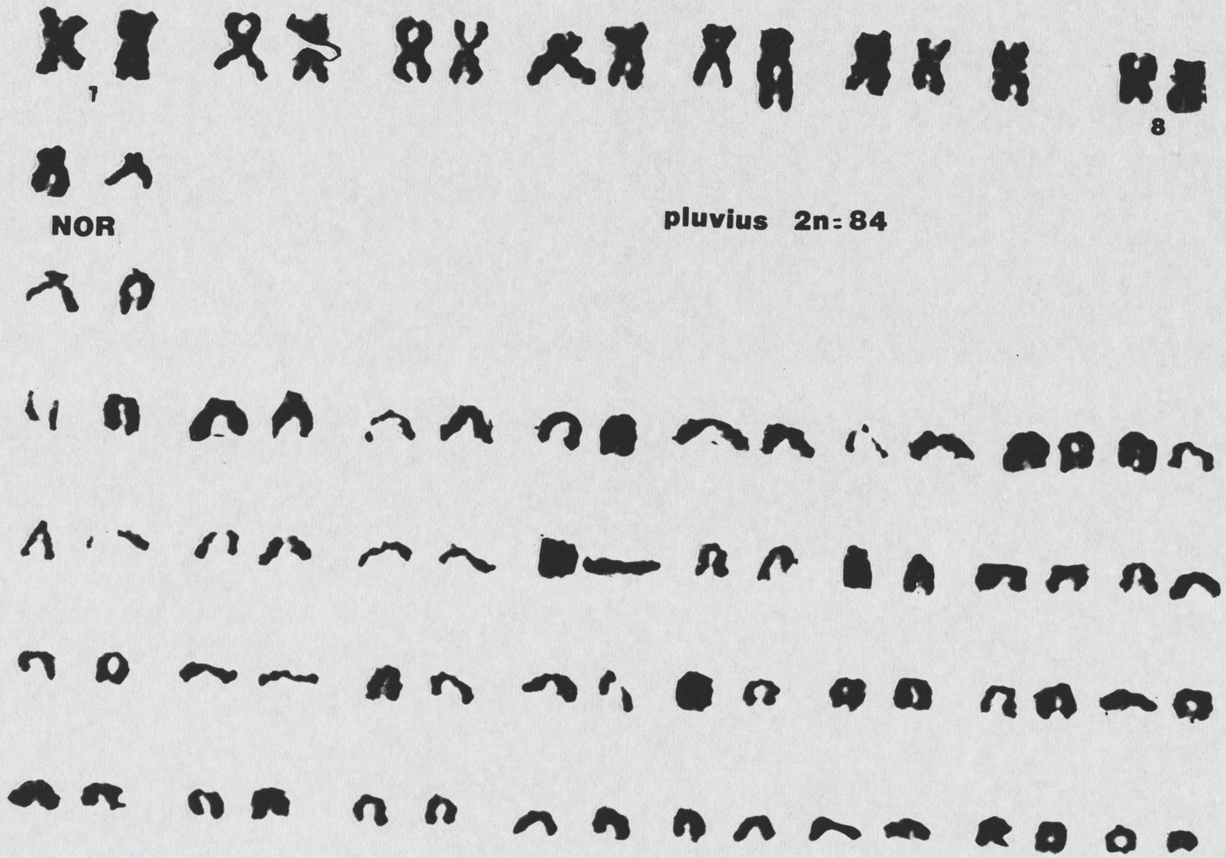


原図返却

横幅を頁いっぱいに縮少

(Fig. 7と組み合わせて
1頁を作って下さい).

Fig. 5



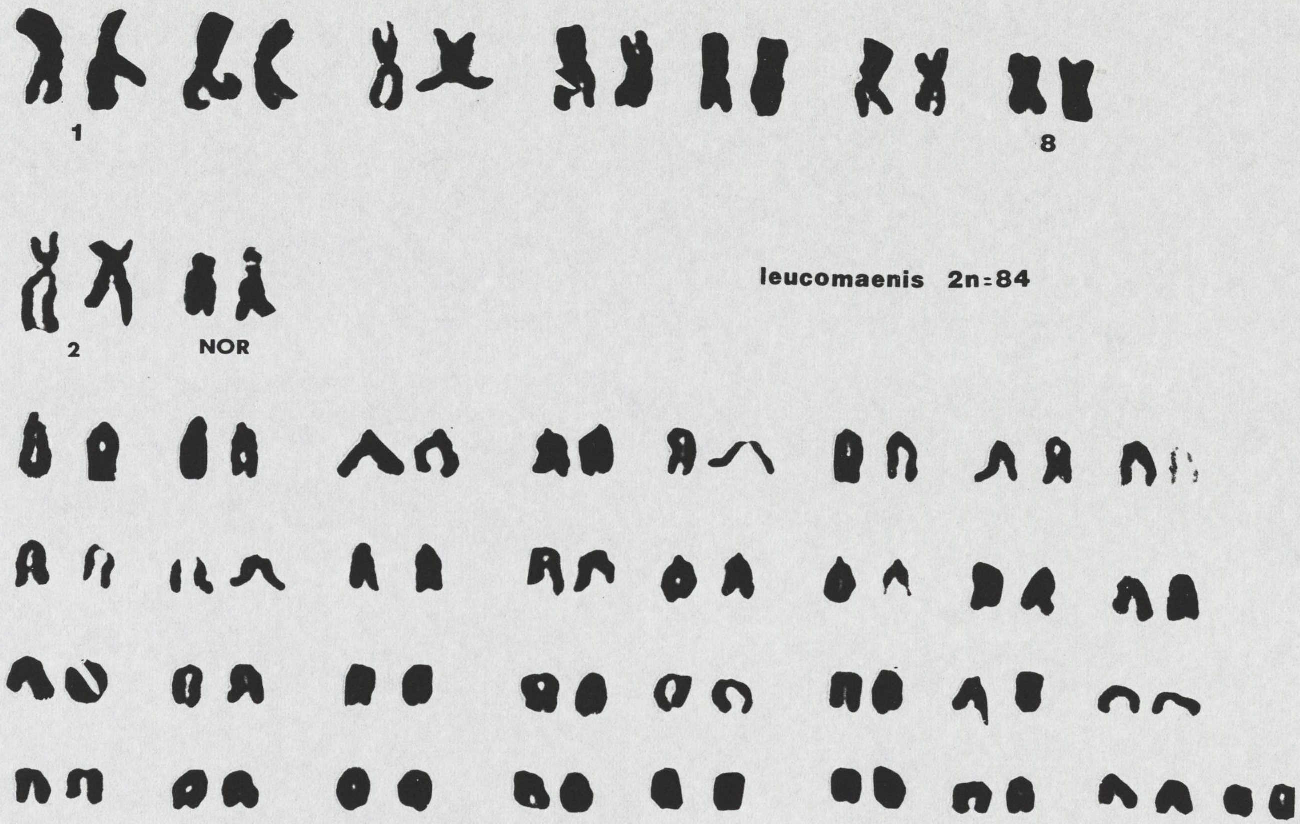
原図返却.

Fig. 5

横幅を頁いっぱいに縮小
(Fig. 6と組み合わせて1頁を作って下さい)

Fig. 5

Fig. 3



原図返却

横幅を頁いっぱいに縮少
(Fig.4と組み合わせて
1頁を作って下さい)

Fig. 4

原図返却

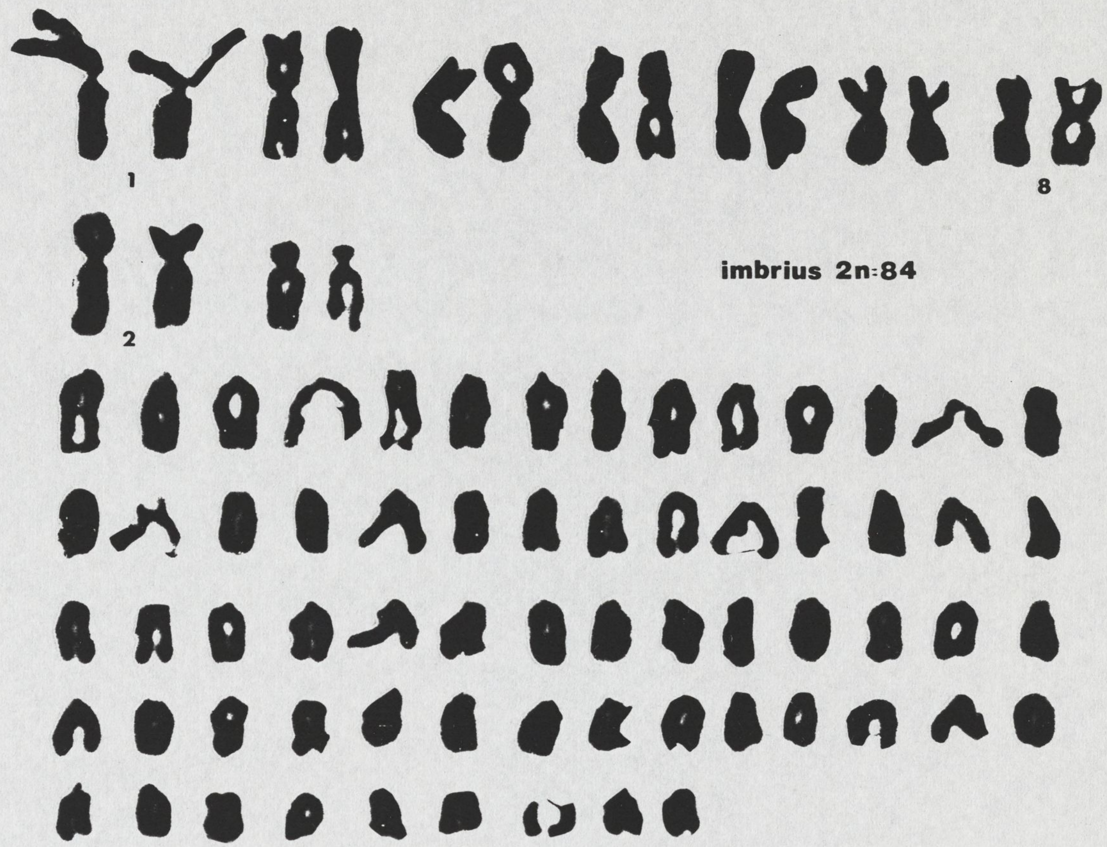
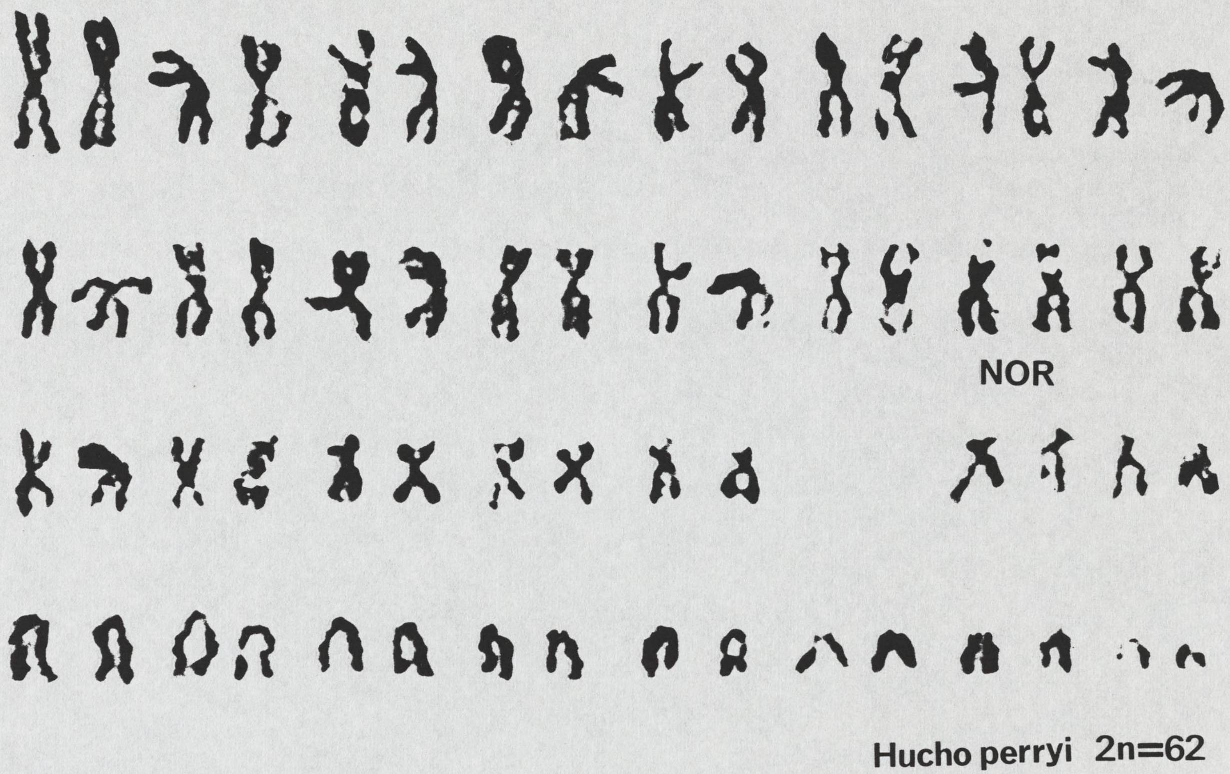


Fig. 4

横幅は頁いっぱいになるように縮少)

(Fig. 3と組み合わせて1頁を作って下さい)

Fig. 4



原図返却

Fig. 1　横幅いっぱいに縮小
(Fig. 2と組み合わせて1頁を作って下さい)

Fig. 1.

Fig. 2

原図返却（木村宛）

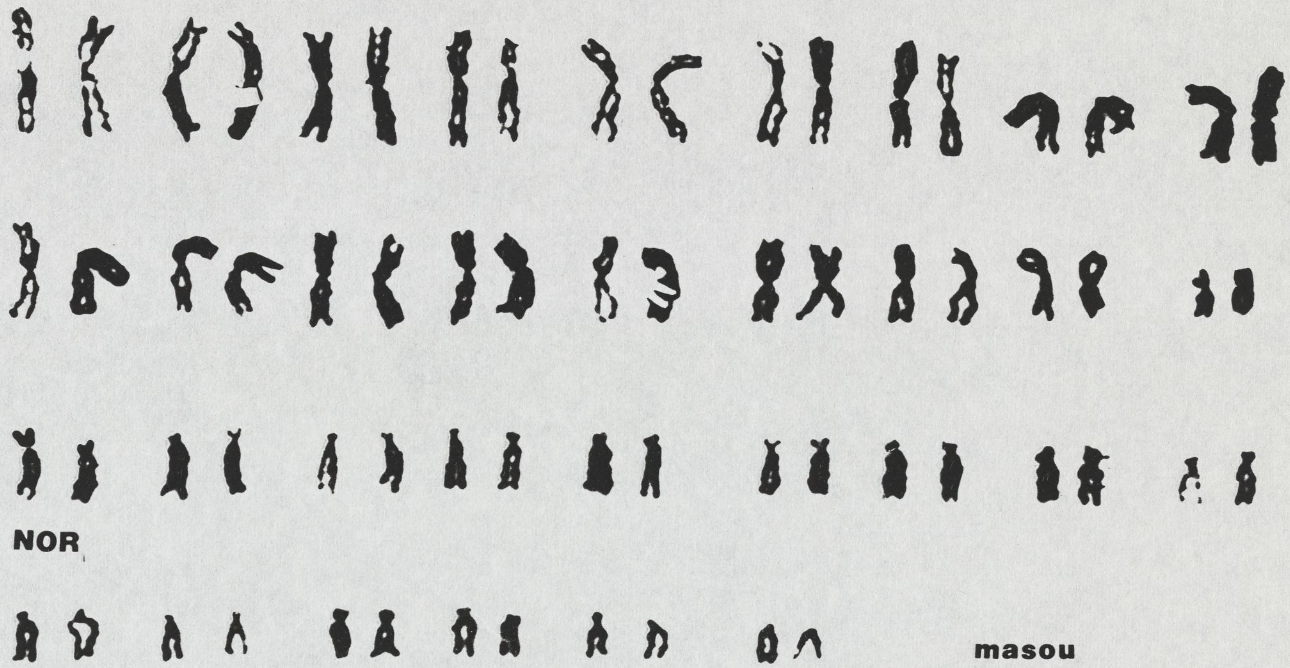


Fig. 2

横幅は頁いっぱいに入るよう縮少
（Fig. 1と組み合わせて1頁を作って下さい）

Fig 2