MORPHOLOGY AND DEVELOPMENT OF GAMETOPHYTES AND EMBRYO IN UNISEXUAL AND ANOMALOUS BISEXUAL FLOWERS OF QUAKING ASPEN

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ABSTRACT

Several populations of dioecious quaking aspen *(Populus tremuloides* Michx.) and four trees with bisexual (perfect) flowers were studied. Flower development in all catkins was acropetal, initiated in winter buds, and completed prior to anthesis. Bisexual flowers were protandrous with one-five stamens. Anther wall development in both staminate, and bisexual flowers conformed to the Dicotyledonous type. The tapetum was of the secretory type and tapetal cells remained uninucleate throughout development. Microsporogenesis was simultaneous, forming tetrahedral tetrads. Pollen was spherical and of variable sizes of which 93 and 86 percent were fertile among staminate and bisexual flowers, respectively. Pollen was shed as single binucleate grains. In both pistillate and bisexual flowers, ovules were anatropous, female gametophyte development conformed to the Polygonum type, endosperm was nuclear, and embryo development was of the Asterad type. The innermost layer of the single integument was sclerified following fertilization, and the hairs associated with seeds were of funicular origin. In five percent of the bisexual flowers, the fertilized ovules either degenerated or were arrested at the zygote stage. In 10 percent of the flowers, only one ovule in the ovary developed while the remaining ovules degenerated. Other abnormalities in the bisexual flowers included degeneration of one to several stamens.

Key words: bisexual flowers, dioecious, embryology, funicular hair, gametophytes, morphology, *Populus tremuloides*, quaking aspen, Salicaceae.

INTRODUCTION

Quaking aspen (*Populus tremuloides* Michx.) is the most widely distributed native North American tree species of commercial and ecological importance (Jones 1985). Variation in form, growth rate, and wood properties as well as clonal habit and the occasional deviation from dioecism have been observed in this species (Einspahr and Winton 1976, Mc Donough 1985). Despite the spotty western distribution in the United States, in two of the Rocky Mountain states, Utah and Colorado, the 404,858 ha (one million ac) of aspen forest comprise more than 25 percent of all commercial forests (Jones 1985). In Montana the 103,239 ha (255,000 ac) of aspen are scattered among the middle-elevation conifer forests and at the lower forest boundaries. In addition to its natural abundance, aspen also is widely cultivated as an ornamental tree because of its foliage and greenish-white bark.

Quaking aspen, included in the Section *Leuce* of the family Salicaceae, exhibits significant phenotypic variation throughout its transcontinental distribution. Several authors have subdivided the species into four species and 13 varieties or forms (Barnes 1969, Beetle 1974). Since much of the total morphological variation within the whole complex may be found in various combinations within single locales, Little (1979) recognizes this taxon to be a single heterogeneous species without subspecific taxa.

Quaking aspen is a deciduous dioecious tree, 6-30 m tall, that may sexually reproduce by 10-20 years of age. Seeds have a short period of viability in which proper moisture and substratum are essential for successful germination (McDonough 1985). However, vegetative propagation through vigorous production of root suckers results in single clones that sometimes cover 40.48 ha (100 ac) or more (Grant 1993). All trees in a clone are pistillate or staminate. However, some perfect flowers mixed with staminate or pistillate flowers and trees with both staminate and pistillate flowers have occasionally been observed in some clones (Erlanson and Hermann 1927, Santamour 1956, Pauley and Mennel 1957, Lester 1963, Strain 1964, Schriener 1974).

Breakdown from dioecy in quaking aspen has generated considerable interest. Whereas a majority of previous studies have focused on description of deviations, some reports described their distribution within the tree and inflorescence. Clones, which alternate between staminate, and pistillate flowers in different years or show combinations of perfect, staminate and pistillate flowers within or between inflorescences on the same tree, also have been reported (Graham et al 1963, Einspahr and Winton 1976). According to Pauley and Mennel (1957) the staminate-pistillate ratio among clones in a given locality may vary considerably ranging from 1:1 to 3:1 or more. Einspahr and Winton (1976) suggested that selfing of bisexual aspen trees may become a useful breeding tool for tree improvement programs emphasizing height and diameter growth and such wood properties as specific gravity and fiber length. Lester (1963) suggested that establishment of distributional patterns within trees and/or within inflorescences could form a basis for speculation and further experimentation on possible genetic, physiological, or environmental influences affecting sex expression.

Although the literature is extensive on various ecological and physiological aspects of the genus *Populus*, embryological studies are far from adequate. Of 35 species, only some aspects of embryology and pollen morphology of a few species have been studied (Graf 1921, Erlanson and Herman 1927, Nagaraj 1952, Kimura 1952, 1955, Gramuglio 1962, Campo 1963, Fechner 1976, Zhu and Li 1989, Palacios-Chavez et al. 1990). No detailed embryological literature is available on dioecious quaking aspen and none exists on bisexual forms. Considering this inadequacy and the variability of deviations from dioecisim, the present study attempts to furnish more comprehensive information on morphology and embryology of quaking aspen in general and the bisexual aspens in particular. It involves several populations of dioecious quaking aspen from Montana, Colorado, Idaho, and Utah and four trees bearing catkins with bisexual flowers. These bisexual trees were discovered by the author among the transplants from naturally-propagated stands near Red Lodge, Montana.

MATERIALS AND METHODS

The material used in the present study consisted of six cohorts that included materials harvested from wild as well as cultivated trees. Individual cohorts are listed below:

1. In spring 1985, 22 trees were transplanted from two wild populations found near Red Lodge, Montana to the west end of the Science building on the campus of Montana State University-Billings (MSU-B). Nineteen of these trees, all approximately 15 years old at the time of this study (14 pistillate, one staminate and four bearing catkins with all bisexual flowers), were used.

2. Six staminate trees (all about 10 years old) were originally obtained from a local nursery and grew at the north side of the science building on the campus of MSU-B.

3. Eight pistillate and eight staminate trees near Red Lodge, Montana; all of undetermined ages were taken from wild populations.

4. Two staminate and two pistillate

trees near Ashton, Idaho were all from wild populations of unknown age.

5. Six pistillate and four staminate cultivated trees near Fort Collins, Colorado were all of unknown age.

6. One staminate and one pistillate tree cultivated in Salt Lake City, Utah both were of unknown age.

I examined 100 catkins from each tree/year for eight consecutive years to determine if the tree was staminate, pistillate, or bore catkins with perfect flowers. Voucher specimens are deposited in the Montana State University-Billings (MSU-B) herbarium.

To determine pollen size, volume, and pollen viability, mature anthers from the staminate flowers of the male trees growing on campus and near Red Lodge, Montana, and from bisexual flowers were randomly collected, lightly crushed and soaked in 1 percent acetocarmine for 8-12 hours. I measured and scanned 2000 pollen grains from each sample to determine fertility. Those appearing dense and darkly stained were considered fertile (Sharma and Sharma 1980).

For embryological studies, 50 reproductive buds, 100 young, and 100 old catkins were collected from all trees in their native habitats or in cultivation at MSU-B campus and fixed in FAA (formalin: acetic acid: 50% ethanol, 1:1:8 v/v) for 24 hours and stored in 70 percent ethanol. Customary methods of dehydration, infiltration and paraffin embedding followed Johansen (1940). Sections 5-15 μm thick were cut and stained in Heidenhain's iron alum hematoxylin with eosin in clove oil as a counter stain and mounted in klearmount. Camera lucida drawings of the developmental stages were made. Fresh ovules and seeds were dissected and stained with cotton blue to study the ovule vasculature and the funicular hair. Bracts were examined from 100 randomly collected reproductive buds and 100 catkins from each tree of the sample growing on campus. I also measured 100 mature catkins from those same trees to obtain average catkin length.

RESULTS

Overview of Flowering Pattern and Morphology of Catkin

Seven of 22 transplanted trees flowered in March 1992 of which two produced catkins with all perfect flowers. Four trees produced all pistillate flowers, and one produced all staminate flowers. All 22 trees flowered in March 1993. The pattern of flowering for seven trees that flowered the previous year remained unchanged. Of the remaining 15, two produced all perfect flowers, and the other 13 produced only pistillate flowers. Three of the trees with all pistillate flowers did not survive to flower in 1994. The pattern of flowering in the remaining 19 trees has been consistent over the last eight years. Thirteen pistillate trees in this population grew between and near the bottom of two very shallow slopes. The four trees that bore catkins with all perfect flowers, one staminate tree, and one pistillate tree were slightly higher on the slope. Two of the trees that bore catkins with perfect flower appeared unhealthy after May 1998 and have produced 17 root suckers since. All other trees examined from Colorado, Idaho, Montana, and Utah were dioecious.

The 13 pistillate trees growing down the shallow slope were about 8 m tall; all others were 4-5 m. The short, straight trunks of all three types of trees bore irregularly bent limbs that made a narrow dome-like crown. The trees, with seemingly no differences in leaf morphology, produced petal-less flowers in catkins of variable length. Stamens and pistils were inserted on a saucer-shaped disc attached to the stalk by a short pedicel and subtended by a brown, hairy cleft bract. In all three types of flowers, development, which begins in the winter buds and is completed prior to anthesis in early spring, was acropetal.

Staminate catkins were 2-3 cm long (2.53±0.51) with 80-90 flowers/catkin. The seven different shaped brown hairy bracts were three- to six-lobed (3- to 4-lobed most common), randomly distributed in the catkin, each with lobes of variable number and length. Number of stamens ranged from six to 16 and had short filaments of equal length with bright red anthers (Plate 1, Figs. 1-3 and Plate 2, Fig. 5).

Pistillate catkins were 4-6 cm long (5.2 ± 1) with 100-120 flowers/catkin. The eight different shaped, randomly distributed brown hairy bracts were three- to eight-lobed (five to eight lobes were most common), each with lobes of variable number and length. Each flower showed two carpels and two bright red stigmas that were deeply cleft into two or more slender lobes (Plate 1, Fig. 4, Plate 2, Figs. 6-8). The fruits were 4-6 mm long with comose seeds.

Catkins bearing perfect flowers were 3-6 cm long (4.91 ± 0.84), each with 90-100 all perfect flowers. The five types of brown

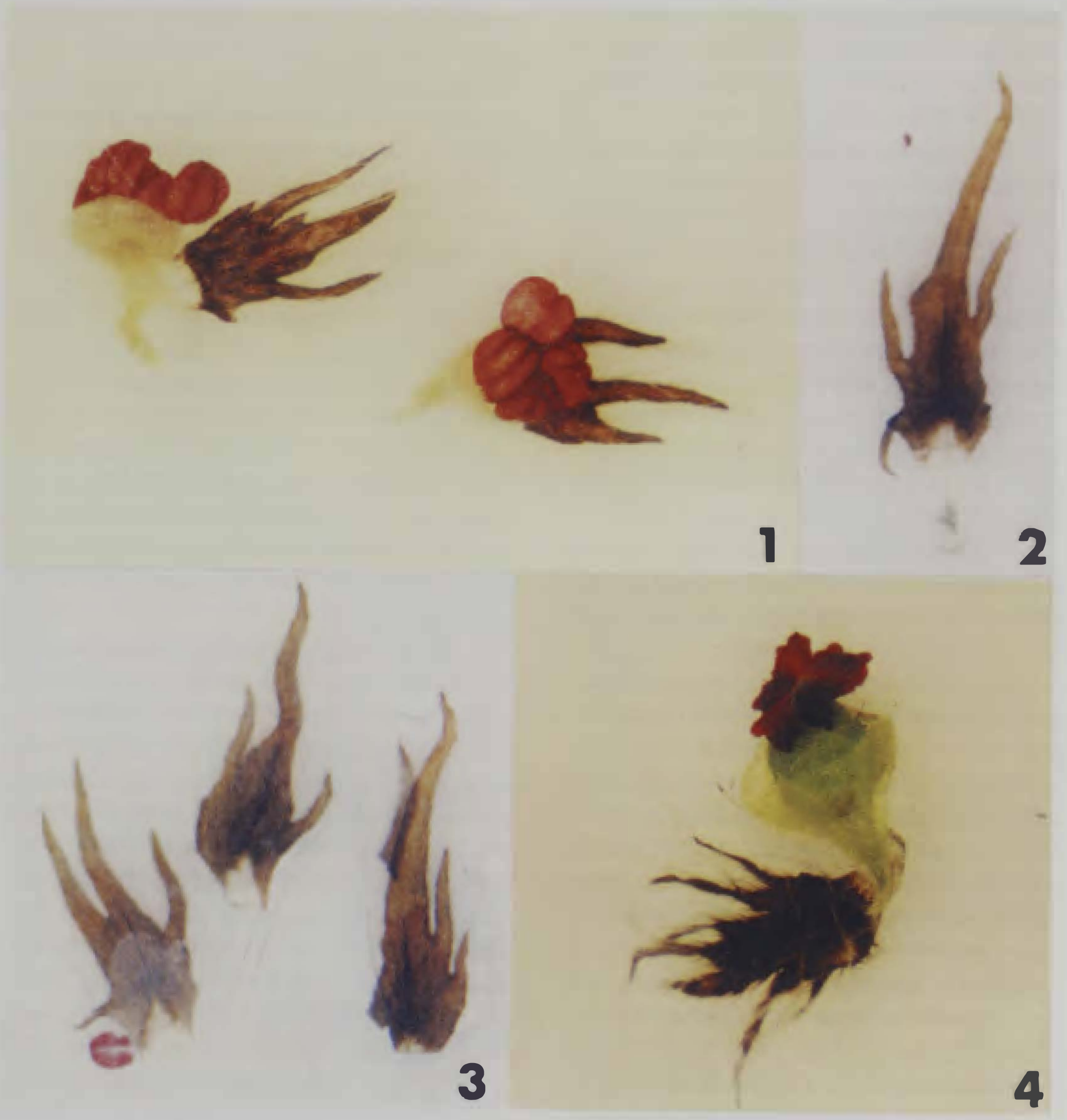

Plate 1 Figs. 1-4. Photographs of flowers and bracts of quaking aspen (*Populus tremuloides* Michx.) from pistillate and staminate inflorescences. All figures X25.

Figure 1. Male flowers showing stamens and two types of bracts.

Figures 2-3. Male bracts.

Figure 4. Female flower with bract.


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Plate 2 Figures 5-8. Photographs of flowers and bracts of quaking aspen (*Populus tremuloides* Michx.) from pistillate and staminate inflorescences.

Figure 5. Male flower with five-lobed bract X45.

Figure 6. Female flower with six-lobed bract X60.

Figure 7. Female flower with eight-lobed bract X45.

Figure 8. Female flower and three, four, five and six-lobed bracts X20.

hairy bracts of perfect flowers bore fewer lobes than those of unisexual flowers. These include four-lobed with two long and two short lobes (Plate 3, Fig. 9), wide and two lobed with one long and one short lobe (Plate 3, Fig. 10), narrow and two lobed (Plate 3, Fig. 11), narrow and entire (Plate 3, Fig. 12), and three lobed with the middle lobe longest (Plate 3, Fig. 13). The ones with three lobes were more frequent, and all types were distributed randomly in the catkin. The number of stamens ranged between 1-5 (Plate 3, Figs. 9-13, and Plate 4, Figs. 14-17), with two being most frequent (one on either side of the ovary). Several variations in the condition of the stamens were observed. The most frequently observed condition was flowers with two healthy stamens (Fig. 14). Several flowers showed two stamens, one healthy and one degenerated; in several others, three or more stamens were observed with one, two, three, or all degenerated ones (Plate 4, Figs. 15-17). In one percent of the catkins, flowers toward the apex had only one stamen, whereas in all other catkins flowers were randomly distributed. Filaments of all stamens were of equal length each with a bright red anther. The ovary was two-carpellate with a short stout style and two deeply cleft bright red stigmas. Fruits were 4-6 mm long and lance-ovate with comose seeds.

Microsporogenesis and Development of Male Gametophytes

Perfect flowers were protandrous. No significant differences were found in the development of the anther wall, microsporogenesis, and the male gametophyte between staminate and perfect flowers. Results for both types of flowers were therefore described together. Both staminate and perfect flowers showed anthers with four lobes. A plate of two to three archesporial cells developed in each lobe. Periclinal divisions in the archesporial cells resulted in an outer layer of primary parietal cells and an inner layer of primary sporogenous cells. The primary parietal cells divided periclinally to form secondary parietal cells. The innermost of these layers became the tapetum while the cells of the outer one divided to form one middle layer toward the tapetum and a layer of endothecial cells toward the epidermis (Plate 5, Fig. 18). The development of the anther wall, therefore corresponded to the Dicotyledonous type (Davis 1966). A young anther wall consisted of an epidermis, an endothecium, one middle layer, and the tapetum surrounding a massive sporogenous tissue (Plate 5, Fig. 18) The tapetum was of the secretory type, and its cells remained uninucleate throughout development. The endothecial cells enlarged considerably and developed bands of fibrillar thickenings at the time pollen grains were formed (Plate 5, Fig. 22). The tapetum and middle layer also began to degenerate about the same time. Primary sporogenous cells divided and enlarged considerably before they transformed into microsporocytes.

Meioisis was accompanied by simultaneous cytokinesis and the resulting microspore tetrads were tetrahedral. Each microspore developed a thick reticulate exine (Plate 5, Fig. 20). The pollen grains

Table 1. Variation in diameter ,volume, percent fertile and average size of pollen grains in quaking aspen (*Populus tremuloides* Michx.)

Diameter	**Male**	**Bisexual**
20μ - 23μ	4	7
24μ - 26μ	461	261
27μ - 29μ	1211	12
30μ - 34μ	271	97
35μ - 39μ	16	1421
40μ - 50μ	37	202
Total	**2000**	**2000**
Average	**28μ**	**40μ**
Volume	**$820\mu^2$**	**$1675\mu^2$**
% Fertile	**93**	**86**

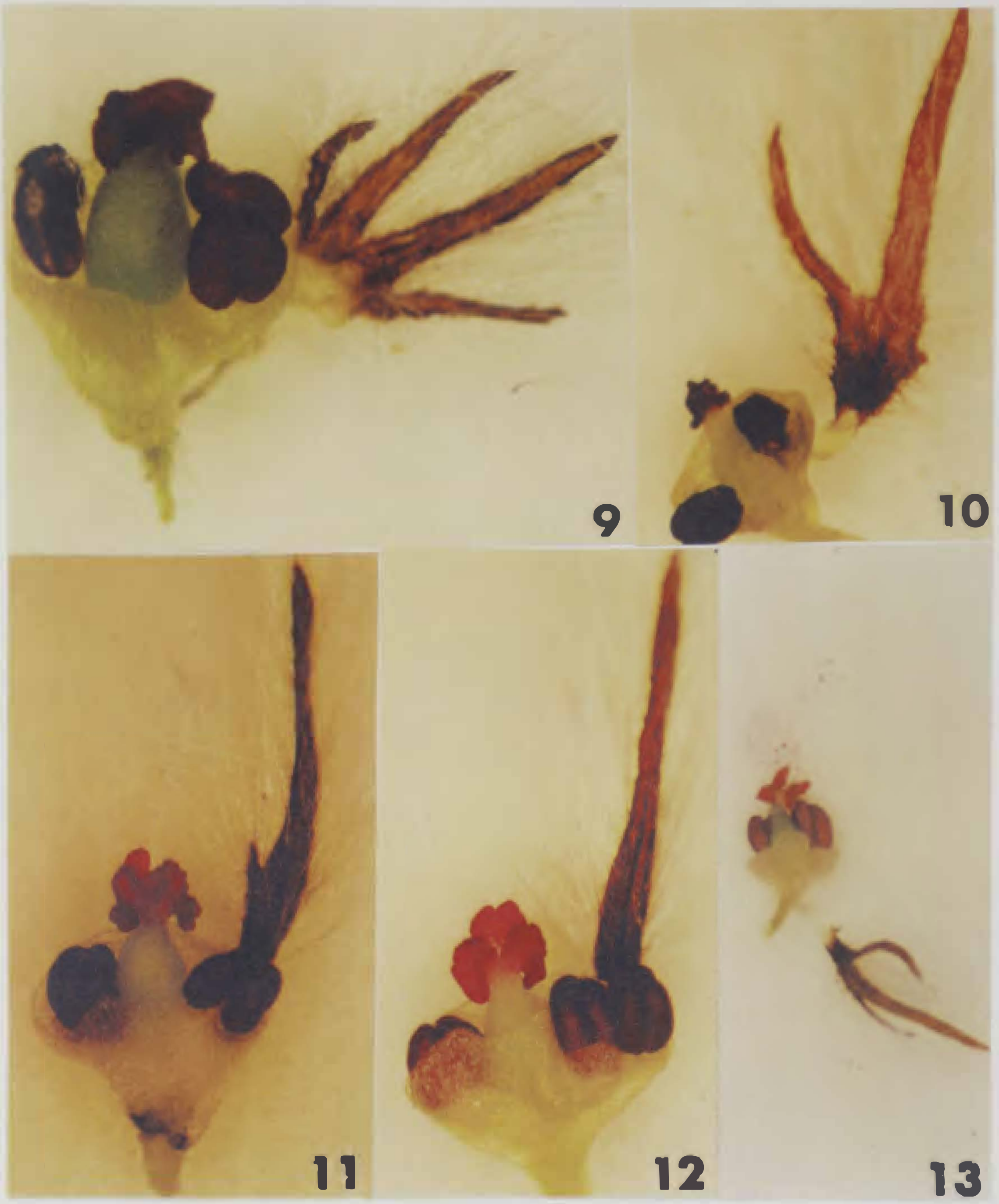

Plate 3 Figures 9-13. Photographs of bisexual flowers and bracts of quaking aspen (*Populus tremuloides* Michx.)

Figure 9. Bisexual flower showing pistil, two stamens (one of the anthers dehiscing) and four-lobed bract X50.

Figure 10. Bisexual flower showing pistil, two stamens (one degenerating) and two-lobed bract X30.

Figure 11. Bisexual flower showing pistil, two stamens and two-lobed bract X45.

Figure 12. Bisexual flower showing pistil, three stamens and unlobed bract X50.

Figure 13. Bisexual flower showing pistil, two stamens and three-lobed bract X15.

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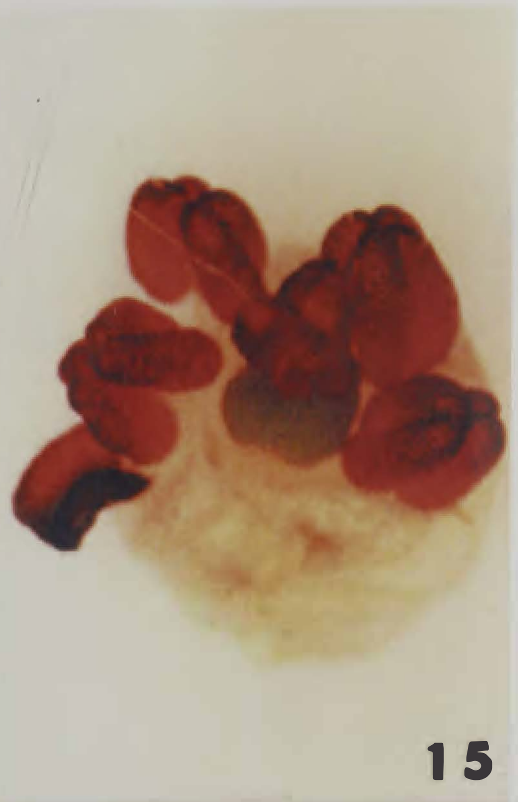

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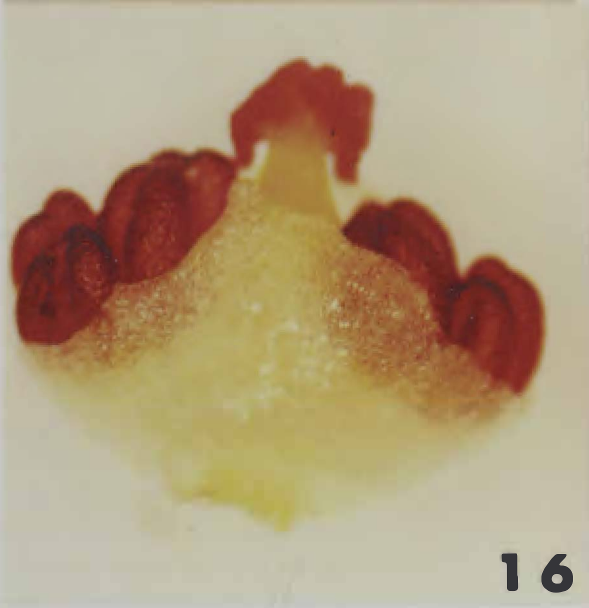

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Plate 4 Figures 14-17. Photographs of bisexual flowers of quaking aspen (*Populus tremuloides* Michx.)

Figure 14. Bisexual flower showing pistil and one stamen X80.
Figure 15. Bisexual flower showing pistil and five stamens X80.
Figure 16. Bisexual flower showing pistil and four stamens X90.
Figure 17. Bisexual flower showing pistil and two stamens X65.

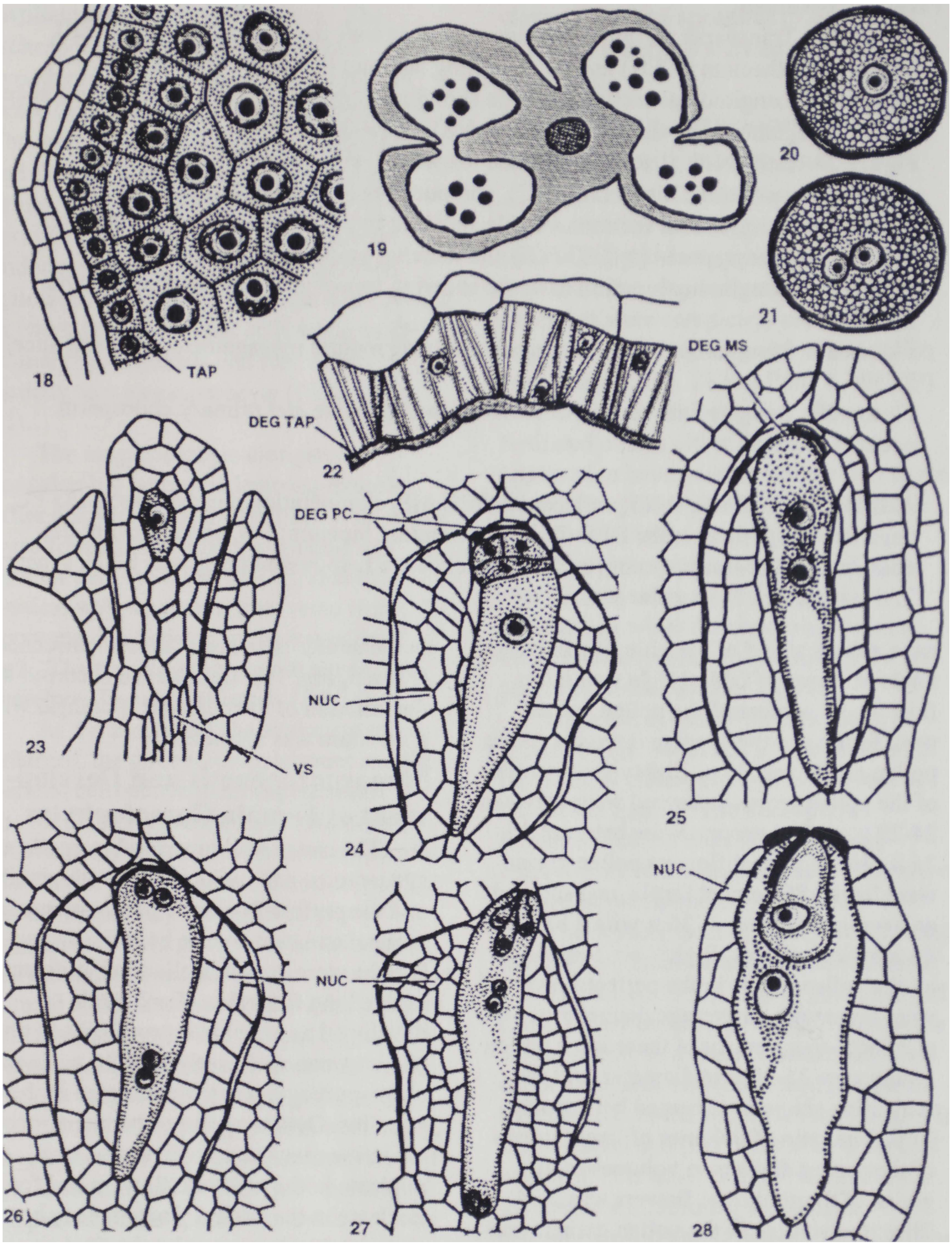

Plate 5 Figures 18-28. Microsporogenesis, development of male gametophyte (Figs. 18-22), megapsorogenesis and development of megagametophyte in bisexual quaking aspen (*Populus tremuloides* Michx.)

Figure 18. Transverse section of portion of anther showing epidermis, endothecium, single middle layer, tapetum and sporogenous tissue (= ST) . X2500.

Figure 19. Transverse section of mature anther showing coalescence of adjacent locules. X160.

Figure 20. Uninucleate pollen grain. X2500.

Figure 21. Two-nucleate pollen grain. X2500.

Plate 5, Continued.

Figure 22. Transverse section of portion of anther wall showing epidermis (= EP), fibrillar endothecium (= EN) and degenerating tapetum (= DEG TAP). X1000.

Figure 23. Longitudinal section of ovule showing megasporocyte (= MSC), parietal cells (= PC), integument and vascular strand (= VS). X1600.

Figure 24. Longitudinal section of ovule showing T-shaped megaspore tetrad, degenerating parietal cells (= DEG PC), and nucellus (= NUC). X1200.

Figure 25. Longitudinal section of ovule showing two-nucleate megagametophyte, degenerating megaspores (= DEG MS) and nucellar epidermis. X1200.

Figure 26. Longitudinal section of ovule showing four-nucleate megagametophyte, X1200.

Figure 27. Longitudinal section of ovule showing mature megagametophyte and nucellus X750.

Figure 28. Longitudinal section of ovule showing zygote and primary endosperm nucleus. X1200.

ABBREVIATIONS: ANT, antipodals; DEG MS, degenerating megaspores; DEG PC, degenerating parietal cells; DEG TAP, degenerating tapetum; EN, endothecium; EP, epidermis; MSC, megasporocyte; NUC, nucellus; ST, sporogenous tissue; SYN, synergids; TAP, tapetum; VS, vascular strand.

were spherical and of variable size in both kinds of flowers (Table 1). In staminate flowers, 93 percent of the pollen grains were fertile and the average diameter of the pollen grains was 28 μ. Eighty-four percent of the pollen grains measured were between 24-29 μ, and 14 percent were between 30-34 μ. In the perfect flowers pollen grains were larger, 86 percent fertile, and showed an average diameter of 36 μ with a bimodal distribution of sizes. Eighty-six percent of all the pollen grains in the perfect flowers were large with an average diameter of 38 μ. Ninety-four percent of these large pollen grains were 35-50 μ in diameter, and the remaining six percent ranged between 30-35 μ. The ratio of volumes of larger grains of the perfect flowers to volumes of all grains of the staminate flowers was 2.04. Thus, the volume of the pollen grains from the perfect flowers tended to be twice that of the volume of pollen grains from the staminate flowers. Fourteen percent of the grains that were small (20-29 μ), in the perfect flowers (mean diameter of 25 μ) also showed variation in size. Of the smaller ones 93.5 percent were 24-26 μ, 4 percent were 27-29 μ, and 2.5 percent were 20-23 μ. In both they were shed at the two-celled stage (Plate 5, Fig. 21). Adjacent sporangia in the anther coalesced before dehiscence (Plate 5, Fig. 19). Dehiscence occurred at the junction of the adjacent sporangia where a stomium was organized.

Megasporogenesis and Development of Female Gametophytes

The ovary was superior, bicarpellate, syncarpous, and unilocular in both pistillate and the perfect flowers. Placentation was parietal and each locule bore six ovules. Ovules were crassinucellar and unitegmic with a long funiculus. Each ovule developed as a globular protuberance on the placenta and was nearly straight when the megasporocyte was differentiated in the nucellus. Ovules appeared hemianatropous when the megagametophyte was two-nucleate in the pistillate flowers and four-nucleate in the perfect flowers; they became completely anatropous by the time an eight-nucleate megagametophyte formed. The integument was differentiated at about the same time as the archesporium in the nucellar dome. It was two-three cells thick and remained so throughout development. The single vascular strand stopped at the base of the nucellus (Plate 5, Fig. 23).

Megasporogensis and the development of megagametophyte was similar in both pistillate and the perfect flowers. The archesporium was single celled and hypodermal. It was recognizable by its dense cytoplasm and conspicuous nucleus. The archesporial cell enlarged considerably and divided periclinally to give rise to a primary parietal cell and a primary sporogenous cell. The primary parietal cell underwent one more periclinal division followed by anticlinal divisions and resulted in two layers of parietal cells whereas the primary sporogenous cell functioned directly as a megasporocyte (Plate 5, Fig. 23).

The megasporocyte elongated considerably before undergoing meiosis. After completion of the first division, dyads were formed. The upper dyad cell was smaller than the lower and divided by a vertical wall whereas a transverse wall accompanied division in the lower dyad cell. The resulting megaspore tetrad was, therefore, T-shaped (Plate 5, Fig. 24).

As the functional chalazal megaspore enlarged, the three micropylar ones and the parietal cells above them degenerated. By the time a two-nucleate megagametophyte was formed, cells of the nucellar epidermis also began to degenerate (Plate 5, Fig. 25). As the developing megagametophyte continued to enlarge toward the micropyle, the remaining nucellar cells on its either side formed a characteristic border simulating an inner integument (Plate 5, Figs. 26 and 27). These lateral nucellar cells persisted until the later stages of embryogeny (Plate 6, Figs. 29,30,36, and 38). Within each ovary, development of the megagametophytes was synchronous in all ovules. In 10 percent of the perfect flowers, only one ovule proceeded with further development while the remaining degenerated.

Mitotic divisions (Plate 5, Figs 25 and 26) in the functional megaspore resulted in an eight-nucleate megagametophyte (Plate 5, Figs. 27) and its development conformed to the Polygonum type (Maheshwari, 1950). A mature megagametophyte was eight-nucleate and consisted of an egg apparatus at the micropylar end, three antipodal cells at the chalazal end and two polar nuclei closer to the egg apparatus (Plate 5, Fig. 27). The synergids showed a filiform apparatus.

Fertilization, Development of Endosperm, Embryo and Seed

Before fertilization, the two polar nuclei fused to form a secondary nucleus and the antipodals degenerated. Fertilization was porogamous. Both synergids were completely crushed and degenerated during the entry of the pollen tube (Plate 5, Fig. 28 and Plate 6, Fig. 29). In five percent of the perfect flowers, fertilized ovules either degenerated or appeared to have been arrested at the zygote stage. The primary endosperm nucleus in those ovules had not undergone any divisions either. Occasionally, only one ovule in the ovary developed past fertilization while the remaining ovules degenerated. No such abnormalities were observed in the pistillate flowers.

Endosperm was of the Nuclear type (Plate 6, Figs. 30 and 31). The division of the primary endosperm nucleus preceded that of the zygote. The endosperm remained nuclear until later stages of embryogeny and eventually cell walls were formed between nuclei.

The development of embryo conformed to the Polygonum variation of the Asterad type (Johansen 1950). The zygote enlarged considerably before it divided. A transverse wall resulting in a short terminal cell (*ca*) and a long basal cell (*cb*) accompanied the first division of the zygote (Plate 6, Fig. 32). The terminal cell (*ca*) divided by a vertical wall while the basal cell (*cb*) divided by a transverse wall forming a T-shaped proembryonal tetrad (Plate 6, Fig. 33). The upper two tiers of the proembryonal tetrad were designated as (*ci*) and *m* (Plate 6, Fig. 33). The two juxtaposed cells formed by the division of the cell (*ca*) divided vertically in a plane perpendicular to the previous one and organized the quadrants *q* (Plate 6, Fig. 34). The middle cell *m* divided vertically and transverse divisions occurred in the cells of *q*. These divisions resulted in a four tiered embryo, *l*, *l'*, *m* and

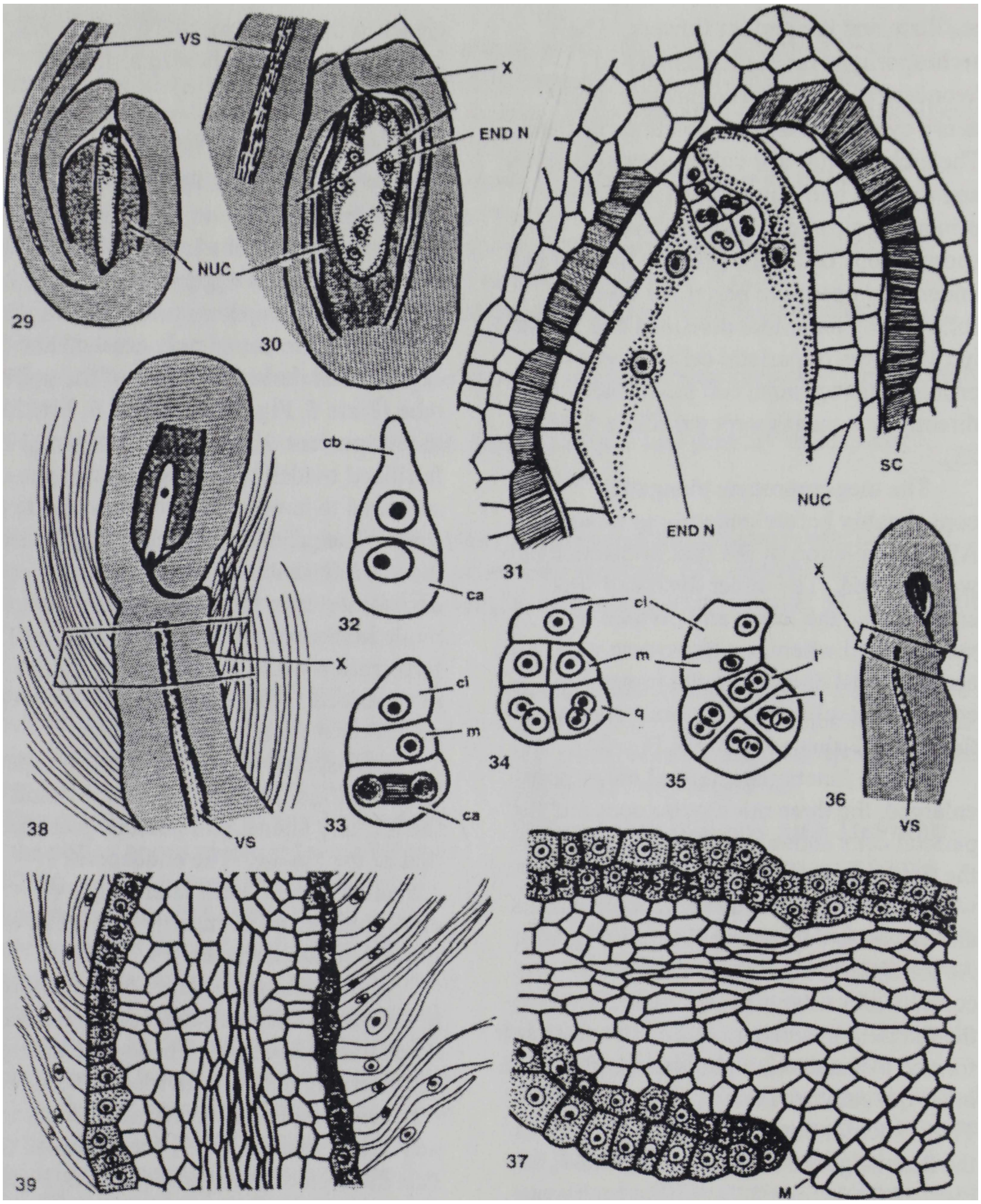

Plate 6 Figures. 29-39. Development of endosperm, embryo, funicular hair and seed coat in bisexual *Populus tremuloides* Michx.

Figure 29. Longitudinal section of ovule showing funiculus, vascular strand Nucellus (= NUC) and mature megagametophyte before fertilization. X300.

Figure 30. Longitudinal section of ovule showing eight-nucleate endosperm (= END N), embryo (= EMB), nucellus (= NUC) and sclerification of inner layer of integument. X300.

Figures 31. Portion marked "X" in Figure 30 enlarged. X1200.

Figure 32-35. Stages in the development of embryo. All X1500

Figure 36. Longitudinal section of ovule showing funiculus, vascular strand (= VS) nucellus and integument. X200.

Figure 37. Portion marked "X" in Figure 36 enlarged to show the initials of hair cells (= HI). X1000.

Plate 6, Continued.

Figure 38. Longitudinal section of ovule showing funiculus, vascular strand (= VS) nucellus, integument and hair cells. X300.

Figure 39. Portion marked "X" in Figure 38 enlarged to show the hair cells. X1000.

ABBREVIATIONS: ca, apical cell; cb, basal cell; ci, lower daughter cell of cb; EMB, embryo; ENDN, endosperm nuclei; HI, initials of hair cells; l, upper tier of octant; l', lower tier of octant; m, middle cell; M, Micropyle; NUC, nucellus; q, quadrant; SC, sclerified cells; VS, vascular strand.

ci (Plate 6, Fig. 35). The cell *m* after two vertical divisions formed four cells which together with the cells of the tier *l'* divided periclinally and formed dermatogen. Further divisions in the embryonic mass after the second periclinal division were irregular. The region l formed the stem tip, the tier *l* gave rise to a part of the hypocotyl while the remaining portion of the hypocotyl was derived from *m*.

Following fertilization, the epidermal and subepidermal cells of the funiculus enlarged showing conspicuous nuclei and dense cytoplasm (Plate 6, Figs. 36 and 37). The epidermal cells were transformed into hair cells and the subepidermal cells then became the outermost layer of the funiciulus (Plate 6, Figs. 38 and 39). The hair cells remained uninucleate and continued to elongate during embryo development and reached the tops of the ovules when the embryo was globular (Plate 6, Fig.38). The funicular hair cells eventually formed the tuft of coma on mature seeds. Simultaneous with these changes, the innermost layer of the integument began sclerification. By the time a proembryonal tetrad was formed, this layer was completely sclerified (Plate 6, Figs. 30 and 31).

DISCUSSION

Erlanson and Hermann (1927) reported the first quaking aspen tree with perfect flowers. Santamour (1956), Pauley and Mennel (1957), Einspahr (1960), Lester (1963), and Maini and Coupland (1964) recorded subsequent combinations of hermaphroditism. Every and Wiens (1971) reported several trees from the Parleys Canyon population of aspens in Utah that were at first characterized as pistillate but on closer inspection were hermaphroditic and producing almost entirely perfect flowers. Deviations from dioeciousness include trees with both staminate and pistillate catkins; trees with catkins having both staminate and pistillate flowers; trees having catkins with some perfect flowers; and trees with various combinations of the above. Although several cases of hermaphroditism existed as defined above, a majority of those trees had flowers that were unisexual in initiation, development, and expression (Lester 1963).

The trees described in this study were different from those described by Erlanson and Hermann (1927) and others. All catkins that I examined from each of the four hermaphrodite trees for eight consecutive years contained only perfect flowers. To the contrary, Erlanson and Hermann (1927) observed perfect flowers in the center section of the catkin with fewer female flowers located distally; even fewer male flowers were found proximally. Although in one percent of the perfect catkins, flowers toward the apex of the catkin had only one stamen, no consistent pattern of an acropetal decrease in the number of stamens was observed in the present study unlike the previous report (Erlanson and Hermann, 1927). However, when compared to staminate flowers, the perfect flowers showed a lower average number of stamens/flower. In 80 percent of the perfect flowers all stamens were normal and fertile. The most frequently observed condition was two healthy stamens, one on either side of the ovary. In 20 percent of the flowers, however, several combinations of normal and degenerating stamens were observed. Several flowers showed two stamens, one healthy and one degenerated; others showed ≥ 3 stamens with one, two, three or all degenerated. Erlanson and Hermann (1927) also reported variable number of stamens in the perfect flowers ranging from one to eight stamens, with three being most frequent. Although Erlanson and Hermann (1927) reported polyspory and other anomalies in microsporogenesis, they did not observe any degenerating stamens in the perfect flowers.

Santamour (1956) examined 67 wild quaking aspen and determined that three of 28 female and two of 39 male trees were hermaphroditic. However, he did not distinguish among trees having all perfect flowers, predominantly female with either male or perfect flowers, and predominantly male with either female or perfect flowers. Santamour (1956) also reported that in progeny of 131 trees from intraspecific crosses, 46.7 percent of the females and 1.4 percent of the males were hermaphroditic. The greatest number of trees were from intraspecific crosses utilizing a known hermaphrodite as the female parent. Of the progeny examined, 54.7 percent of female and 2.9 percent of male trees showed evidence of hermaphroditism. In a controlled cross between a known hermaphroditic quaking aspen and bigtooth aspen (*P. grandidentata)*, one of two female hybrids was hermaphroditic. Santamour (1956) concluded that the tendency to exhibit hermaphroditism may be inherited, and since the phenomenon was found primarily in female trees, perhaps the trait is X-linked; the self-fertile hermaphrodites may provide a tool for forest tree improvement.

In the present study, all dioecious trees consistently produced catkins with only unisexual flowers and the bisexual trees produced all perfect flowers over the eight consecutive years they were examined. Lester (1963) recorded varying degrees of perfectity and deviant flowers in 44.8 percent of the 138 trees he examined in two consecutive years. Deviations included perfect flowers, vestigial sex structures, and catkins with both staminate and pistillate flowers or with staminate, pistillate, and perfect flowers. For some trees the second-year sample revealed that many new deviants had arisen in trees that were unisexual the first year. He also corroborated earlier reports of an acropetal change from male to female character in the catkins. Lester (1963) concluded that hermaphrodites are not typically found in stands but in one or a few isolated trees and deviation from dioecism in quaking aspen is a phenomenon of considerable variability with an uncertain basis.

In addition to differences in the number of stamens and percentage of fertile pollen, the present study also revealed differences in the number and appearance of bracts in perfect flowers. Whereas male flowers showed three- to six-lobed bracts of seven different types, and female flowers were associated with three- to eight-lobed bracts of eight different types, the perfect flowers were subtended by one of five distinct types of brown hairy bracts. Linear, non-cleft and two- cleft bracts with one very small and the second long lobe were unique to perfect flowers. The three-, four-, and five-lobed bracts, although similar in the number of lobes, were morphologically distinct from those of the male and the female flowers. Likewise, bracts associated with male and female flowers were morphologically distinct and specific to each type of flower. Despite differences in the shape and number of lobes, no correlation occurred between a specific type of bract and its position on the catkin. There also was no correlation between a specific type of bract and the number of stamens in perfect flowers.

In this study microsporogenesis and anther wall development in both perfect and male flowers conformed to the Dicotyledonous type even though Davis (1966) reported a Monoctyledonous type of anther wall development for the family Salicaceae. The tapetal cells remained uninucleate through out development and only one middle layer was observed in anthers of both male and perfect flowers. Nagaraj (1952), however, reported binucleate tapetal cells and two-three middle layers in the anther wall of eastern cottonwood (*Populus deltoides)* . In this study both male and perfect flowers showed only tetrahedral tetrads unlike eastern cottonwood, where tetrahedral and isobilateral microspore tetrads were observed (Nagaraj 1952). Results of this study showed variation in size and fertility of pollen grains in both male and perfect flowers. The volume of pollen grains from perfect flowers tended to be twice the volume of the pollen grains from male flowers and suggested (at least as a first approximation) that perfect flowers may be tetraploid. The bimodal distribution of pollen diameters in the perfect flowers further emphasized that the four trees included one diploid and three tetraploids. Erlanson and Hermann (1927) also reported that pollen grains in the perfect flowers included several sizes ranging from dwarf spherical grains to exceptionally large grains; pollen of male flowers was markedly smaller and dwarf grains were rarely found. Every and Wiens (1971) reported pollen from several diploid trees to show a wide variation in size and a few cases exhibited a bimodal size distribution (30 μ and 50 μ). They considered the large size class (50 μ) to represent the unreduced (2n) pollen.

Megasporogenesis, development of female gametophyte, and ovule in both perfect and female flowers, conformed to other previously studied species of *Populus* (Nagaraj, 1952). However, in the present study, ovules appeared hemiantropous when the megagametophyte was two-nucleate in female flowers and four-nucleate in perfect flowers and became completely anatropous by the time an eight-nucleate megagametophyte was formed. Nagaraj (1952) observed a hemianatropous condition at the megasporocyte stage and a complete anatropous condition before the megagametophyte attained a two-nucleate stage in eastern cottonwood and quaking aspen. Fertilization and development of endosperm was similar in both female and perfect flowers and consistent with previous reports for *Salix* and *Populus* (Davis, 1966). Results of this study showed Polygonum variation of the Asterad type of development in both female and perfect flowers. Nagaraj (1952) reported an Onagrad type in eastern cottonwood, and although no details were given, he alluded to a similar development in quaking aspen as well. Zhu and Li (1989) described a Solanad type of embryogenesis in *P. lasiocarpa*. An Asterad type of embryogenesis has also been reported in *Salix* (Davis 1966).

The hair cells that eventually formed the tuft of coma on mature seeds were funicular in origin in both female and perfect flowers in this study. These observations were consistent with those of Fechner (1976) for female flowers of quaking aspen. Nagaraj (1952), however, reported hair of placental origin in both in eastern cottonwood and quaking aspen.

The present study revealed some striking abnormalities in perfect flowers that point to their polyploid nature. In five percent of these flowers, the fertilized ovules either degenerated or apparently had been arrested at the zygote stage. The primary endosperm nucleus in those ovules had not undergone any divisions either. In 10 percent of the flowers, only one ovule in the ovary had developed whereas the remaining ovules had degenerated. These abnormalities, when combined with the pattern of degeneration of stamens, relatively lower number of stamens, and lower pollen fertility were significant in light of suggestions made by several authors (Santamour 1956, Lester 1963, Einspahr and Winton 1976) regarding use of bisexual aspens in breeding programs for forest tree improvement.

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