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Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine, *Pinus banksiana* Lamb.

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Summary. Segregation ratios of allozymes in haploid female gametophytes obtained from viable seed were studied in a natural population of jack pine, *Pinus banksiana*. Stability of these ratios was assessed for three levels of the sexually reproductive crown as well as for four years of natural fertilization. Analyses of observed segregation ratios of four of five polymorphic isozyme loci showed good correspondence to the overall 1 : 1 ratios expected for simple Mendelian inheritance. Allozymes of glucose-6-phosphate dehydrogenase did not segregate in the expected 1 : 1 ratio. In addition, there were significant deviations from the expected segregation ratio for all the loci at some sampling positions on individual trees. Heterogeneity of segregation among trees, strata and years could be the result of pollen pool heterogeneity, segregation distortion and/or recessive lethal and semi-lethal gene combinations resulting in early embryo abortion. These types of segregation deviations in viable seed can affect the estimation of allele frequencies from bulked samples of a small number of individuals, the inference of heterozygosity/homozygosity of parental trees, and estimates of selfing rates.

Key words: Segregation distortion – Allozymes – *Pinus banksiana* Lamb. – Lethal allelism

Introduction

The utility of protein electrophoresis for studying population genetic structure and mating systems has been demonstrated for diverse organisms (Allard 1975; Brown 1979; Lewontin 1974; Nevo 1978; Rick et al. 1977; Shaw et al. 1981). However, the utility of the method for genetic studies requires knowledge of the inheritance of electrophoretically detectable polymorphism. Ideally their linkage relationships also should be known.

Inheritance and linkage of electrophoretically detectable allozyme polymorphisms have been studied in a number of coniferous species (for a recent summary see Cheliak and Pitel 1984). The nature of the life cycle in conifers permits inheritance and linkage studies to be performed simultaneously. Unlike angiosperms, the endosperm (female gametophyte or megagametophyte) of gymnosperms is haploid in the mature seed. This condition allows the genotype of an individual (the female parent) to be determined probabilistically by analysis of haploid megagametophytes without the necessity of performing crosses. Furthermore, since one haploid megaspore mitotically gives rise to all other cells within the ovule, barring mutation, the embryo will inherit a haploid genotype identical to the megagametophyte.

In this paper, the inheritance of five polymorphic enzyme systems of megagametophytes of jack pine (*Pinus banksiana* Lamb.) is described. In addition, open-pollinated progeny arrays were assayed to determine if the polymorphic enzyme loci surveyed were inherited in a simple Mendelian fashion. This forms part of a larger study, the main objective of which was to estimate parameters of the mixed mating system model for this species. Thus, to be considered for inclusion a gene had to be co-expressed in both the megagametophytic tissue and its corresponding embryo.

Materials and methods

Thirty sexually mature jack pine trees were sampled from a stand near Bruderheim, Alberta (Fig. 1). The average age of

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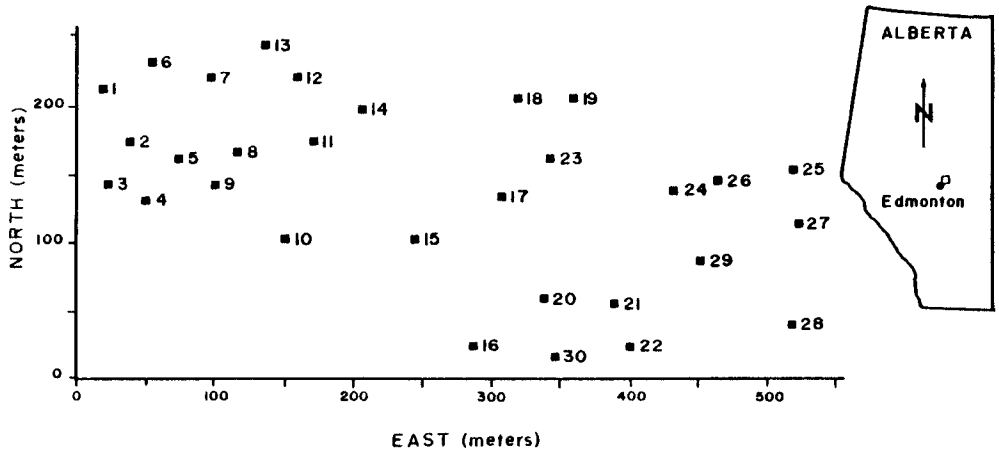

Fig. 1. Geographical loction of the population sampled and the distribution of the trees sampled within the stand. The numbers refer to the identification number assigned to each tree. The stand (□) is located near Bruderheim, Alberta

the trees in this stand in 1980 was 38 years. However, there were two distinct age classes in the stand (24 and 44 years). In addition to the disturbance giving rise to the younger age class, there was evidence of selective cutting and an additional fire approximately 28 years ago (ca.1952).

To reduce the possibility of sampling closely related individuals (i.e., full or half siblings), a minimum of 30 m spacing between sample trees was maintained. To assess stratification of segregation (spatial variation), the active female reproductive crown of each tree was arbitrarily stratified into three strata: low (L), middle (M) and high (H). Serotinous cones, representing pollinations of 1975, 1976, 1977 and 1978, were harvested from each *stratum* of each tree from a randomized cardinal direction in the late fall of 1980 to assess possible temporal variation. A sampling position will be designated as a particular combination of year and crown stratum. Thus, there are 12 sampling positions per tree.

Open-pollinated seeds were extracted from the serotinous cones after reflexing the ovuliferous scales by heating. Seeds were then removed from the cones by lightly tapping the cones on a hard surface.

For maternal genotyping, seeds were allowed to imbibe distilled water for 24 h and either were assayed immediately or refrigerated for up to one week before assay. Embryos for the progeny arrays were germinated for about 72 h before being assayed. There was, however, a great deal of inter-family variation in 72-h germination of the seeds. Therefore, it was decided on the basis of a preliminary ontogenetic study to allow the radicle to emerge approximately 2–3 mm from the seed before embryo assay.

Seed coats and embryos were removed for maternal genotyping. Individual megagametophytes were crushed in a scintillation cup (0.5 ml) with one drop of an extraction buffer (Yeh and O'Malley 1980). Up to 40 megagametophytes were assayed per tree for preliminary genotyping. In the next experiment, individual embryos, with 2–3 mm of radicle, were prepared in the same manner. The female gametophyte and the corresponding embryo were placed at adjacent positions in the gels. Generally, 10 embryos were assayed per sampling position (12 positions per tree). Details of the electrophoretic conditions, buffers and description of enzyme recipies are given in Cheliak (1983).

The log-likelihood ratio test statistic (asymptotic χ^2) was used to test goodness of fit to 1 : 1 segregation of alleles in the megagametophytes (Sokal and Rohlf 1969). A heterogeneity G was calculated to test the homogeneity of the observed segregation ratios among females and among sampling positions within a tree. A test for linkage was made for multiply heterozygous trees. The test criterion was a G analysis conditional on the null hypothesis of a 1 : 1 : 1 : 1 distribution of gametes at two heterozygous loci within a tree. A deviation from the expected ratio is judged statistically significant if it exceeds that of chance expectation at $\alpha = 0.05$ or less.

Results

Five loci (Aph-2, G6p, Mdh-3, Mdh-4 and 6Pgd-2) were inferred to be segregating in this population sample from analysis of female gametophytes. Variation at additional enzyme loci was detected in aconitase (E.C. 4.2.1.3), adenelate kinase (E.C. 2.7.4.3), phosphoglucose isomerase (E.C. 5.3.1.9), 6-phosphogluconic dehydrogenase-1 (E.C. 1.1.1.44), leucine aminopeptidase (E.C. 3.4.11.1), aspartate aminotransferase-2 (E.C. 2.6.1.1), and diaphorase (NADH dependent) (E.C. 1.6.4.3) using only the haploid megagametophytic tissue. However, problems in consistently resolving these systems or in interpretation using the diploid embryos precluded their inclusion in this study. For each variable locus, a discussion of the allozyme variants detected and detailed analysis of segregation of allozymes summarized by female, year and crown stratum follow.

Acid phosphatase (Aph) E.C. 3.1.3.2

Two zones of activity are observed on gels scored for acid phosphatase. Both zones segregate in the megagametophytes of trees heterozygous for single-band allozyme variants. However, problems in consistently resolving the more anodal zone under the electrophoretic conditions of this study precluded its inclusion.

Zone 2 (called Aph-2) embryos show either a single band at the same position as the megagametophyte, or three bands with one or two bands coincident with one or both bands observed in the megagametophytes. An intermediate band of approximately double the intensity is observed in embryos displaying the three-band phenotype. This observation strongly suggests that Aph enzymes are functionally dimeric, as has been reported in *Picea abies* (L. [Karst.]) by Lundkvist and Rudin (1977).

In the single tree heterozygous for Aph-2, the average segregation ratio does not fit the expected 1 : 1 distribution of gametes ($G_1 = 8.78$, where the subscript represents the degrees of freedom; Table 1). There is no detectable heterogeneity of the pooled distributions among crown strata ($G_2 = 0.74$) or among years ($G_2 = 0.25$). Only one sampling position has a statistically significant deviant segregation ratio M-1975 ($G_1 = 7.36$). The significant average deviation of the segregation ratio is attributable to a consistent deficiency of the *2* allele at this locus.

Malate dehydrogenase (Mdh) E.C. 1.1.1.37

Two zones of activity are observed on gels scored for Mdh. While there is evidence of four loci in this system, under the electrophoretic conditions for this study, only the two most cathodal loci could be scored reliably in the progeny. Both zones, Mdh-3 and Mdh-4, segregate in megagametophytes of trees heterozygous for single-band variants. Two alleles are observed at each of these loci. For both zones, embryos show either a single band at the same position as the megagametophyte or three bands with one or two bands coincident with one or both bands observed in the megagametophytes. There is an intermediate band of approximately double intensity. This strongly suggests that both Mdh enzymes are functionally dimeric. Dimeric forms of Mdh have been reported in pitch pine (*Pinus rigida* Mill.) by Guries and Ledig (1978).

Mdh-3. The average segregation ratio for the three trees heterozygous for alleles *1* and *2* fits the Mendelian expectation of a 1 : 1 ratio of gametes well ($G_1 = 0.003$; Table 1). There is, however, significant heterogeneity among trees ($G_2 = 9.98$). Megagametophytes of trees 5 and 27 have an excess of the *1* allele while those of tree 10 have an excess of the number *2* allele. For tree 10, the average segregation ratio deviates significantly from a 1 : 1 ratio ($G_1 = 6.89$). Significant excess of the allele *2* is also observed in the low crown stratum in 1977 and 1978, middle crown stratum in 1978 and high crown stratum in 1976. Since four out of twelve sampling positions of this tree have significantly deviant segregation ratios in the same direction, it appears that the megagametophytes from this tree are not segregating in a 1 : 1 ratio, spatially or temporally. Megagametophytes from trees number 5 and 27 are segregating temporally and spatially as expected under the null hypothesis of a 1 : 1 segregation ratio. However, there is significant heterogeneity of the segregation ratio (pooled over years) among all the trees for the middle crown stratum ($G_2 = 6.70$).

Mdh-4. The average segregation ratio for the four trees (numbers 4, 7, 11 and 29) heterozygous at Mdh-4 fits expectations of a 1 : 1 segregation ratio ($G_1 = 0.84$), with no detectable heterogeneity of the average segregation ratio among heterozygous trees ($G_3 = 3.60$; Table 1). Trees 7 and 29 both exhibit one significant deviation from the null hypothesis, which is not unexpected. However, trees 4 and 11 have three and four significant comparisons, respectively. There is no consistent trend for the direction of the deviation among the three significant comparisons for tree 4. For tree 11, there is significant heterogeneity among the segregation ratios of the middle crown stratum across the years ($G_2 = 8.92$). While no heterogeneity is evident among the trees for the segregation ratio in the high crown stratum, there is an overall lack of fit to a 1 : 1 ratio ($G_1 = 8.40$). This lack of fit is due to an overall deficiency of the *2* allele. Most of the deviant segregation ratios for Mdh-4 tend to occur in years 1977 and 1978. The segregation ratios of crown strata (pooled over years) also are significantly heterogeneous ($G_2 = 7.18$).

6-Phosphogluconic dehydrogenase (6Pgd) E.C. 1.1.1.44

Two zones of activity are observed on gels scored for 6Pgd. While both zones were variable, only zone 2 provided consistent resolution in diploid embryos. Thus, discussion will center only on variants detected in zone two (designated 6Pgd-2). Megagametophytes exhibit segregation in zone two of trees heterozygous for single-band variants. Three allozymes are observed in this zone. Embryos have either a single band at the same position as the megagametophyte or three bands, with the alternate band coincident with the variant observed in the megagametophytes and an intermediate band of approximately double intensity. These results strongly suggest that the enzyme 6Pgd-2 is functionally dimeric, as has been reported in loblolly pine (*Pinus taeda* L.) (Adams and Joly 1980), and pitch pine (Guries and Ledig 1978).

6Pgd-2, allele 2. The average segregation ratio of the six trees heterozygous for this gene locus fits the expected distribution of a 1 : 1 ratio ($G_1 = 0.43$; Table 1). There is no heterogeneity of the average segregation ratio among the trees ($G_5 = 0.49$).

Table 1. Observed spatial and temporal segregation of allozymes from heterozygous jack pine trees from a natural population

Locus	Alleles	Female	Segregation summary															
			Stratum						Year									
			Low		Middle		High		1975		1976		1977		1978		Total	
			1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Aph-2	1, 2	2	18	10	25	11	24	16	20	10	17	11	19	10	11	6	67	37
Mdh-3	1, 2	5	17	19	20	10	19	16	14	13	17	12	12	13	13	7	56	45
		10	15	20	12	21	13	26	13	14	12	15	11	19	4	19	40	67
		27	22	17	24	16	21	19	15	15	18	11	17	13	17	13	67	52
	Summary		54	56	56	47	53	61	42	42	47	38	40	45	34	39	163	164
Mdh-4	1, 2	4	33	26	18	21	18	15	19	18	25	15	9	21	16	8	69	62
		7	16	20	16	24	22	15	13	16	16	12	10	20	15	11	54	59
		11	20	17	20	20	29	11	16	14	16	14	17	13	20	7	69	48
		29	14	25	20	17	21	16	18	10	14	15	13	15	10	18	55	58
	Summary		83	88	74	82	90	57	66	58	71	56	49	69	61	44	247	227
6Pgd-2	1, 2	1	21	15	24	16	19	17	17	13	17	13	19	11	11	11	64	48
		6	15	19	18	14	18	22	8	21	16	10	9	14	18	10	51	55
		9	22	17	24	18	21	16	15	20	16	11	17	10	19	10	67	51
		14	22	18	5	14	23	14	8	9	12	8	16	13	14	16	50	46
		18	17	22	23	23	17	21	12	15	24	16	12	18	9	17	57	66
		29	15	24	18	19	22	15	15	13	13	16	16	12	11	17	55	58
	Summary		112	115	112	104	120	105	75	91	98	74	89	78	82	81	344	324
6Pgd-2	1, 4	7	20	16	30	27	32	24	18	16	19	14	23	20	22	17	82	67
G6p	1, 2	15	23	10	26	10	19	11	23	4	18	11	16	10	11	6	68	31
		27	22	19	23	14	34	6	21	9	19	10	17	13	22	7	79	39
	Summary		45	29	49	24	53	17	44	13	37	21	33	23	33	13	147	70

Trees 1, 9 and 29 have gamete distributions that fit the expected segregation ratio. There is no heterogeneity among the observed distributions of gametes for the crown strata or years sampled for these trees.

Tree 6 exhibits two distorted segregation ratios in the low and high crown strata in 1975. The average segregation ratio for years for this tree is significantly heterogenous ($G_3 = 10.70$); years 1975 and 1977 have an excess of the *2* allele while 1976 and 1978 have a deficiency of this allele. The average segregation ratio for 1975 deviates significantly from a 1 : 1 ratio ($G_1 = 6.04$). This lack of fit is attributable to a deficiency of the *1* allele.

Tree 14 has three deviant segregation ratios. While there is no consistent trend in the direction of the deviation, positions L-1978 and H-1977 both have deficiencies of the *2* allele. When pooled over years, the observed gamete distribution in the middle crown stratum does not fit the expected 1 : 1 distribution ($G_1 = 4.43$). This is attributable to a deficiency of the *1* allele. As well, there is significant heterogeneity among crown strata ($G_2 = 6.88$).

Tree 18 has two significant segregation distortions (L-1975 and H-1978). The lack of fit for both of these is due to a deficiency of the *1* allele. There is no heterogeneity or lack of fit for the year on crown stratum average segregation ratios.

When all trees are considered, there is a tendency for aberrant segregation ratios to occur in 1977 and 1978. This trend is essentially independent of crown stratum, although the low and high crown strata deviated more often than did the middle crown stratum.

6Pgd-2, allele 4. Only one tree (number 7) among the 30 tested is heterozygous for alleles designated *1* and *4*. There are no significantly deviant segregation ratios. As well, there is no detectable heterogeneity of the average segregation ratios among crown strata or years sampled for this tree.

Glucose-6-phosphate dehydrogenase (G6p) E.C. 1.1.1.49

One main zone of activity is observed on gels stained for G6p activity. There is, however, an ontogenetic modification of this zone of activity. After day four of

germination, apparent activity in this zone progressively decreases and the apparent activity of another zone (anodal) increases at approximately the same rate. By day ten, most detectable activity in the scored zone had disappeared, and the main region of activity has shifted to the more anodal zone.

In trees apparently heterozygous for G6p, the megagametophytes show segregation for single-band variants. Embryos have either a single band in the same position as the megagametophyte or exhibit one of two other phenotypes. Embryos heterozygous for alleles *1* and *2* (= *12* embryos) commonly occur with unresolved bands with the top and bottom of the zone of activity coincident with the relative migration distances observed for the two alleles in the megagametophytes. When clear banding patterns occurred they reveal a three-band phenotype in heterozygous embryos, suggesting that the enzyme G6p is functionally dimeric. The other embryo phenotype, occasionally observed, appears as an elongated, unresolved zone of weak activity extending to the origin. There is no evidence that the bands have migrated in a cathodal direction. No instances of clear banding patterns were observed for this embryo phenotype. Also, none of these phenotypes are observed in heterozygous females. Therefore, this phenotype is designated as *13*.

The average segregation ratio for both trees heterozygous at G6p does not fit the expected 1 : 1 ratio ($G_1 = 27.93$; Table 1). As well, each tree (numbers 15 and 27) exhibits an average segregation ratio significantly different from a 1 : 1 ratio ($G_1 = 14.17$ and 13.83, respectively). There is no heterogeneity between these two trees for the average segregation ratio ($G_1 = 0.07$). In both cases, the lack of fit to a 1 : 1 ratio is attributable to a marked deficiency of the *2* allele. This deficiency is primarily due to samples M-1977 and L-1975 for tree 15, and H-1975, H-1976 and H-1978 for tree 27. However, there is only one instance in 23 observations where the number of the *2* allele exceeds the number of the *1* allele (tree 15 H-1977). Therefore, we conclude that these trees are not segregating in a 1 : 1 fashion for this locus.

Linkage analysis

Two trees (numbers 7 and 27) were multiply heterozygous for Mdh-4 and 6Pgd-2 (allele *4*), and Mdh-3 and G6p, respectively. The goodness-of-fit tests of a 1 : 1 : 1 : 1 distribution of gametes reveal no evidence of close linkage between the loci of either pair.

Discussion

The four enzyme systems studied here in the megagametophytes and embryos of viable seed from jack pine reveal allozyme variation at five gene loci. Direct evidence of genetic control is presented for these loci based on the observed segregation of allozyme variants in haploid megagametophytic tissues of heterozygous trees. Enzyme band patterns of the megagametophytes for these loci are similar to patterns reported in different pine species.

Band patterns in the embryos indicate that the same locus codes for allozymes in both the haploid megagametophyte and diploid embryo tissues. Inference of the functional form of the enzyme from the gel phenotype of heterozygous embryos agrees with that reported for many other plant and animal species.

Although most heterozygotes, on the average, segregate as expected, segregation distortion is observed for some trees and/or pooled distributions of megagametophytes in specific years and/or crown strata. These types of segregation distortion appear to be a relatively common phenomonon in forest tree species (Adams and Joly 1980; Eckert et al. 1981; Lundkvist 1974; O'Malley et al. 1979; Rudin 1975, 1977; Rudin and Ekberg 1978).

Segregation distortion, as evidenced by a significant departure from the expected 1 : 1 recovery of alternative alleles in megagametophytes from seeds of heterozygous trees, has effects and ramifications at the individual and population level.

Inference of individual heterozygosity using the sampling theory of Morris and Speith (1978) is contingent upon equal survival of alternative gametophytes of heterozygous trees. Equal frequencies of alternative alleles are most likely to be true at meiosis. However, studies of segregation using mature conifer seeds do not assess this parameter at meiosis, but at a time considerably later. Pollination and fertilization of the ovule are separated by approximately 17 months in *Pinus* (Owens et al. 1981), so there is ample opportunity for intervening gametic selection. After fertilization, maturation of a viable seed depends upon survival of the embryo. Thus, ascertainment of a megagametophyte ultimately depends on the fitness of the genotype of the embryo to which it has contributed. We shall briefly discuss some of the potential mechanisms leading to segregation distortion in gametophytes from mature seeds.

Documented meiotic irregularities, such as precocious disjunction and failure of chiasma terminalization (Saylor and Smith 1969), should not produce segregation distortions, given that they occur at random. Meiotic disturbances such as segregation preference or meiotic drive mechanisms are known to occur in plants, but not in gymnosperms (Rhoades 1952). Furthermore, meiotic disturbances and irregularities can not account for heterogeneity among trees, or among sampling positions within a tree.

One mechanism for the observed segregation distortion would be the interaction between genetic composition of the pollen and of the ovule determining the fitness of the embryo, hence, maturation of the seed. The pollen pool must be considered in most of these discussions since maternal segregation was indirectly assayed after fertilization. Conifers typically

have a high embryonic-lethal genetic load (Fowler 1965; Koski 1971; Sorensen 1969). In natural populations of Douglas-fir, the mean number of lethal equivalents is nine to ten per zygote (Sorensen 1969). Estimates for animal species range from one to two per zygote in *Drosophila pseudoobscura* (Dobzhansky et al. 1963) and *Tribolium* (Levene et al. 1965) to three to five per zygote in man (Morton et al. 1965). In forest trees, the majority of this load is expressed in early embryonic development, particularly upon self-fertilization, although some is continually expressed from seed germination through all stages to maturity (Orr-Ewing 1957, 1965). However, it is also conceivable that embryonic lethality would occur, albeit less commonly, with more distant inbreeding as well as in a very small proportion of random matings due to lethal allelism.

Controlled self-fertilization in conifers results in a higher proportion of empty seeds than cross- or wind-fertilization (Hadders and Koski 1975; Rudolph 1976), although a large proportion of empty seeds can also be observed under normal field conditions (Anderson 1965). After controlled self-pollination, the progress of fertilization and subsequent embryogenesis generally proceed normally until shortly after the suspensor stage (i.e., the beginning of the embryo proper) in Norway spruce and Scots pine (*Pinus sylvestris* L.) (Koski 1971). Orr-Ewing (1957) showed that development of the embryo following self-fertilization continued normally until two or three weeks after fertilization in *Pseudotsuga menziessii* (Mirb.) Franco. Thus, an explanation for the aberrant segregation ratios observed in this study would be either linkage of the isozyme marker with a recessive embryonic lethal or deleterious effects of the isozyme itself. Since both types of homozygous progeny were observed, the linkage cannot be absolute or the deleterious effects would not be that of a recessive lethal.

In situations where there is no heterogeneity among females (or sampling positions within a tree), we may hypothesize that either the pollen pool was homogenous (for outcrossing) or that the genetic background of these females did not differ with respect to linked recessive lethals or semi-lethals upon selfing.

When heterogeneity among trees or among sampling positions within a tree is observed, it would seem reasonable to infer that the pollen pool is genetically heterogeneous in space and time. Alternatively for inter-female differences, different genetic backgrounds could be responsible for the observed departure from 1 : 1 segregation ratios. Homogeneity and heterogeneity of the pollen pool also refers to the proportion of embryos derived as a direct result of self-fertilization, as well as gene frequency changes in the external pollen pool. From these analyses, it is not possible to unambiguously identify an embryo resulting from cross fertilization with a pollen allele electrophoretically identical to the allele of the mother (identical in type or homozygous) or from a self-fertilization event (identical by descent or autozygous).

To estimate parameters of the mixed mating system model, several assumptions about the fitness of zygotes and the underlying distribution of gametes in the pollen pool are required (Brown and Allard 1970). Whether the segregation distortions observed in this study are attributable to direct distortion in the "female" gametophytes, or due to lethal or semi-lethal mediated allelism from inbreeding (thus, not all zygotes have the same fitness), or perhaps due to heterogeneous distributions of lethal alleles in pollen pools can not be determined. However, the net effect, observed segregation distortions, affects estimates of mixed mating system parameters. If the distortion is strictly a phenomenon of the female gametophyte, the bias can be in both directions; over- and under-estimates of $\hat{t}$, $\hat{p}$, (where $\hat{t}$ is the outcrossing rate and $\hat{p}$ is the estimate of the allele frequency in the outcrossed pollen pool), depending upon allele frequencies in the pollen.

For example, suppose that in the population, the true outcrossing rate and allele frequencies are $\hat{t}$ and $\hat{p}_i$, $i = 1, \ldots, k$ with the alleles ordered from high to low frequency (i.e., $\hat{p}_i > \hat{p}_{i+1}$). If the segregation distortion is toward alleles of high frequency in the population, an apparent assortative mating system will result. This would result in an overestimate of the common allele frequency and an underestimate of $\hat{t}$. Opposite results would occur if the distortion is towards alleles of low-frequency in the population.

There are many other situations possible. However, if non-random segregation distortions are a real effect in plant populations, estimates of population parameters can be compromised. Segregation distortion can effect the estimates of population allele frequency made from bulked samples of megagametophytes, particularly when a small number of individuals are represented in the sample. Furthermore, these aberrant segregation ratios affect the binominal probability statement about detection of heterozygosity of an individual plant. Especially for inference of individual heterozygosity, it may be necessary to include one or two additional megagametophytes to compensate for these sources of bias.

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