What Can Be Inferred from Open-Pollination Progenies about the Source of Observed Segregation Distortion? – A Case Study in Castanea sativa Mill.

By Elizabeth Gillet and H.-R. Gregorius

Abteilung für Forstgenetik und Forstpflanzenzüchtung,
Georg-August-Universität, Büsgenweg 2,
DW—3400 Göttingen

(Received 15th July 1991)

Summary

A systematic discussion of the primary sources of segregation distortion is presented together with methods of analysis which allow discrimination between them, particularly for the study of open-pollination progenies lacking distinguishability of maternal and paternal gametic contributions. In such progenies, considerations are necessarily restricted to segregation disortion in the female gametic sex. This discussion was motivated by the observation of considerable and quite erratic deviations from regular segregation among female gametes, including "reversal" of direction, at the AP-A-locus in the seed from open pollination of several sweet chestnut trees (*Castanea sativa* Mill.) (Fineschi *et al.*, 1990). In spite of the limited available data (a situation typical for the study of trees), two genetic hypotheses can be advanced to explain the data. In the one, AP-A is linked to a female gametic viability locus but does not have any effect of its own on viability, while in the other it is assumed to have a modifying effect involving a null allele on a viability locus. Both hypotheses are shown to have a paradigm in the Segregation Distorter (SD) system, which causes sperm dysfunction in *Drosophila melanogaster*.

Key words: segregation distortion, open-pollination progeny, gametic selection, post-zygotic selection, null allele.

Introduction

Segregation distortion has been observed in most of the "traditional" model organisms of genetics, and the elucidation of the various causal mechanisms in these organisms has long been the objective of intense study (cf. Crow, 1988, for a review). Since these organisms were originally chosen for reasons other than observation of segregation distortion, it can be assumed that this phenomenon is widespread throughout the plant and animal kingdoms. In coniferous tree species, for example, whose seeds contain a primary endosperm (megagametophyte) representing the genetic information of the female gamete, deviations from regular segregation have been observed by a number of authors, e.g. Neale et al. (1984), Cheliak *et al.* (1984), Boyle and Morgenstern (1985), Strauss and Conkle (1986) and references therein, Cheliak *et al.* (1987), Geburek and von Wuehlisch (1989), and Adams *et al.* (1990). However, its discovery has been less frequent in organisms, such as broad-leaved trees, whose reproductive characteristics cause them to be less amenable to genetic analysis. In angiospermous trees, it has been observed in controlled crosses in e. g. *Camellia japonica* L. (Wendel and Parks, 1982), *Citrus* (Torres *et al.*, 1985), and avocado (Torres *et al.*, 1986).

In investigations of broad-leaved forest trees, the performance of controlled crosses, if at all possible, is typically difficult and time-consuming. It would be desirable to have methods of genetic inference based on single-tree progenies from open pollination, since these are relatively easy to collect in considerable numbers. However, an obvious disadvantage for segregation analysis is that the paternal genotype of an offspring is not known. In addition, fully developed seeds of the widespread order *Fagales* contain no sexually differentiated tissue (i. e., no secondary endosperm), nor can the maternal and paternal gametic contributions be otherwise directly distinguished at any ontogenetic stage of an individual. Thus, for offspring from open pollination of heterozygous trees, the ordered genotype can be inferred only if the pollen contributed an active allele not possessed by the female parent.. As a result, segregation proportions cannot be observed for all successful female gametes but rather only for those which are associated with a particular male gametic type. Nevertheless, the relative ease of obtaining such data justifies and necessitates the development of applicable methods of genetic inference.

This situation is typical for studies in natural populations of angiospermous plants, especially trees. It explains and justifies our subsequent approach to an analysis of the phenomenon of segregation distortion with particular reference to the case where the offspring genotypes are unordered and the paternal genotype of an offspring is not known. This approach was conceived to serve three purposes:

(1) to present a systematic discussion of the primary sources of segregation distortion together with methods of analysis which allow discrimination between them,
(2) to demonstrate problems that may typically arise during the derivation and analysis of genetic hypotheses on segregation distortion, and
(3) to communicate an interest in the applicability of the results to an observed case of segregation distortion in single-tree seed progenies from open pollination of sweet chestnut (*Castanea sativa* Mill.). To our knowledge, this type of observation together with our attempt to explain it differ from all previous studies.

Primary sources of segregation distortion

Basically, segregation distortion is defined by significant deviations among the gametic types produced by an individual from the proportions expected under regular meiosis. Since in almost all cases segregation distortion is observable only after fusion of the gametes, the definition is probably more operational when applied to successful gametes in place of any stage prior to fusion. This also accounts more directly for the evolutionary significance of segregation distortion.

Along the pathway extending from meiosis to the fusion of gametes, three phases can be distinguished in which forces leading to segregation distortion may become active:

(i) The process of meiosis may be disturbed, thus resulting in unequal representation of complementary gametic types (meiotic drive).

(ii) The gametic types present in the meiotic products differ in their ability to survive to the stage of fertilization (gametic viability selection).

(iii) Among those of an individual's gametes which are available for fertilization (after gametic viability selection), differential fertilization success of gametic types may occur as a consequence of specific fusion relations determined by the mating system (gametic mating success).

Meiotic irregularities usually result in gones that are not completely reduced or lack parts of the genome. This situation is not considered to be a case of segregation distortion, since it is associated with changes in ploidy rather than with preferential production of certain genetically completely reduced ("perfect") gone types. In fact, it is hard to imagine how meiosis can produce perfect gones carrying certain genetic types in excess. However, even if this were somehow possible, the situation would be tantamount to regular meiosis with subsequent viability selection among the gones or gametes (this also includes "preferential segregation" as reviewed e. g. in GRANT, 1975, p.228ff). Hence, with respect to segregation distortion, the phases (i) and (ii) can be treated as being the same.

Gametophytic incompatibility appears to be the only type of mating system that has been explicitly studied for its effects on segregation frequencies among gametes. With special reference to self-incompatibility, LEACH (1988) demonstrated that this "heterophasic" system of mating (GREGORIUS, 1989) produces segregation distortion as a consequence of differential fertilization success among the male gametic types in a cross. Clearly, in systems of gametophytic incompatibility, segregation distortion can occur in the male gametic sex only. The possibility of segregation distortion in both gametic sexes exists in so-called "haplo-homophasic" mating systems, where the fusion of a pair of gametes is solely determined by haplophase characteristics of these gametes (GREGORIUS, 1989). Evidence for the activity of such haplo-homophasic systems of mating (sometimes called "selective fertilization") is given in studies of *Oenothera* species (see e. g. HAUSTEIN, 1967), but hitherto observations of this kind appear to be very rare and limited to a small number of species.

Consequently, there are three basic classes of causes for segregation distortion which have to be distinguished, one referring to gametic viability selection (including the above phases (i) and (ii)) and two (gametophytic incompatibility or heterophasic mating, and haplo-homophasic mating) referring to mating success in phase (iii). Segregation distortion in the female gametic sex cannot result from gametophytic incompatibility. Determination of the effects of any of the other two classes requires observations of pairwise associations of gametic types either directly before fertilization or after fertilization in zygotes or any other early diplontic stage. Given such information for mating types, each male gametic type can be characterized by its *fertilization distribution*, i. e. the set of frequencies of female gametic types with which it is associated. By definition, segregation distortion in the female gametic sex can then be stated if, for at least one male gametic type, its fertilization distribution differs significantly from the expectation of regular segregation among the female gametes. Haplo-homophasic mating can be ruled out as a cause of this segregation distortion if the fertilization distributions of all male gametic types are (statistically) identical. In this case viability selection among the female gametes can be accepted as the cause for the segregation distortion. Otherwise, if at least two male gametic types differ in their fertilization distributions, haplo-homophasic mating must be involved. It follows that gametophytic incompatibility does not per se preclude segregation distortion in the female gametic sex as long as the fertilization distributions of all male gametic types are identical.

By analogy, interchanging the roles of male and female gametes, fertilization distributions can be specified for female gametic types, thus allowing distinction between gametic viability selection and haplo-homophasic mating as causes for segregation distortion among male gametes.

Post-zygotic viability selection

Probably the majority of experiments concerned with studies of segregation distortion are based on observations obtained for post-zygotic (diplontic) stages. In this situation, an analysis of segregation frequencies among (successful) gametic types must account for the possibility of selective deaths occurring between the zygotic and the census stage. Post-zygotic viability selection may thus subsequently annul or modify the gametic fertilization success, so that the problem of how to distinguish between the two effects arises.

It is easy to demonstrate that such distinction is impossible if the inference must proceed from relative frequencies (e. g. of diplo-genotypes) among offspring; any observation can be explained solely by models of post-zygotic viability selection. In a strict sense, post-zygotic viability selection can be ruled out as a possible cause for segregation distortion only if no reduction in number occurred between the zygotic and the census stage. Since the formation of zygotes (via the fusion of gametes) can hardly ever be fully recorded, one is forced to find indirect evidence of additional effects which would allow one to conclude that post-zygotic viability selection alone cannot explain the observations. For example, it is unlikely that postzygotic selection is the sole determinant of an observed segregation distortion if the number of offspring shows no correlation with the degree of segregation distortion.

On the other hand, if correlations between number of offspring and degree of segregation distortion do exist, this does not suffice to exclude the involvement of gametic viability selection or haplo-homophasic mating. For example, the number of offspring of an individual may be directly proportional to the number of female gametes surviving to the stage of fertilization. Hence, the number of offspring would be strongly correlated with the degree of segregation distortion among female gametes, even though no post-zygotic deaths occur. The concept of fertilization distribution introduced above may yet help to solve this dilemma in that it provides conditions under which gametic and post-zygotic viability selection and haplo-homophasic mating are effectively equivalent.

As was emphasized, pure viability selection among gametic types as the sole cause of segregation distortion implies identity among the fertilization distributions either of all female or all male gametic types. Then, segregation

distortion in the female gametic sex is identically repeated in the fertilization distributions of all male gametic types, and the analogous statement applies to segregation distortion in the male gametic sex. In this way both gametic sexes can be considered separately or jointly. Post-zygotic forms of viability selection that are equivalent to gametic viability selection are realized, for example, if the survival probabilities of each (diploid) offspring genotype result from multiplication of two numbers, one representing the (haploid) genetic contribution of the female and the other that of the male gamete. These numbers can alternatively be conceived of as gametic viabilities or parameters of gametic fertilization success. On the basis of post-zygotic census data it is therefore operational to attribute an observed segregation distortion to gametic or post-zygotic viability selection according to whether the above fertilization distributions are or are not identical, respectively. For example, in the case discussed by HEDRICK and MUONA (1990), the highly significant heterogeneity among both the female and the male fertility distributions in a Scots pine selfing is a clear indication of post-zygotic viability selection, which accords with their postulation. Separation of post-zygotic viability selection from effects of haplo-homophasic mating requires additional information on frequency distributions of gametic types prior to fertilization (or fusion).

A case study in Castanea sativa Mill.

In a study of FINESCHI *et al.* (1990) on sweet chestnut (*Castanea sativa* MILL.), significant deviation from regular segregation among the female gametes in several trees was observed at the locus AP-A. This locus controls the fastest-migrating enzyme of the aminopeptidase (AP) system, that is, leucine and alanine aminopeptidase, E.C. 3.4.11.1 and E.C. 3.4.11.12, respectively. (Staining solutions were stated to be not specific for either of the two systems in this species.) The material used consisted of 20 trees growing in an isolated seed orchard comprising ca. 500 grafted trees and a seed sample from open pollination of each of the 20. In some cases a "tree" had more than one trunk; the observation that different trunks of a "tree" can differ in isoenzyme phenotype but that more than two phenotypes per isoenzyme were never observed indicates that some trunks have grown out of the root. For each of the 20 trees in this study, all seeds were harvested from only one trunk to ensure the identity of the maternal genotype (S. FINESCHI, personal communication). No two of the investigated trees (resp. trunks) belong to the same clone. Three active alleles, A_1, A_2, A_3, and a null allele A_0 were found. All data relevant here is given in *table 1*.

Female gametic segregation in heterozygous trees can be investigated in those seven of the thirteen heterozygous trees, for which a sufficient number of seeds were found to contain a non-maternal active allele and thus could be assigned an ordered genotype (*Table 1*). The respective female gametic segregation ratios are given in *table 2*. The hypothesis of homogeneity of these segregation ratios for maternal trees of the same genotype (A_0A_2: $X^2 = 8.46^*$, A_0A_3: $X^2 = 20.29^*$) must be rejected, suggesting involvement of the individual genetic backgrounds. In *table 2* it is seen that, in the single tree of genotype A_1A_3, no significant deviation of the female gametic frequencies from the 1:1 ratio expected from regular segregation was observed. Statistically significant

Table 1. — **Maternal and progeny genotypes (or phenotypes) observed at the AP-A-locus after open pollination in a clonal seed orchard of sweet chestnut (*Castanea sativa* MILL.). Data from FINESCHI et al. (1990).**

Maternal tree		Progeny genotype or phenotype						
No.	Type	Σ	A_1-	A_1A_2	A_2-	A_1A_3	A_2A_3	A_3-
			$A_0^{\varphi}A_1^{\sigma}$	$A_2^{\varphi}A_1^{\sigma}$	A_2-		$A_2^{\varphi}A_3^{\sigma}$	$A_0^{\varphi}A_3^{\sigma}$
5	A_0A_2	78			43		14	21
172	A_0A_2	73			56		13	4
523	A_0A_2	99	1	2	60		12	24
					$A_0^{\varphi}A_2^{\sigma}$		$A_3^{\varphi}A_2^{\sigma}$	A_3-
84	A_0A_3	97			37		2	58
93	A_0A_3	99			26		25	48
511	A_0A_3	51			11		7	33
			$A_1^{\varphi}-$	$A_1^{\varphi}A_2^{\sigma}$		A_1A_3	$A_3^{\varphi}A_2^{\sigma}$	$A_3^{\varphi}-$
100	A_1A_3	52	7	13		6	12	14
					$A_2^{\varphi}-$		A_2A_3	$A_3^{\varphi}-$
4	A_2A_3	50			15		21	14
22	A_2A_3	53			20		19	14
320	A_2A_3	43			16		23	4
472	A_2A_3	41			19		11	11
488	A_2A_3	52			26		20	6
515	A_2A_3	46			24		13	9

deviation was, however, observed in three of the remaining six trees, all of which are heterozygous for the null allele A_0: In one of the three trees of genotype A_0A_3 (No. 84), the statistically highly significant ratio of 37:2 in favor of A_0 indicates extreme distortion. Furthermore, for the three trees of genotype A_0A_2, all three classes of segregation are realized, namely lack of significant deviation from regular segregation (No. 5) as well as statistically significant deviation in favor of A_0 (No. 523) and against A_0 (No. 172). Thus, the fact that three of the seven trees showed significant deviation from the 1:1 ratio, together with the extreme heterogeneity of the segregation ratios, seemingly cannot be explained by random effects.

Admittedly, this data base seems quite narrow. Actually, the number of offspring sampled per tree was quite large. However, among a total of 20 investigated trees only thirteen were heterozygous, and in only seven of the

Table 2. — **Male fertilization distributions for non-maternal alleles at the gene locus AP-A, as observed in the seeds of heterozygous trees (derived from *table 1*).**

Maternal tree		Frequencies of ordered genotype $A^{\varphi}A^{\sigma}$ among offspring	Female gametic segregation ratio [1]
No.	Type		
			$(A_0^{\varphi} : A_2^{\varphi})$
5	A_0A_2	$21 \times A_0^{\varphi}A_3^{\sigma}$ $14 \times A_2^{\varphi}A_3^{\sigma}$	$(.600 : .400)^{n.s.}$
172	A_0A_2	$4 \times A_0^{\varphi}A_3^{\sigma}$ $13 \times A_2^{\varphi}A_3^{\sigma}$	$(.235 : .765)^{*}$
523	A_0A_2	$24 \times A_0^{\varphi}A_3^{\sigma}$ $12 \times A_2^{\varphi}A_3^{\sigma}$	$(.667 : .333)^{*}$
		$1 \times A_0^{\varphi}A_1^{\sigma}$ $2 \times A_2^{\varphi}A_1^{\sigma}$	$(.333 : .667)^{n.s.}$
			$(A_0^{\varphi} : A_3^{\varphi})$
84	A_0A_3	$37 \times A_0^{\varphi}A_2^{\sigma}$ $2 \times A_3^{\varphi}A_2^{\sigma}$	$(.949 : .051)^{***}$
93	A_0A_3	$26 \times A_0^{\varphi}A_2^{\sigma}$ $25 \times A_3^{\varphi}A_2^{\sigma}$	$(.510 : .490)^{n.s.}$
511	A_0A_3	$11 \times A_0^{\varphi}A_2^{\sigma}$ $7 \times A_3^{\varphi}A_2^{\sigma}$	$(.611 : .389)^{n.s.}$
			$(A_1^{\varphi} : A_3^{\varphi})$
100	A_1A_3	$13 \times A_1^{\varphi}A_2^{\sigma}$ $12 \times A_3^{\varphi}A_2^{\sigma}$	$(.520 : .480)^{n.s.}$

[1] Levels of significance for deviation from regular segregation under χ^2-test are n.s.) not significant, *)0.05, ***)0.001

thirteen were offspring found whose ordered genotypes were inferable (see *Table 1*). Thus, only relatively few (224) of the 1167 investigated seeds were utilizable for an examination of female gametic segregation proportions.

In order to derive genetic hypotheses which accord with our observations, we shall apply the classification of causes and basic methods of analysis of segregation distortion discussed in the last section. First of all, it was not possible to obtain any reliable estimates for the number of seed (offspring) produced by single trees. Moreover, the quality and structure of the data allow for an analysis of segregation in the female gametic sex only, and, with a single and insignificant exception, it provides fertilization distributions only for one male gametic type among the seed of each individual tree. This is a consequence of the fact that the genotypes observable in the seed cannot be ordered in all cases with respect to their female and male gametic contributions. The fertilization distributions are given in *table 2* and can directly be inferred from *table 1*. According to the above explanations, effects of post-zygotic viability selection therefore cannot be studied. In addition, since segregation is considered in the female gametic sex, gametophytic incompatibility cannot explain the observations.

On the other hand, the fact that per tree a male gametic fertilization distribution could be specified among the seed for essentially only one allele does not allow distinction between female gametic viability selection and haplo-homophasic mating as causes for the observed segregation distortion. However, since the experimental verification of haplo-homophasic systems of mating appears to have been successful only in a very small number of studies and species, we shall postulate female gametic viability selection as the present working hypothesis.

In our case, segregation distortion in the female gametic sex is realized, since two of the $A_3\,♂$ fertilization distributions in A_2A_0 maternal trees and the $A_2\,♂$ fertilization distribution in an A_3A_0 maternal tree deviate significantly from regular segregation (see *Table 2*). The fact that in two A_2A_0 maternal trees the distortions point in opposite directions rules out a sole effect of the AP-A-locus on gametic viability. Under this condition the probably simplest genetic model is provided by

Hypothesis 1:

The viability of a female gamete is solely determined by the allele it carries at gene locus D, say, and this locus is linked to the AP-A-locus. Gametic viability is not modified by the (diplo-) genotype of the maternal tree.

All maternal trees showing no segregation distortion at the A-locus (No. 5 being A_0A_2, Nos. 93 and 511 both A_0A_3, and No. 500 with A_1A_3) can then be assumed to be homozygous at the D-locus. Now suppose that D_1 confers higher viability to the gametes carrying this allele than does D_2, and that in tree No. 172 genes A_2 and D_1 are located on one chromosome and A_0 and D_2 on the other. The genotype of the maternal tree can thus be written as A_2D_1/A_0D_2. Hence, through linkage, A_2 is expected with higher frequency than A_0 among the successful female gametes, as is the case. The association A_2D_2/A_0D_1 would explain the excess of A_0-gametes in maternal tree No. 523, while the excess of A_0-gametes in tree No. 84 would accord with the maternal genotype A_3D_2/A_0D_1.

Since the recombination fraction among the A- and D-locus is likely to be same in all three maternal trees, the degrees of segregation distortion can also be expected to be the same. Yet, with high statistical significance, this is not the case. Hence, in order to retain the hypothesis, more than two alleles differing in gametic viability must be assumed to exist at the D-locus. In fact, the two A_0A_2 maternal trees (Nos. 172, 523) do not differ significantly in their degrees of segregation distortion (0.765 versus 0.667), so that both can be assumed to be heterozygous for the same pair of alleles at the D-locus as suggested above. The A_0A_3 maternal tree (No. 84) is then heterozygous at the D-locus for a third allele which must be assumed to cause the strongest reduction in gametic viability.

It is, of course, unsatisfactory that the data on the third heterozygous maternal genotype A_2A_3, which is one of the two most frequent genotypes, do not allow verification of the hypothesis, since fertilization distributions cannot be inferred for any male gametic type (cf. *Table 1*). We thus do not know whether the patterns of segregation realized by the carriers of this genotype are compatible with hypothesis 1. The only situation in which the hypothesis could be falsified with high probability would arise if all of the A_2A_3 maternal trees showed regular segregation among their female gametes. Hence, this situation, if it were realized, would require a different genetic model, and it will be considered in the following.

Again, because of the opposite segregation distortions observed for genotype A_0A_2, at least one additional gene locus must be assumed to affect gametic viability. As in the previous model, this locus will be denoted by D. Whether D-alleles result in differential viability of the gametes in which they are present now depends on the genotype realized at the AP-A-locus. Hence, one obtains

Hypothesis 2:

The viability of a female gamete is determined by the allele it carries at a gene locus D together with the maternal genotype at the linked locus AP-A. The AP-A-locus acts as a modifier of gametic viability selection at the D-locus in such a way that allelic differences at the D-locus become effective only in maternal trees carrying the allele A_0 at the AP-A-locus.

Thus the A-locus has two functions. One consists in realizing the potential for gametic viability selection at the D-locus, and the other consists in allowing inference of the segregation distortion produced at the D-locus. The effect of A_0 in the maternal tree is similar to that of an environmental condition which turns a previously neutral genetic polymorphism into a selective one (here referring to the D-locus). This phenomenon appears to emerge particularly under stress conditions (cf. e. g. Stebbins and Hartl, 1988). It is interesting in this context to note that null alleles are generally considered to be non-functional, so that carriers of A_0 might be conceived of as providing internal "stress" conditions for the development of germ cells. The fact that individuals homozygous for the A_0-allele were found neither among the maternal trees nor among their seed might be taken as further evidence supporting this suggestion.

More explicitly, the maternal heterozygotes A_2A_0 and A_3A_0 are assumed to induce gametic viability selection at the D-locus, while the heterozygotes A_1A_3 and A_2A_3 are assumed not to induce such selection. Consequently, all of the above explanations obtained for hypothesis 1 can be retained for the trees of genotype A_2A_0 and A_3A_0. In particular, this includes the location of A- and D-alleles on the same chromosome and the resulting opposite direc-

tions in segregation distortion, homozygosity at the D-locus, and the number of alleles at this locus. The only difference arises with respect to the possible interpretations of the regular segregation among the female gametes of genotype A_1A_3, since homozygosity at the D-locus is not required in order to explain this observation under the present hypothesis.

Discussion

The present study probably differs from the majority of studies on segregation distortion or meiotic drive in that offspring from open pollination rather than from controlled crosses were used. The disadvantage of this type of observation lies in the necessary restriction of the analysis to one gametic sex, the female, since the contributions of individual male parents cannot be distinguished. On the other hand, the use of single plant offspring from open pollination has the advantage that the female gametic types of a single individual appear in association with a large number of male gametic types. Thus, post-zygotic effects of the genetic background on the female gametic segregation frequencies observable at marker gene loci have a higher probability of detection than can be expected from crosses of a small number of paternal parents with one maternal parent. Yet, for the above data, we could not take advantage of this opportunity, since the offspring genotypes are unordered (male and female contributions to a zygote are indistinguishable), and since only four alleles were found among which one (a so-called null allele) was completely recessive. The latter situation (four alleles, one of which was rare and another completely recessive) prohibited an analysis of post-zygotically acting effects, so that gametic viability selection remained as the object of derivation and testing of genetical hypotheses.

The concept of "fertilization distributions", which does not seem to have been explicitly applied in earlier work on the subject, proved to be a very effective means of analysis, even under the restrictive conditions set by our data. The concept is particularly suited to enable discrimination between effectively pre-zygotic and effectively post-zygotic impacts on the observed segregation frequencies, provided male and female gametic contributions to the offspring can be distinguished. If the genotypes of all offspring can be ordered in this sense, such discrimination is possible among offspring from both controlled crosses (including multiple crosses) and open pollination. However, the only technique known to yield ordered genotypes for all offspring from open pollination is based on endosperm-embryo comparisons in seed of gymnosperms (introduced by MÜLLER[-STARCK], 1976). The technique was used, for example, by CHELIAK *et al.* (1987) to infer post-zygotic viability selection as a possible explanation for the segregation distortion observed at one of the enzyme loci in Norway spruce. By studying both the maternal and paternal contributions to controlled cross seed of Douglas-fir, ADAMS *et al.* (1990) determined that in cases in which distortion was consistent in more than one parent tree, it occurred in only one gametic sex. Application of the above-described concept of fertilization distributions to their data could provide more detailed insight.

The two genetic hypotheses suggested as possible explanations of the present observations were inspired by the detection of "reversed" segregation distortion in the seed of two maternal trees with the same genotype at the AP-A gene locus. This locus was therefore postulated to be strongly, though not completely, linked to a second locus (D), the alleles of which are involved in gametic viability selection. The one genetic hypothesis assumes that AP-A has no effect on gametic viabilities, while the other assumes that it has a modifying effect. Although there is some additional evidence in support of the second hypothesis, the data did not suffice to give clear priority to either of the two.

Both Hypothesis 1 and 2 appear to have paradigms in the technical literature. In the following we will consider one of the most extensively studied systems of segregation distortion, the SD-system in *Drosophila melanogaster* first reported by SANDLER et al. (1959). These authors observed that, for several crosses between males which were chromosomally heterozygous for a "normal" and variant second chromosome and chromosomally "normal" homozygous females, the variant paternal chromosome was found in 83%—98% of the progeny instead of in only 50% as expected under regular segregation. In the reciprocal crosses between chromosomally heterozygous females and homozygous males, the two chromosomes were found in (statistically) equal proportions. Additional experiments led them to attribute this observation to dysfunction of sperm containing the "normal" chromosome. Further efforts led to the formulation of several models to explain the distortion (see reviews in e. g. CROW, 1979; HIRAIZUMI, 1990). Without going into more detail here, two gene loci, termed Sd and Rsp, located on the second chromosome are postulated as being mainly responsible for the distortion, with additional loci modifying the extent of distortion. The paternal genotype at Sd appears to have a modifying effect on spermiogenesis in that certain Sd-genotypes disrupt the maturation of sperm containing certain allelic variants at Rsp. This principle remains essentially unaltered, despite the recent observation of additional phenomena which seem to require even more complex genetic models (HIRAIZUMI, 1990; GOLIC, 1990; TEMIN, 1991).

In the last section, two hypotheses were advanced as possible explanations for the observed deviations from regular segregation among female gametes of heterozygous sweet chestnut trees. The question now arises as to whether a hypothetical system of segregation distortion formally analogous to the SD-system but operating during female instead of male gamete development can be shown to conform to one or both of these hypotheses. We shall see that the SD-system can indeed be used as a paradigm for both hypotheses.

Comparison of the SD-system with Hypothesis 1: The Sd and Rsp loci are located on the same chromosome. From the point of view of a single linked marker locus, the effects of the two-locus SD-system on sperm viability could not be separated from male gametic viability selection attributable to different alleles at single (artificial) "SD"-gene locus. If we call the artificial gene locus D and suppose that the AP-A-locus is the linked marker locus, then it is seen that the SD-system provides an example of Hypothesis 1.

Comparison of the SD-system with Hypothesis 2: As opposed to Hypothesis 1, Hypothesis 2 postulates the direct action of AP-A as a modifier of gametic viability selection at the D-locus, such that allelic differences at D become effective only if the null allele A_0 is present in the genotype of the maternal tree. This hypothetical system of segregation distortion is observable due to the additional

function of AP-A as a marker locus. It parallels the SD-system if AP-A is assigned the role of the Sd-locus and D acts as the Rsp-locus. In this case, A_0 would correspond to one of the Sd-alleles and A_1, A_2, and A_3 would be allelic variants of the other, while D_1 would correspond to the "insensitive" allele Rsp^i and D_2 to the "sensitive" allele Rsp^s. With the help of this analogy, we can explain all of our observations. For one, the reversal in the direction of distortion with respect to A_0 can be reasoned by the fact that, in a male heterozygous for Sd/Sd+ and Rsp^s/Rsp^i, the Sd-allele which happens to be located on the Rsp^s-chromosome is the one to be distorted. In addition, the observation of Rsp^s-alleles of differing sensitivity (corresponding in our case to D_2 and D_3) can be drawn upon to explain the heterogeneity in the amount of distortion observed. In fact, one of the amounts is of the same extreme order of magnitude as that observed in the SD-system. Furthermore, the necessarily two-fold function of the AP-A-enzyme as modifier and marker locus is quite credible, since the Sd-locus is assumed to produce a regulatory protein (perhaps an enzyme?) which could, at least in principle, be made visible by means of electrophoresis. Finally, just as A_0 can be thought to cause internal "stress" conditions for megasporogenesis, as mentioned above, the modifying effect of the allele Sd on Rsp^s can be considered as a similar condition for spermatogenesis.

References

Adams, W. T., Neale, D. B., Doerksen, A. H. and Smith, D. B.: Inheritance and linkage of isozyme variants from seed and vegetative bud tissues in coastal Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco]. Silvae Genetica **39**: 153—167 (1990). — Boyle, T. J. B. and Morgenstern E. K.: Inheritance and linkage relationships of some isozymes of black spruce. Can. J. For. Res. **15**: 992—996 (1985). — Cheliak, W. M., Morgan, K., Dancik, B., Strobeck, C. and Yeh, F. C.: Segregation of allozymes in megagametophytes of viable seed from a natural population of jack pine *Pinus banksiana* Lamb. Theor. Appl. Genet. **69**: 145—151 (1984). — Cheliak, W. M., Skrøppa, T. and Pitel, J. A.: Genetics of the polycross. 1. Experimental results from Norway spruce. Theor. Appl. Genet. **73**: 321—329 (1987). — Crow, J. F.: Genes that violate Mendel's rules. Scientific American **240** (2): 104—113 (1979). — Crow, J. F.: The ultraselfish gene. Genetics **118**: 389—391 (1988). — Fineschi, S., Gillet, E. and Malvolti, M. E.: Genetics of sweet chestnut (*Castanea sativa* Mill.). III. Genetic analysis of zymograms of single tree offspring. Silvae Genetica **39**: 188—194 (1990) — Geburek, T. and von Wühlisch, G.: Linkage analysis of isozyme gene loci in *Picea abies* (L). Karst. Heredity **62**: 185—191 (1989). — Golic, K. G.: Examination of a mutable system affecting the components of the Segregation Distorter meiotic drive system of *Drosophila melanogaster*. Genetics **125**: 51—76 (1990). — Grant, V.: Genetics of Flowering Plants. Columbia University Press, New York and London (1975). — Gregorius, H.-R.: Characterization and Analysis of Mating Systems. Ekopan Verlag, Witzenhausen (1989). — Haustein, E.: Die selektive Befruchtung. In: Ruhland, W. (ed): Handbuch der Pflanzenphysiologie. Band XVII. Springer-Verlag, Berlin, Heidelberg, New York (1967). — Hedrick, P. and Muona, O.: Linkage of viability genes to marker loci in selfing organisms. Heredity **64**: 67—72 (1990). — Hiraizumi, Y.: Negative segregation distortion in the SD system of *Drosophilla melanogaster*: A challenge to the concept of differential sensitivity of *Rsp* alleles. Genetics **125**: 515—525 (1990). — Leach, C. R.: Detection and estimation of linkage for a co-dominant structural gene locus linked to a gametophytic self-incompatibility locus. Theor. Appl. Genet. **75**: 882—888 (1988). — Müller [-Starck], G.: A simple method of estimating rates of self-fertilization by analyzing isozymes in tree seeds. Silvae Genetica **25**: 15—17 (1976). — Neale, D. B., Weber, J. C. and Adams, W. T.: Inheritance of needle tissue isozymes in Douglas-fir. Can. J. Genet. Cytol. **26**: 459—468 (1984). — Sandler, L., Hiraizumi, Y. and Sandler, I.: Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion. Genetics **44**: 233—250 (1959). — Stebbins, G. L. and Hartl, D. L.: Comparative evolution: Latent potentials for anagenetic advance. Proc. Natl. Acad. Sci. USA **85**: 5141—5145 (1988). — Strauss, S. H. and Conkle, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483—493 (1986). — Temin, R. G.: The independent distorting ability of the Enhancer of Segregation Distortion, E(SD), in *Drosphila melanogaster*. Genetics **128**: 339—356 (1991). — Torres, A. M., Mau-Lastovicka, T., Vithanage, V. and Sedgley, M.: Segregation distortion and linkage analysis of hand pollinated avocados. J. Heredity **77**: 445—450 (1986). — Torres, A. M., Mau-Lastovicka, T., Williams, T. E. and Soost, R. K.: Segregation distortion and linkage of *Citrus* and *Poncirus* isozyme genes. J. Heredity **76**: 289—294 (1985). — Wendel, J. F. and Parks, C. R.: Genetic control of isozyme variation in *Camellia japonica* L.. J. Heredity **73**: 197—204 (1982).

Genetic Control and Inheritance of Isoenzymes in Poplars of the Tacamahaca Section and Hybrids

By G. Müller-Starck*)

Abteilung Forstgenetik und Forstpflanzenzüchtung,
Universität Göttingen, DW-3400 Göttingen, Germany

(Received 1st August 1991)

Abstract

Eleven full sib families were analysed by means of starch gel electrophoresis or isoelectric focussing in order to verify the genetic control and the mode of inheritance of the polymorphism of 15 enzyme systems. The study includes a check on environmental, ontogenetic and tissue specific variation of isoenzymes with inclusion of in vitro stages and also on the localization of certain enzyme systems within cell compartments. Intra-individual modification of isoenzymes can occur in the systems ACP, EST, and PER, which therefore were not included in the genetic analyses.

Segregation within full sib families revealed condominant expression of the studied enzyme types. It is concluded that 12 enzyme systems are controlled genetically by at least 18 polymorphic gene loci. For a set of 39 *Tacamahaca* clones and hybrids the average number of alleles per locus is 3.4. Recombination analyses were performed on the basis of 62 different two-locus-combinations. Highly significant deviations from random segregation are indicated for each of two pairs of loci: GOT-A/GOT-B and NDH-A/PGM-A. The loci IDH-B, 6PGDH-B,

*) Since 1991: Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft, CH-8903 Birmensdorf-ZH, Switzerland