GENETIC RELATEDNESS AMONG DEVELOPING SEEDS AND INTRA FRUIT SEED ABORTION IN *DALBERGIA SISSOO* (FABACEAE)[1]

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Dalbergia sissoo, a wind-dispersed tropical tree, exhibits high intrafruit seed abortion. Of the four to five ovules in the flower, generally one and occasionally two or three develop to maturity. It has been proposed that the seed abortion is a consequence of intense sibling competition for maternal resources and that this competition occurs as an inverse function of the genetic relatedness among the developing seeds. Accordingly, developing seeds compete intensely when they are genetically less related but tend to develop together when genetically more related. We tested this hypothesis by comparing the genetic similarity among the pairs of seeds developing within a pod with that among (a) random pairs from the pool of all seeds, (b) random pairs from single-seeded pods, and (c) random pairs from two-seeded pods, using both randomly amplified polymorphic DNA (RAPD) and isozymes in five trees. We found that the pairs of seeds developing within a pod are genetically more similar than any random pairs of seeds in a tree. Thus the formation of two-seeded pods appear to be associated with increased genetic relatedness among the developing seeds. We discuss the results in the context of possible fitness advantages and then discuss the possible mechanisms that promote tolerance among related seeds.

Key words: *Dalbergia sissoo*; Fabaceae; genetic relatedness; inclusive fitness; kin selection; seed abortion; sibling rivalry.

Intrafruit seed abortion, wherein only a proportion of fertilized ovules matures into seed, is a widespread phenomena in multiovulated species. The abortion may range from as high as 93% (e.g., in *Kleinhovia hospita*; Uma Shaanker, Ganeshaiah, and Bawa, 1988) to none (in some members of the Euphorbiaceae; Ganeshaiah and Uma Shaanker, 1991). In *Cassia fasiculata* though 95% of the ovules are fertilized, only ~53% mature into seeds (Stephenson, 1981). The abortion of the seeds within fruit could be random or systematic with respect to the position of the aborted embryo (Stephenson, 1981). In species with linearly arranged ovules, such as in members of Leguminosae, embryos at either the peduncular (Bawa and Webb, 1984; Guth and Weller, 1986; Ganeshaiah and Uma Shaanker, 1988; Arathi, 1990; Mohan Raju, 1993), stigmatic (Meinke, 1982; Donnel and Bawa, 1993), or at both ends (Hedley and Ambrose, 1981) are reported to systematically abort. However, in species with nonlinearly (e.g., radially) arranged ovules, it is often difficult to characterize the spatial pattern of abortion (Casper and Weins, 1981; Arathi, 1990; Rigney, 1995). Based on the observed patterns in the extent of abortion, seed abortion could be either variable or stringent. Species such as *Dalbergia sissoo* exhibit a consistently highly positively skewed distribution of seed number per pod but variable over trees and season (Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, 1993). However, in species such as *Syzygium cuminii,* the abortion is invariant and stringent with all trees over all locations maturing only one of the

30–35 ovules (Arathi, 1990). Several ecological correlates of seed abortion have been identified in plants. Seed abortion tends to increase with habit from herbs to trees and with breeding system from self to outbred (Bawa and Webb, 1984; Weins, 1984; Weins et al., 1987; Cumaraswamy and Bawa, 1989). Species with fruit as a unit of dispersal that are dispersed through wind, water, or animals show on average higher seed abortion than those with seeds as the unit of dispersal (Uma Shaanker, Ganeshaiah, and Bawa, 1988).

Several proximate mechanisms have been put forth to explain the low seed to ovule ratio in plants. Lack of sufficient pollen loads on the stigma to fertilize all (Wilson and Schemeske, 1980; Petersen, Brown, and Kodric-Brown, 1982; Snow, 1982; Gross and Werner, 1983; Schemske and Pautler, 1984; Weins, 1984; Zimmerman and Pyke, 1988) and the lack of maternal resources to provide for seed development (Willson and Schemske, 1980; Stephenson, 1981; Wyatt, 1981; Lee and Bazzaz, 1982, 1986; Weins, 1984; Zimmerman and Pyke, 1988) have been most commonly attributed as causes for the low seed set in plants. However, these hypotheses have not always stood the test of critical experimentation. Thus, in a number of species, supplemental pollination (Casper, 1983; Uma Shaanker and Ganeshaiah, 1984; Guth and Weller, 1986) and or enhancement of resources status of the plants (Bawa and Webb, 1984; Ho, 1988) have failed to inhibit seed abortion. The suggestion that abortion could be due to the expression of developmentally recessive lethals (Weins, 1984; Weigel and Hughes, 1986; Weins et al., 1987; Bawa et al., 1989) also does not seem to explain the observed seed abortion. Studies have shown that embryos that are otherwise aborted could be rescued in vitro or by removing the effect of dominance among the developing ovules (Weigel and Hughes, 1986; Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, 1993). None of these hypotheses satisfactorily explain the nonrandom pat-

terns of seed abortion observed in plants, over seasons and location. Further, they also do not explain the ecological correlates of seed abortion in plants (Bawa and Webb, 1984; Weins et al., 1987).

Uma Shaanker, Ganeshaiah, and Bawa (1988) proposed a paradigm shift in the explanation of the observed patterns of seed abortion in plants by extending the principles of sociobiology to plants. They argued that the consistent patterns of seed abortion in plants can be best viewed as a consequence of intrafruit sibling competition to be the lone survivors in the fruit and thus to maximize their individual fitness. In plants several components of offspring fitness, such as dispersal efficiency, escape from predation, and postdispersal seedling survival decreases with increase in the number of seeds packed per fruit (brood size). For instance, in species in which the entire fruit is dispersed as a unit through wind, water, or animals, the dispersal efficiency of the fruits decreases with the seediness of the fruits (Ridley, 1930; Janzen, 1982; Augspurger and Hogan, 1983; Ganeshaiah and Uma Shaanker, 1988, 1991; Hegde, Uma Shaanker, and Ganeshaiah 1991a, b). Furthermore, seeds in a large brood are more likely to be preyed upon than those in a small brood. Ganeshaiah and Uma Shaanker (1988) argued that under these conditions, selection would favor fratricide on the part of each sibling in a fruit, thus enhancing individual fitness. In this sense, they argued that fruits in plants are akin to a clutch in birds (Nakamura, 1980; Kress, 1981), such that sibs developing within a fruit would be selected to be "selfish" and competitive, a situation often leading to intense fratricide (Ganeshaiah and Uma Shaanker, 1988; Uma Shaanker, Ganeshaiah, and Bawa, 1988; Ganeshaiah, Uma Shaanker, and Joshi, 1991; Uma Shaanker and Ganeshaiah, 1998; Arathi et al., 1996; Mock and Parker, 1997, 1998).

Ganeshaiah and Uma Shaanker (1988) showed that the sibling-rivalry-induced seed abortion in plants could be mediated through a process wherein the "dominant" embryo developing within a fruit usurps resources leading to the selective starvation of the young "subordinate" embryos or in the death of the subordinate embryos. For example, in *Syzygium cuminii* (Krishnamurthy, Uma Shaanker, and Ganeshaiah, 1997) and *Dalbergia sissoo* (Mohan Raju, Uma Shaanker, and Ganeshaiah, 1996), it was shown that the dominant embryo within the fruits inhibited the translocation of resources to the subordinate embryos. Such dominant-embryo-induced abortion of the remaining embryos in the ovary has also been reported in a few other species (in *Quercus*—Mogenson, 1975; in *Macadonia*—Sedgley, 1981; and in *Kleinhovia hospita*—Uma Shaanker and Ganeshaiah, 1989). However, if such "dominance" effect were to be removed (by experimental excision of the dominant embryos), the inhibition is totally relieved (Mohan Raju, 1993). Extracts and diffusates of the dominant embryos significantly inhibited the uptake of resources by the subordinate embryos compared to those of control tissues (maternal tissue). In other words, abortion of embryos seems to be mediated by sibling rivalry (fratricide) and not due to maternal intervention in these species (infanticide; Arathi, 1990; Krishnamurthy, 1995; Krishnamurthy, Uma Shaanker, and Ganeshaiah, 1997).

Based on inclusive fitness models, Uma Shaanker, Ganeshaiah, and Bawa (1988) argued that the extent of sibling rivalry among the developing ovules will be a function of the genetic relatedness between them, and accordingly, genetically more related embryos would tolerate their mutual develop-

ment. Though this might increase seediness of the fruits and thus reduce the dispersal advantage of individual seeds, Uma Shaanker, Ganeshaiah, and Bawa (1988) argued that the inclusive fitness accrued through the joint survivorship of the genetically related sibs (kins) could compensate for the loss in dispersal advantage. Uma Shaanker, Ganeshaiah, and Bawa (1988) showed that for relatively small benefits due to seed abortion, an offspring would be more selected to favor killing its siblings when they are half-sibs ($r = 0.25$) than when they are full sibs ($r = 0.50$). Thus, they predicted that sibling-rivalry-driven seed abortion should be more intense in outbred compared to inbred conditions.

Though there are no direct tests of the above prediction, anecdotal evidence exists that offers support for this view. In pigeon pea, intrafruit seed abortion was positively correlated with the extent of outcrossing (Cumaraswamy and Bawa, 1989). In *Epilobium*, congeneric species that were outbred had a higher degree of seed abortion than those that were inbred (Weins et al., 1987). In *Phaseolus latheroides*, the extent of seed abortion increased with increase in the number of pollen donors used for pollination (Vasudeva, 1995). Kress (1981) predicted that competition among embryos would be most severe when the seeds within fruits have several fathers.

Several studies at the whole-plant level suggest that interactions among plants could have a kin selection basis. Cheplick (1992) reported that the degree of relatedness among the offspring is likely to be increased in plants that have a self-fertilized breeding system and that this could enhance the likelihood of interactions between the genetically related individuals. In *Phytolacca americana*, plants growing with their siblings had a greater growth (synergistic effect) compared to plants growing with nonsiblings (antagonistic effect; Nakamura, 1980). Under intraspecific competition in the glass house, the number of *Plantago lanceolata* plants flowering per pot increased with the genetic relatedness from nonsibs to half sibs to full sibs (Tonsor, 1989).

In this paper, we provide a test of the argument that seed abortion in fruits of *Dalbergia sissoo*, a wind-dispersed tropical tree species, is a function of the genetic relatedness among the sibs developing within a fruit. In *Dalbergia*, predominantly only 1 of the 4–5 ovules per ovary develops to maturity. Following fertilization, usually 1 and occasionally 2 or 3 of the 4–5 ovules dominate and kill the rest (Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, 1993; Mohan Raju, Uma Shaanker, and Ganeshaiah, 1995, 1996). Uma Shaanker, Ganeshaiah, and Bawa (1988) hypothesized that the extent of rivalry, and hence the abortion, are a function of the genetic relatedness among the developing seeds. Accordingly, genetically related embryos should exhibit less competition in a pod resulting in low seed abortion, while genetically less related siblings exhibit severe competition resulting in high seed abortion. A testable prediction of this hypothesis is that the mean genetic relatedness among developing seeds chosen at random from the population should be less than the genetic relatedness among two or more seeds matured together in a pod.

MATERIALS AND METHODS

Study system—*Dalbergia sissoo* Roxb. ($2n = 20$, $x = 10$; Fabaceae) is an economically important, wind-dispersed timber tree species in the tropical deciduous of south and central India (Troup, 1986). The trees flower during March–April and are outcrossed primarily by bees. Twenty-five trees located in and around the University of Agricultural Sciences, G.K.V.K. campus, at

TABLE 1.   Number of seeds and ovules and seed to ovule ratios of five trees of *Dalbergia sissoo* used in the study.

Tree	Number of seeds X ± SD (N)	Number of ovules X ± SD (N)	Seed to ovule ratio	Pods with >1 seed (%)
T-35	1.12 ± 0.365 (100)	4.67 ± 0.565 (125)	0.239	11.12
T-27	1.72 ± 0.919 (161)	4.77 ± 0.640 (161)	0.360	44.73
T-10	1.69 ± 0.051 (100)	4.59 ± 0.091 (150)	0.368	29.30
T-20	1.18 ± 0.025 (100)	4.57 ± 0.104 (150)	0.258	17.35
T-24	1.27 ± 0.475 (152)	5.26 ± 0.617 (152)	0.241	18.37

Bangalore, India, were censused for ovule number per ovary and seed number per pod. Of them, four trees (T-35, T-27, T-10, and T-20) with widely differing mean seed number per pod were selected for studies using isozyme analysis, and two trees (T-35 and T-24) were used for RAPD analysis (Table 1).

Estimation of genetic relatedness between seed pairs—In *Dalbergia sissoo,* the abortion of embryos occur at a very early stage, within a week after fertilization (Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, Uma Shaanker, and Ganeshaiah, 1995, 1996). Consequently, it is not practically feasible to obtain sufficient amounts of embryonic tissue for extracting isozymes and reasonable quantities of DNA for the estimation of genetic relatedness among the developing seeds, though, in certain other systems, the paternity of the aborted seeds at early stages has been successfully established (Rigney, 1995).

The kin-selection-based argument proposed by Uma Shaanker, Ganeshaiah, and Bawa (1988) states that if two competing embryos are less genetically related, then generally the first fertilized dominates and aborts others. Thus, seeds surviving in the single seeded pods represent a random subset of the range of possible genotypes in the species and hence, genetic relatedness among them should represent the average expected among the developing seeds. Accordingly, if two or more seeds develop in a pod owing to their increased genetic relatedness, then the genetic similarity among them can be expected to be greater than that among seeds from single-seeded pods. We tested this prediction by comparing the genetic similarities between seeds developing in two-seeded pods with those between randomly chosen seeds to obviate the problem of recovering the samples from very young developing seeds.

Isozyme analysis—Five-day-old seedlings from one- and two-seeded pods grown in petri dishes were extracted in vegetative buffer I at pH 6.7 (Cheliak and Pitel, 1984). The samples were electrophoresed in histidine buffer (0.125 mol/L Tris, pH 7.0 with 1 mol/L citric acid) using a 10% starch gel. The enzymes were assayed by activity staining (Cheliak and Pitel, 1984) using an agar overlay technique (Uma Shaanker and Ganeshaiah, 1997). The enzymes included in the study are ADH, MDH, MR, PGI, 6 PGDH, ALD, EST, G6PDH, IDH, and PGM (for details see Appendix). Since the genetic interpretation of the isozyme banding patterns could not be done unambiguously, we scored for each enzyme the individual band presence (=1) and absence (=0) for further analysis of the genetic similarity (Wendel and Weeden, 1989; Chung et al., 1991; Dolan, 1994).

DNA analysis—Five-day-old germinated seeds from one- and two-seeded pods grown in petri dishes were used for extraction of DNA following the method of Doyle and Doyle (1987). The amplification of DNA was done using nine selected randomly designed oligonucleotide primers viz. OPT 17, OPQ 04, OPQ 08, OPM 05, OPC 06, OPC 14, OPC 05, OPM 17, OPQ 14 (Operon Technologies, Alameda, California, USA) following the protocol of Williams et al. (1990). The amplified products were electrophoresed on 1% agarose gels, stained with ethydium bromide, and bands were scored using a binary code.

Statistical analysis—*Similarity index*—Genetic similarity between pairs of seeds was estimated as the squared Euclidean distance following Ludwig and Reynolds (1988) using the formula

$$SI = \sqrt{\sum_{i=1}^{n} (X_{ij} - X_{ik})^2}$$

where n = the number of bands recovered from all enzymes and X_{ij} and X_{ik} are the values (1 or 0) for ith band of jth and kth seeds. Accordingly, four estimates of the similarity index (SI) were computed for each tree: (1) pooled SI: average similarity index among all possible pairs of seeds from one- and two- seeded pods; (2) single-seeded pod SI: average similarity index among all possible pairs of seeds from one-seeded pods; (3) two-seeded pod SI (random): average similarity index among all possible pairs of seeds from two-seeded pods; and (4) two-seeded pod SI (intra-pod): average similarity index between pairs of seeds within two-seeded pods.

In addition, we also computed the above similarity indices (referred to as trimmed similarity index) by excluding those bands that were invariant in the group studied. We excluded bands whose frequencies were either <10% (nearly completely absent) and >90% (nearly completely present).

A bootstrapping analysis was conducted by randomly selecting 25 samples within each group (pooled, single-seeded, and two-seeded random) with replacement. The mean similarity index over all possible pairs derived from the 25 samples (300 pairs) was computed and compared with the observed similarity index of two-seeded pods. The iteration was repeated 500 times.

The standard deviation for each estimate was also computed. Similarity indices were computed for seeds of each tree separately. To avoid the effect of intertree genetic variation in our analysis we did not pool data across trees (Appendix).

The similarity indices based on the pooled, single-seeded, and two-seeded random sample bootstrap values were compared with the observed similarity index of the two-seeded pods using Student's t test (Siegel and Castellan, 1988). The frequency distribution of the similarity indices of the single-seeded and two-seeded (intrapod) pods was analyzed and examined for its deviation from normality following the G test (Siegel and Castellan, 1988). Further, the two distributions were compared using the Kolmogorov-Smirnov test (Siegel and Castellan, 1988).

Clustering—Based on the calculated SIs, a cluster analysis was performed using the Multivariate Statistical Package (MVSP87) to examine whether the seeds from the single-seeded and two-seeded pods differ in their genetic configurations. We computed the squared Euclidean distances among all possible pairs of seeds among the various groups (as mentioned above), and following a minimum variance algorithm we performed a clustering analysis and constructed a dendrogram of the clusters (Ludwig and Reynolds, 1988).

Segregation of seeds from one- and two-seeded pods into clusters was tracked to test whether the two seeds from each of the two-seeded pods have a tendency to cluster together (termed ''aligned'' into the same cluster). The expected number of such ''aligned'' two-seeded pods, assuming that seeds segregated randomly, was estimated based on the relative size of each cluster, the number of clusters, and the number of pods. For example, the number of two-seeded pods expected to ''align'' in the ith cluster (A_i) was computed as

$$A_i = (n_i/N)^2$$

where n_i is the number of seeds from two-seeded pods in the ith cluster and N is the total number of seeds from two-seeded pods. The total number of two-seeded pods expected to be ''aligned'' in all the clusters was obtained by summing these A_i values over all clusters. The deviation between expected and observed number of ''aligned'' pods was tested using the χ^2 test (Siegel and Castellan, 1988).

RESULTS

The mean intrapod genetic similarity of seeds in two-seeded pods was significantly higher compared to that of random pairs selected from pooled seeds, from single-seeded pods, or from two-seeded pods; in only one case involving T_{24} was the similarity index significantly different between the two-seeded (random pairs) and the intra-pod pairs (Tables 2 and 3). This

TABLE 2. Mean ± SD and ranges (in parentheses) of the similarity indices (SI) among different categories of seeds.

Tree	Pooled				Single-seeded pods				Two-seeded (random) pods				Two-seeded (intrapod) pairs			
	N	NI	Original	Trimmed	N	NI	Original	Trimmed	N	NI	Original	Trimmed	N	NI	Original	Trimmed
Isozyme																
T-35	169	14196	0.73 ± 0.13 (0.27–1.0)	0.62 ± 0.17 (0.08–1.0)	75	2775	0.73 ± 0.13 (0.27–1.0)	0.60 ± 0.17 (0.09–1.0)	94	4371	0.73 ± 0.13 (0.33–1.0)	0.63 ± 0.18 (0.16–1.0)	94	47	0.88 ± 0.13 (0.61–1.0)	0.83 ± 0.17 (0.41–1.0)
T-27	144	10296	0.81 ± 0.08 (0.43–1.0)	0.65 ± 0.14 (0.23–1.0)	60	1770	0.81 ± 0.09 (0.53–1.0)	0.58 ± 0.190 (0.16–1.0)	84	3486	0.82 ± 0.08 (0.46–1.0)	0.61 ± 0.16 (0.16–1.0)	84	42	0.93 ± 0.07 (0.73–1.0)	0.86 ± 0.13 (0.58–1.0)
T-10	67	2211	0.74 ± 0.09 (0.43–1.0)	0.62 ± 0.14 (0.07–1.0)	29	406	0.81 ± 0.08 (0.56–1.0)	0.68 ± 0.13 (0.33–1.0)	37	703	0.73 ± 0.10 (0.43–1.0)	0.58 ± 0.16 (0.07–1.0)	38	19	0.88 ± 0.10 (0.65–1.0)	0.823 ± 0.14 (0.46–1.0)
T-20	60	1770	0.78 ± 0.07 (0.55–1.0)	0.68 ± 0.10 (0.39–1.0)	24	276	0.83 ± 0.07 (0.65–1.0)	0.66 ± 0.13 (0.32–1.0)	36	630	0.77 ± 0.07 (0.58–0.9)	0.675 ± 0.10 (0.41–0.97)	36	18	0.88 ± 0.18 (0.75–0.97)	0.84 ± 0.08 (0.63–0.95)
DNA																
T-35	30	435	0.71 ± 0.14 (0.36–1.0)	0.63 ± 0.14 (0.28–1.0)	16	120	0.71 ± 0.05 (0.36–0.97)	0.62 ± 0.19 (0.2–0.96)	14	91	0.78 ± 0.10 (0.56–1.0)	0.63 ± 0.16 (0.34–1.0)	14	7	0.94 ± 0.09 (0.75–1.0)	0.90 ± 0.14 (0.59–1.0)
T-24	28	378	0.69 ± 0.08 (0.51–0.9)	0.60 ± 0.14 (0.31–1.0)	12	66	0.70 ± 0.07 (0.55–0.86)	0.60 ± 0.12 (0.32–0.81)	16	120	0.82 ± 0.08 (0.56–1.0)	0.58 ± 0.12 (0.30–1.0)	16	8	0.83 ± 0.08 (0.65–0.92)	0.614 ± 0.10 (0.43–0.78)

Note: N = number of seeds, NI = number of pairs compared, and trimmed refers to the data in which invariant alleles were removed.

TABLE 3. Computed Student *t* values for the differences in similarity indices between different categories of seeds.

	Trees	Single-seeded pods	Two-seeded (random) pods	Two-seeded (intrapod) pairs
Pooled	T-35	1.11	1.77	7.94**
	T-27	1.42	3.18**	9.70**
	T-20	15.27**	2.24	6.55**
	T-10	10.37**	2.46	6.11**
	T-35	0.23	4.27**	4.23**
	T-24	0.95	15.74**	4.96**
Single-seeded pods	T-35		0.31	7.73**
	T-27		3.28	8.79**
	T-20		14.11**	3.31**
	T-10		10.88**	2.90**
	T-35		6.55**	11.14**
	T-24		10.39**	4.96**
Two-seeded pods (random pairs)	T-35			7.71**
	T-27			9.27**
	T-20			6.23**
	T-10			6.24**
	T-35			4.07**
	T-24			0.34

** $P < 0.01$.

pattern was seen in all trees for both isozyme and DNA data. The statistical differences in the mean similarity indices between the intrapod pairs and the other groups persisted even after the invariant bands were excluded from the analysis (Table 2).

A bootstrap analysis of data from both the original and the trimmed set (with invariant bands removed) also indicated nearly always a significant difference in the similarity index between the intrapod pairs and the other groups (Table 4). For instance, in all the iterations (100%), the similarity index of the pooled seeds (for T-35, T-27, T-10, and T-20), single-seeded pods (for T-35 and T-27), and two-seeded pods (random pairs; for T-35, T-27, T-10, and T-20) was significantly lower than that of the respective intrapod pairs. Thus, genetic similarities between seeds in two-seeded pods were significantly higher than those between any two randomly selected seeds.

Intrapod SI values were negatively skewed (skewness test; Snedecor and Cochran, 1994), while those among the single-seeded pods were normally distributed in most cases (Fig. 1, for T-35; Table 5). Further, the frequency distributions of intrapod SI values differed significantly from those of random pairs from the single-seeded pods for all trees (Table 5).

Clustering of seeds based on their isozyme and DNA banding patterns showed that there are distinctly identifiable genetic groups based on the band sharing and their frequencies among the seed pools of each tree (Fig. 2; for tree T-35). However, the seeds from each of the two seeded pods segregated nonrandomly into these clusters, indicative of their high degree of genetic similarity. The pairs of seeds from each pod exhibited a tendency to align together (Table 6) into a cluster though the segregation of the aligned pairs (i.e., pods) into these clusters was random.

DISCUSSION

The results suggest that in *Dalbergia sissoo,* seed pairs developing in a given pod are genetically more similar than pairs of seeds chosen randomly from a tree. In fact, they are more similar even when compared to random pairs of seeds from other two-seeded pods. The skewness coefficients for the SI

TABLE 4. Mean similarity index (SI) of 500 bootstrapped samples for pooled, single-seeded, and two-seeded (random) pods in different trees and percentage of those means significantly different from that of two-seeded (intrapod) pairs.

		Original						Trimmed				
Treatment	Tree	No. of bands	N	$N1$	$N11$	X500	Pr*	No. of bands	N	$N1$	$N11$	X500	Pr*
Pooled	T-35	18	169	25	300	0.732	100	12	169	25	300	0.632	100
	T-27	30	144	25	300	0.808	100	13	144	25	300	0.659	100
	T-10	23	67	25	300	0.751	100	13	67	25	300	0.626	100
	T-20	35	60	25	300	0.779	100	23	60	25	300	0.688	100
	T-35	43	30	25	300	0.723	100	30	30	25	300	0.647	100
	T-24	67	28	25	300	0.734	82	41	28	25	300	0.616	0
Single-seeded pods	T-35	18	75	25	300	0.735	100	11	75	25	300	0.611	100
	T-27	30	60	25	300	0.810	100	12	60	25	300	0.590	100
	T-10	23	29	25	300	0.820	90	12	29	25	300	0.692	99
Two-seeded (random) pods	T-35	18	94	25	300	0.735	100	12	94	25	300	0.637	100
	T-27	30	84	25	300	0.817	100	12	84	25	300	0.623	100
	T-10	23	38	25	300	0.738	100	13	38	25	300	0.596	100
	T-20	35	36	25	300	0.778	100	23	36	25	300	0.684	100

Note: Trimmed = data in which invariant alleles were removed; N = samples size; $N1$ = number of seeds sampled from "N" while bootstrapping; $N11$ = number of pairs compared in each bootstrapping; X500 = mean of 500 SI values computed by randomly selecting "N" from "$N1$" seeds; Pr* = percentage of SI values (computed by bootstrapping in each category) significantly different from that of a pair (intrapod) of seeds.

values suggest that the pairwise SI values are almost always negatively skewed between the two seeded (intrapod) pairs while they were not so for the single-seeded pods. Hence, seeds of two-seeded pods are in general more genetically similar than random seeds from the single-seeded pods. Thus, the data support the prediction based on parent–offspring conflict and kin selection theory (Trivers, 1974; Haig and Westoby, 1988; Uma Shaanker, Ganeshaiah, and Bawa, 1988) that developing seeds in two-seeded pods of *Dalbergia sissoo* have been selected to reduce the competition with genetically highly related sibs, despite reduced dispersal advantage and increased post-dispersal competition for the survivors associated with two-seeded pods.

Although the observed pattern does conform to the prediction proposed by Uma Shaanker, Ganeshaiah, and Bawa (1988) based on inclusive fitness theory, the increased similarity between seeds within a pod can also arise in other ways as well. For instance, the genotypes of the developing embryos might differ in their competitive ability or "greediness" in drawing maternal resources (Joshi, 1992). If the first few embryos fertilized in a pod happen to be, purely by chance, "less

greedy" genotypes, then the reduced competitiveness among them leads to less of a dominance hierarchy. This would eventually result in the development of pods with seeds that are of more similar genotypic constitution (weak competition) relative to randomly selected seeds.

But seeds from two-seeded pods do not appear to belong to such specific genotypes. They segregated randomly into four to six genetic groups derived by hierarchical clustering. However, seed pairs from these pods showed a tendency to "align" into clusters. The genetic similarity between random pairs of seeds from two-seeded pods was low relative to intrapod seed pair similarity. In other words, the genetic similarity of intrapod seeds in two-seeded pods is greater than that of randomly selected seeds from two-seeded pods.

The observed patterns of similarities among seeds could arise by other independent processes as well. In insect-pollinated plants such as *Dalbergia sissoo,* ovules of a pod have a

TABLE 5. Skewness coefficient values for similarity indices among seeds from single-seeded pods and among pairs of seeds within each of two-seeded pods. The last column gives D_{max} values from Kolmogorov-Smirnov (KS) test between these two sets of seeds.

	Skewness coefficient[a] of pairs from		
Tree no.	Single-seeded pods	Two-seeded pod (intrapod)	D_{max} values[b]
Isozyme data			
T-35	−0.14*	−0.73*	0.45*
T-27	0.07[NS]	−1.20*	0.53*
T-10	0.04[NS]	−1.16*	0.44*
T-20	0.13[NS]	0.42[NS]	0.04*
DNA data			
T-35	−0.78*	—	0.79*
T-24	0.53*	—	0.66*

[a] The asterisks in these two columns refer to the significance of skewness coefficient values in them; —means a value could not be computed due to limited sample size (see Table 2).

[b] Asterisks in this column refer to the significance of differences in the frequency distribution of similarity indices among single-seeded pods and among pairs of seeds within two-seeded pods. D_{max}: maximum value of the difference between the frequencies of the two categories cumulated over different class ranges.
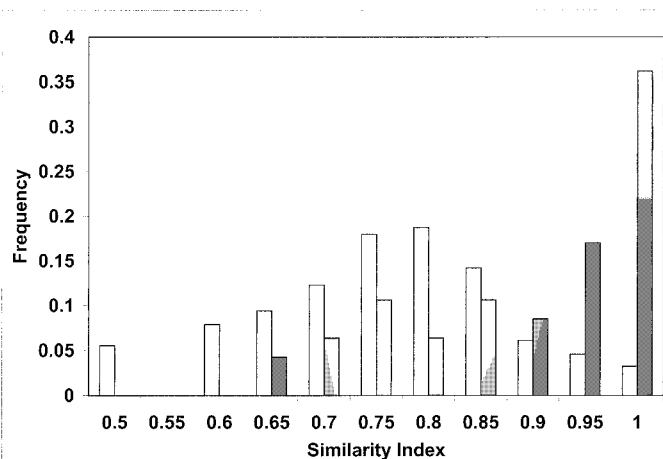
* $P < 0.05$. NS = not significant.

Fig. 1. Distribution of the similarity index among single-seeded pods (open) and pairs of seeds within each of the two-seeded pods for tree T-35.
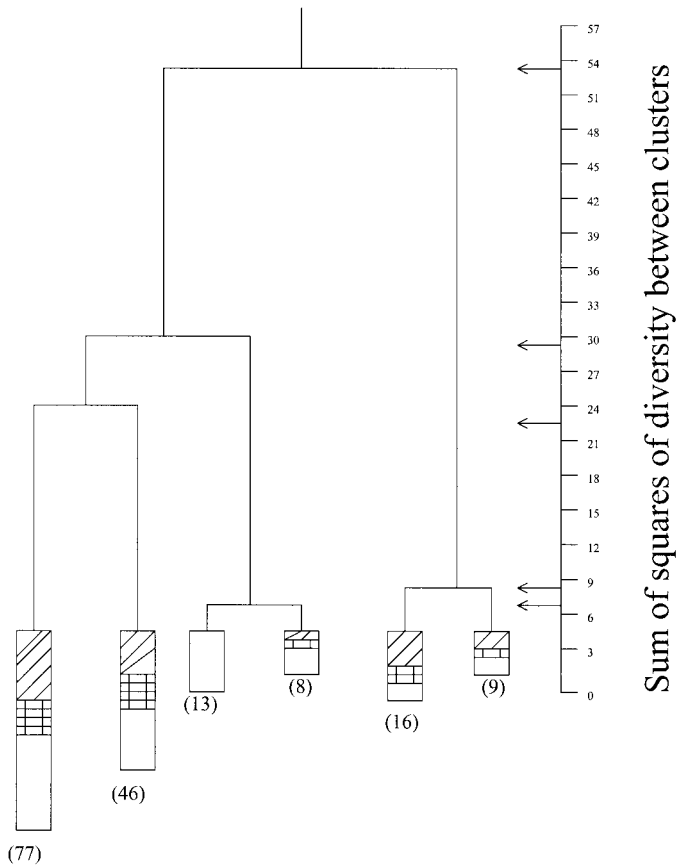
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Fig. 2. Dendrogram of clusters formed based on squared Euclidean distances for tree T-35. The size of each cluster box refers to the number of seeds grouped in it. Different shades represent the proportion of single-seeded (open), aligned two-seeded (cross-hatched), and nonaligned two-seeded (brick-hatched) pods.

TABLE 6. Observed and expected (in parentheses) numbers of aligned and nonaligned pods into different clusters formed in different trees and test of goodness of fit.

Tree no.	Aligned pairs	Nonaligned pairs	Chi-square value
Isozyme data			
T-35	32 (14)	15 (33)	32.9**
T-27	33 (7)	9 (35)	115.8**
T-10	14 (3)	5 (16)	47.8**
T-20	15 (4)	3 (14)	38.8**
DNA data			
T-35	7 (1)	0 (6)	42.0**
T-24	7 (2)	1 (6)	16.6**

** $P < 0.05$.

1983) it has been shown that members of a colony "tolerate" relatives to a greater degree than nonrelatives. In animals, such kin favoring among relatives has been argued to be advantageous to the perpetrators of the behavior (Hamilton, 1964; Greenberg, 1979; Waldman and Adler, 1979; Wu et al., 1980; Breed, 1981; O'Hara and Blaustein, 1981; Holmes and Sherman, 1982; Packer et al., 1991; Reeve, 1992; Manning, Wakeland, and Potts, 1992). In plants also, Uma Shaanker and Ganeshaiah (1988) argued that the inclusive fitness accrued by the surviving kin can overcompensate the reduced dispersal advantage and increased post-dispersal competition associated with such tolerance shown to the related sibs. In *Dalbergia sissoo*, the intrafruit seed abortion is considered to be due to the diffusion of certain chemicals by the dominant ovules, which in turn prevent the subordinate embryos from drawing further resources (Mohan Raju, Uma Shaanker, and Ganeshaiah, 1996). Similar mechanisms are seen to operate in *Syzygium cuminii* and *Derris indica* as well (Arathi, 1990; Arathi et al., 1996; Krishnamurthy, Uma Shaanker, and Ganeshaiah 1997), suggesting that sibling-competition-mediated abortion of embryos is not a rare phenomenon in plants. However, if competition (or favoring) among sibs is to be manifested as a function of genetic relatedness as it appears to be from the present data, then there should be a fine-tuned mechanism through which the gradient of genetic relatedness between sibs would be sensed. It is not clear how such a mechanism would operate in plants. In fact, operation of kin selection requires that sib recognition must occur according to a gradient of genetic relatedness. Surprisingly, even in animal systems where kin selection is well documented, sib discrimination or recognition appears to be mostly binary (kin vs. nonkin; Greenberg, 1979; Breed, 1981, 1983) and rarely according to a gradient of genetic relatedness (Manning, Wakeland, and Potts, 1992). It would be interesting to explore the processes that could be involved in such gradient-based recognition of kins.

higher probability of being fertilized by pollen grains from the same donor since the insect vectors collect and deposit pollen grains en masse (Kress, 1981; Uma Shaanker, Ganeshaiah, and Bawa, 1988; Uma Shaanker and Ganeshaiah, 1990; Uma Shaanker, Ganeshaiah, and Radhamani, 1990). Thus, even if the development of two-seeded pods is merely a random process, it is possible that the intrapod genetic similarity among the two-seeded pods will be higher by default. However, if the development of two-seeded pods is independent of kin favoring, then unrelated seeds would also develop within a pod and hence one would expect seeds from two-seeded pods to exhibit a wide range of genetic similarities as those between the random pairs of single-seeded pods. This will increase the range of genetic similarity values in two-seeded pods, but the mean SI would still be higher than random pairs of seeds from different fruits. Our results show that the SI values among the intrapod seed pairs were always higher and the range narrower than those among the single-seeded pods (Fig. 1; Table 2). The latter showed SI as low as 0.27, while the lowest SI among the intrapod seed pairs was 0.61 (Table 2).

Thus, the data appear to suggest the operation of a genetic-relatedness-based mutual favoring among developing seeds within a pod. The results are akin to those observed in a few unique and interesting systems in animals where kins are favored and nonkins are discriminated against. For instance, in sweat bees (Greenberg, 1979) and honey bees (Breed, 1981,

LITERATURE CITED

ARATHI, H. S. 1990. Proximate causes of embryo abortion in plants with reference to *Derrisindica* [Lam.] and *Syzygium cumini* [L.] Skeels. M.Sc.(Ag.) thesis, University of Agricultural Sciences, Bangalore, India.

———, K. N. GANESHAIAH, R. UMA SHAANKER, AND S. G. HEGDE. 1996. Factors affecting embryo abortion in *Syzygium cuminii* (L.) Skeels (Myrtaceae). *International Journal of Plant Sciences* 157: 49–52.

AUGSPURGER, C. K., AND K. P. HOGAN. 1983. Wind dispersal of fruits with variable seed number in a tropical tree [*Lonchocarpus pentaphyllus*; Leguminosae]. *American Journal of Botany* 70: 1031–1037.

BAWA, K. S., S. G. HEGDE, K. N. GANESHAIAH, AND R. UMA SHAANKER. 1989. Embryo and seed abortion in plants. *Nature* 342: 625–626.

———, AND C. J. WEBB. 1984. Flower, fruit and seed abortion in tropical

forest trees: implications for evolution of paternal and maternal reproductive patterns. *American Journal of Botany* 71: 736–751.

Breed, M. D. 1981. Individual recognition and learning of queen odors by worker honey bees. *Proceedings of the National Academy of Sciences, USA* 78: 2635–2637.

———. 1983. Nestmate recognition in honeybees. *Animal Behaviour* 31: 86–91.

Casper, B. B. 1983. The efficiency of pollen transfer and rates of embryo initiation in *Cryptantha*. *Oecologia* 59: 262–268.

———, and D. Wiens. 1981. Fixed rates of random ovule abortion in *Cryptantha flava* [Boraginaceae] and its possible relation to seed dispersal. *Ecology* 62: 866–869.

Cheliak, W. M., and J. A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information report PI-X-42, Petawava National Forestry Institute. Canadian Forestry Service, Ottawa, Ontario, Canada.

Cheplick, G. P. 1992. Sibling competition in plants. *Journal of Ecology* 80: 567–575.

Chung, M. G., J. L. Hamrick, S. B. Jones, and G. S. Derda. 1991. Isozyme variations within and among populations of *Hosta* (Liliaceae) in Korea. *Systematic Botany* 16: 667–684.

Cumaraswamy, A., and K. S. Bawa. 1989. Sex allocation and mating systems in Pigeon pea (Fabaceae). *Plant Systematics and Evolution* 168: 59–69.

Dolan, R. W. 1994. Patterns of isozyme variation in relation to population size, isolation and phytogeographic history in royal catchfly (*Silene regia*; Caryophyllaceae). *American Journal of Botany* 81: 965–972.

Donnel, M. E., and K. S. Bawa. 1993. Gametic selection and patterns of ovule and seed abortion. *Current Science* 65: 214–219.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

Ganeshaiah, K. N., and R. Uma Shaanker. 1988. Seed abortion in wind dispersed pods of *Dalbergia sissoo*: maternal regulation or sibling rivalry? *Oecologia* 77: 135–139.

———, and ———. 1991. Seed size optimization in a wind dispersed tree *Butea monosperma*: a trade-off between seedling establishment and pod dispersal efficiency. *Oikos* 60: 3–6.

———, ———, and N. V. Joshi. 1991. Evolution of polyembryony: consequences to the fitness of the mother and the offspring. *Journal of Genetics* 70: 103–127.

———, and ———. 1992. Frequency distribution of seed number per fruit in plants: a consequence of the self-organizing process? *Current Science* 62: 359–365.

Greenberg, L. 1979. Genetic component of bee odour in kin recognition. *Science* 206: 1095–1097.

Gross, R. S., and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed set of individuals: experimental studies on four co-occuring species of golden rod [*Solidago*: compositae]. *Ecological Monographs* 53: 95–117.

Guth, C. J., and S. G. Weller. 1986. Pollination, fertilization and ovule abortion in *Oxalis magnifica*. *American Journal of Botany* 73: 246–253.

Haig, D., and M. Westoby. 1988. Inclusive fitness, seed resources and maternal care. *In* J. Lovett-Doust and L. Lovett-Doust [eds.], Plant reproductive ecology, 60–79. Oxford University Press, New York, New York, USA.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. *Journal of Theoretical Biology* 7: 1–16.

Hedley, C. L., and M. J. Ambrose. 1981. Designing "leafless" plants for improving yields of the dried pea crop. *Advances in Agronomy* 34: 225–277.

Hegde, S. G., R. Uma Shaanker, and K. N. Ganeshaiah. 1991a. Evolution of seed size in the bird dispersed tree *Santalum album*: a trade-off between the seedling establishment and pod dispersal efficiency. *Evolutionary Trends in Plants* 5: 131–135.

———, ———, and ———. 1991b. Fruit preference criteria by avian frugivores: their implications in the evolution of clutch size in *Solanum pubescens*. *Oikos* 60: 3–6.

Ho, L. C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 355–378.

Holmes, W. G., and P. W. Sherman. 1982. The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist* 22: 491–517.

Lee, T. D., and F. A. Bazzaz. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology* 63: 1363–1373.

———, and ———. 1986. Maternal regulation of fecundity: non-random ovule abortion in *Cassia fasciculata*. *Oecologia* 68: 439–465.

Janzen, D. H. 1982. Variation in average seed size and fruit seediness in a fruit crop of a Gunacaste tree (Leguminosae) *Enterolobium cyclocarpum*. *American Journal of Botany* 69: 1169–1178.

Joshi, N. V. 1992. Sibling rivalry between seeds with in a fruit: some population genetic models. *Journal of Genetics* 71: 105–119.

Kress, W. J. 1981. Sibling competition and evolution of pollen unit, ovule number and pollen vector in angiosperms. *Systematic Botany* 6: 101–112.

Krishnamurthy, K. S. 1995. Mechanisms of seed abortion in *Syzygium cuminii* (L.) Skeels, and the role of self organised flow of resources to sinks in differential development of seeds in plants. M.Sc.(Ag.) thesis, University of Agricultural Sciences, Bangalore, India.

———, R. Uma Shaanker, and K. N. Ganeshaiah. 1997. Seed abortion in an animal dispersed species, *Syzgium cuminii* (L.) Skeels (Myrtaceae): the chemical basis. *Current Science* 73: 869–873.

Ludwig, J. A., and J. F. Reynolds. 1988. Statistical ecology: a primer on methods and computing. John Wiley and Sons, New York, New York, USA.

Manning, E. J., E. K. Wakeland, and W. K. Potts. 1992. Communal nesting patterns in mice implicate MHC gene in kin recognition. *Nature* 360: 581–584.

Meinke, D. W. 1982. Embryo-lethal mutants of *Arabidopsis thaliana*: evidence for gametophytic expression of the mutant genes. *Theoretical and Applied Genetics* 63: 381–386.

Mock, D. W., and G. A. Parker. 1997. The evolution of sibling rivalry. Oxford University Press, Oxford, UK.

———, and ———. 1998. Siblicide, family conflict and the evolutionary limits of selfishness. *Animal Behaviour* 56: 1–10.

Mogenson, H. L. 1975. Ovule abortion in *Quercus*. *American Journal of Botany* 62: 160–165.

Mohan Raju, B. 1993. Embryo abortion in *Dalbergia sissoo* Roxb.: proximate mechanisms and ultimate causes. M.Sc.(Ag.) thesis, University of Agricultural Sciences, Bangalore, India.

———, R. Uma Shaanker, and K. N. Ganeshaiah. 1995. Differential rates of seed abortion among trees of *Dalbergia sissoo*: role of post-dispersal sibling competition. *Current Science* 68: 1114–1118.

———, ———, and ———. 1996. Intrafruit seed abortion in a wind dispersed tree, *Dalbergia sissoo*: proximate mechanisms. *Sexual Plant Reproduction* 9: 273–278.

Nakamura, R. R. 1980. Plant kin selection. *Evolutionary Theory* 5: 113–117.

O'Hara, R. D., and A. R. Blaustein. 1981. An investigation of sibling recognition in *Rana cascadae* tadpoles. *Animal Behaviour* 29: 1121–1126.

Packer, C., D. A. Gilbert, A. E. Pusey, and S. J O'Brien. 1991. A molecular genetic analysis of kinship and cooperation in African lions. *Nature* 51: 562–565.

Peterson, C., J. H. Brown, and A. Kodric-Brown. 1982. An experimental study of floral display and fruit set in *Chilopsis linearis*. *Oecologia* 55: 7–11.

Reeve, H. K. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole rat. *Nature* 358: 47–149.

Ridley, H. N. 1930. Dispersal of plants throughout the world. Reeve, Ashford, UK.

Rigney, L. P. 1995. Postfertilization causes of differential success of pollen donors in Erythronium grandiflorum(liliaceae): non random ovule abortion. *American Journal of Botany* 82: 578–584.

Schemske, D. W., and L. P. Pautler. 1984. The effect of pollen composition on fitness components in a neotropical herb. *Oecologia* 62: 31–36.

Sedgley, M. 1981. Early development of the *Macadamia* ovary. *Australian Journal of Botany* 29: 185–193.

Siegel, S., and N. J. Castellan. 1988. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, New York, USA.

Snedecor, G. W., and W. G. Cochran. 1994. Satistical methods, 8th ed. Iowa State University Press, Ames, Iowa, USA.

Snow, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. *Oecologia* 55: 231–237.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and

ultimate functions. *Annual Review of Ecology and Systematics* 12: 253–279.

TONSOR, S. J. 1989. Relatedness and intraspecific competition in *Plantago lanceolata. American Naturalist* 134: 897–906.

TRIVERS, R. L. 1974. Parent–offspring conflict. *American Zoologist* 14: 249–264.

TROUP, R. S. 1986. *"Dalbergia sissoo." In* Silviculture of Indian trees, vol. 1, 294–318. International Books, Dehradun, India.

UMA SHAANKER, R., AND K. N. GANESHAIAH. 1984. Does pollination efficiency shape pollen grain to ovule ratio? *Current Science* 53: 751–752.

———, AND ———. 1988. Bimodal distribution of seeds per pod in *Caesalpinia pulcherrimma*—parent offspring conflict? *Evolutionary Trends in Plants* 2: 91–98.

———, AND ———. 1989. Stylar plugging by fertilized ovules in *Kleinhovia hospita* [Sterculiaceae]—a case of vaginal sealing in plants? *Evolutionary Trends in Plants* 3: 59–64.

———, AND ———. 1990. Pollen grain deposition patterns and stigma strategies in regulating seed number per pod in multiovulated species. *In* K. S. Bawa and M. Hadley [eds.], Reproductive ecology of tropical forest plants, 165–178. Parthenon Press, Carnforth, Lancashire, UK.

———, AND ———. 1997. Conflict between parent and offspring in plants: predictions, processes and evolutionary consequences. *Current Science* 72: 932–939.

———, AND ———. 1998. Fruit and seed set in tropical trees: evolutionary constraints and proximate mechanisms. *In* A. K. Mandal and G. L. Gibson [eds.], Forest genetics and tree breeding, 205–216. Oxford University Press, Oxford, UK.

———, ———, AND K. S. BAWA. 1988. Parent offspring conflict, sibling rivalry and brood size patterns in plants. *Annual Review of Ecology and Systematics* 19: 177–205.

———, ———, AND T. R. RADHAMANI. 1990. Association among the modes of pollination and seed dispersal: ecological factors and phylogenetic constraints. *Evolutionary Trends in Plants* 4: 107–111.

VASUDEVA, R. 1995. Consequences of increased gametophyitc competition on seed development and hybrid vigour in plants with special reference to *Hibiscus cannabinus* L. and *Phaseolus latheroides* L. Ph.D. dissertation, University of Agricultural Sciences, Bangalore, India.

WALDMAN, B., AND K. ALDER. 1979. Toad tadpoles associate preferentially with siblings. *Nature (London)* 282: 611–613.

WEIGEL, R. C., AND K. W. HUGHES. 1986. In vitro ovule culture of a seedless persimmon. *Journal of Heredity* 7: 213.

WEINS, D. 1984. Ovule survivorship, brood size, life history, breeding systems and reproductive success in plants. *Oecologia* 64: 47–53.

———, C. I. CALVIN, C. A. WILSON, C. I. DAVERN, D. FRANK, AND S. R. SEAVEY. 1987. Reproductive success, spontaneous embryo abortion and genetic load in flowering plants. *Oecologia* 71: 501–509.

WENDEL, J., AND N. WEEDEN. 1989. Visualization and interpretation of plant isozymes. *In* D. Soltis and P. Soltis [eds.], Isozymes in plant biology, 5–45. Dioscorides Press, Portland, Oregon, USA.

WILLIAMS, J. G. K., A. R. KUBELIK, K. J. LIVAK, AND J. A. RAFALSKI. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

WILLSON, M. F., AND D. W. SCHEMSKE. 1980. Pollinator limitation, fruit production and floral display in Pawpaw [*Asimina triloba*]. *Bulletin of the Torrey Botanical Club* 107: 401–408.

WU, H. M. H., W. G. HOLMES, S. R. MEDINA, AND G. P. SACKETT. 1980. Kin preference in infant *Macaca nemstrina. Nature* 285: 225–227.

WYATT, R. 1981. The reproductive biology of *Asclepias tuberosa*. II. Factors determining fruit set. *New Phytologist* 88: 375–385.

ZIMMERMAN, M., AND G. H. PYKE. 1988. Reproduction in *Polemonium*: assessing factors limiting seed set. *American Naturalist* 131: 723–738.

APPENDIX. Enzymes studied, primers used, and the number of corresponding bands obtained for each tree.

Tree	Enzymes assayed	Total no. of bands scored	Primers assayed	Total no. of bands scored
T-35	MDH, MR, PGI, G6PDH, 6PGDH, IDH, PGM, ADH	30	OPT17, OPQ04, OPQ08, OPM05, OPC06, OPC14, OPCO5, OPM17, OPQ14	51
T-27	ADH, MDH, MR, PGI, PGM, G6PDH, IDH, G6PDH	30	—	
T-10	ADH, ALD, MDH, PGI, MR, EST, PGM	44	—	
T-20	ADH, ALD, MDH, EST, PGI, MR, PGM	44	—	
T-24	—	—	OPT17, OPQ04, OPQ08, OPM05, OPC06, OPC14, OPC05, OPM17, OPQ14	61

Note: ADH: alcohol dehydrogenase; ALD: aldolase; G6PDH: glucose-6-phosphate dehydrogenase; IDH: isocitrate dehydrogenase; MDH: malate dehydrogenase; MR: menadione reductase; 6PGDH: 6-phosphogluco dehydrogenase; PGI: phosphogluco isomerase; PGM: phosphoglucomutase; EST: esterase.