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VIABILITY SELECTION AT THREE EARLY LIFE STAGES OF THE TROPICAL TREE, *PLATYPODIUM ELEGANS* (FABACEAE, PAPILIONOIDEAE)

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Abstract.—Given the enormous number and high mortality of fertilized ovules in plants, it is possible that selection during the earliest stages of the life cycle plays an important role in shaping the genetic composition of plant populations. Previous research involving selection component analyses found strong evidence for viability selection in annual plant species. Yet despite this evidence, few attempts have been made to identify the magnitude and timing of viability selection as well as the mechanisms responsible for mortality among genotypes. *Platypodium elegans*, a Neotropical tree with high rates of early fruit mortality, represents an opportunity to study viability selection at a level of discernment not previously possible. Microsatellite markers were used to analyze the genetic composition of aborted embryos, as well as mature seeds and seedlings of the same cohort. While selection resulted in an overall decrease in self-fertilized progeny across each life stage, the greatest change in the genetic composition of progeny occurred between mature seeds and established seedlings. This suggested that inbreeding depression, and not late-acting self-incompatibility, was responsible for early selection. An investigation of the mature seed stage revealed that self-fertilized seeds weigh significantly less than outcrossed seeds. The result of this early selection conceals the mixed-mating system and high levels of inbreeding depression in *Platypodium elegans*, resulting in an apparently outcrossed adult population that does not differ significantly from Hardy-Weinberg expectations.

Key words.—Fabaceae, fruit abortion, inbreeding depression, microsatellites, *Platypodium elegans*, viability selection.

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The potential for competition and selection during zygote formation and seed maturation has long been recognized in flowering plants due to the high mortality rates observed at early life stages (Darwin 1859, 1876). Enormous amounts of pollen and ovules filter through an environmental ''sieve'' to result in a relatively small number of viable seeds and seedlings. Early investigations of seedling mortality using allozyme markers revealed a strong viability component of selection in annual species (Clegg and Allard 1973; Allard et al. 1977; Clegg et al. 1978). While the basis for selection was unknown, differences in survival from fertilization to reproduction were attributed to an often two-fold survival advantage of heterozygotes.

Traditionally, selection component analyses have measured viability selection in plant populations only for the transition between seedlings and adults (Weir 1996). However, selective differences in survival may occur at any point from fertilization through reproductive maturity. Furthermore, viability components quantify selection among genotypes but often do not identify the mechanism responsible for selection. High rates of selective embryo mortality in the earliest life-cycle stages may result, in part, from two possible mechanisms: late-acting self-incompatibility or inbreeding depression (Stephenson 1981; Seavey and Bawa 1986). In both cases, selection acts against self-fertilized and inbred progeny in favor of outcrossed progeny (e.g., Stephenson and Winsor 1986; Bush and Smouse 1992; Hardner and Potts 1997).

Several examples of late-acting self-incompatibility (SI) have been documented, including long-lived woody species like *Theobroma cacao* (Seavey and Bawa 1986). In these examples of postzygotic rejection, selection against self-fertilized ovules occurs prior to seed maturation. It is difficult to distinguish between late-acting SI and early episodes of inbreeding depression as the cause of viability selection. However, Seavey and Bawa (1986) suggest that postzygotic rejection should result in uniform failure of self-fertilized ovules, while failure due to inbreeding depression should result in the mortality of embryos at various stages of development. Self-incompatibility is primarily considered a means of avoiding the disadvantages of inbreeding depression by ensuring outcrossed fertilization (but see Charlesworth and Charlesworth 1987).

Inbreeding depression is the reduced fitness of self-fertilized progeny due to either the loss of overdominant effects of heterozygotes or increased expression of recessive deleterious alleles (Wright 1921; Charlesworth and Charlesworth 1987). Fitness differences due to inbreeding depression may affect progeny survivorship at any stage of the life cycle. In contrast to single estimates of viability components, the magnitude and timing of inbreeding depression have been examined in numerous plant species at several developmental stages (see review by Husband and Schemske 1996). Models and empirical observations suggest that predominantly outcrossing species experience high levels of inbreeding depression early in the life cycle (Lande and Schemske 1985; Schemske and Lande 1985). In these populations, large numbers of deleterious recessive alleles that maintain levels of inbreeding depression above a threshold level are predicted to prevent the evolution of self-fertilization. Examples of this are apparent in gymnosperms where high rates of selective seed abortion suggest a large genetic load (e.g., Sorensen 1969; Koski 1973; Sorensen 1982).

Given that levels of inbreeding are linked to the mating system, predictions of the effects and trajectory of inbreeding

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depression depend on accurate estimates of the outcrossing rate in plant populations. However, outcrossing rates are commonly estimated in seedlings, possibly after early selection during seed set and germination has occurred (Ritland 1986). This limitation is primarily due to technical difficulties in the collection and analysis of early life stages (e.g., the stage of seed abortion) in natural populations. Several methods attempt to distinguish the outcrossing rate at fertilization from the realized outcrossing rate in seedlings. These include embryo rescue prior to abortion, experimental hand pollinations, and greenhouse or common garden experiments (Schemske and Lande 1985; Charlesworth 1988; Mont et al. 1993). However, these methods may not be feasible in studies of long-lived plant populations in their natural environment. Recent advances in genetic technology provide another possibility for the analysis of aborted embryos: DNA extraction and amplification by polymerase chain reaction (PCR). Polymerase chain reaction analysis requires only minute amounts of DNA and amplification of marker loci is often possible for degraded DNA (Jackson et al. 1994). Analyses of the transition between fertilization and germination are therefore possible in species where seed abortion results in some remaining embryo tissue.

Platypodium elegans J. Vogel is a Neotropical tree species with a geographic range from Panama to Brazil (Croat 1978). Previous research with this species resulted in an estimated outcrossing rate in seedlings of 92%, suggesting that populations of *P. elegans* are predominantly outcrossing (Hamrick and Murawski 1990; Murawski and Hamrick 1991). However, reproductive adults exhibit high levels of fruit abortion unusually late in fruit development and mortality remains high throughout early life stages. If fruits are selectively aborted, the outcrossing rate estimated in *P. elegans* may not represent the outcrossing rate at fertilization. In addition, subsequent seed and seedling mortality may also result from the differential survival of genotypes. If early mortality in *P. elegans* results from viability selection, estimates of the mating system based on seedlings would reflect only net outcrossing rates and miss significant episodes of selection during previous life-cycle stages.

In this paper, we report results of an investigation of the cause of fruit abortion and the effects of viability selection at three early life stages in *P. elegans*. These life stages include the immature fruit stage (in the form of aborted fruit), mature seeds, and seedlings. We recognize that aborted fruit are not representative of all immature fruit, but for the purposes of this paper we will refer to aborted fruit as one of three life stages. The late timing of fruit abortion in this species resulted in sufficient remaining embryo tissue for analyses via PCR amplification of microsatellite loci (Hufford et al. 2000).

Specific objectives of this study were to determine the genetic composition of aborted and mature seeds and seedlings, and to document changes in the mating system at the three early life stages. Once evidence of selection was discovered, we partitioned life stages to determine both the magnitude and timing of selective events as well as the mechanism of selection. This allowed the following questions: Is *P. elegans* predominantly outcrossed or do differences in early survival mask a mixed-mating system? Does fruit abortion represent late-acting SI, inbreeding depression, or random mortality? What factors influence the differential survival of progeny in this population? The results are then discussed in light of current models of inbreeding depression and present data on population genetic dynamics in tropical tree populations.

Materials and Methods

Study Site and Species

Barro Colorado Island (BCI), Republic of Panama, is the site of a field station operated by the Smithsonian Tropical Research Institute. Island vegetation is moist, semi-deciduous, tropical forest and annual rainfall (2600 mm) is seasonal (Croat 1978). In 1980, Hubbell and Foster (1983) established a 50-hectare Forest Dynamics Plot (FDP) on BCI in which all stems greater than 1 cm in diameter and 1 m in height were mapped and identified by species. As a result, the locations of many *P. elegans* reproductive adults are readily available, greatly facilitating seed and seedling collections for genetic analyses.

Platypodium elegans J. Vogel (Fabaceae, Papilionoideae) is a large canopy tree common to BCI (Croat 1978). Flowers are hermaphroditic and bee-pollinated and the winged fruits or samaras typically hold single seeds. Trees flower synchronously from April to June and mature fruits are dispersed by wind from February to April of the next year. Fruit abortion begins shortly after flowering, peaks in August, and continues through October. Aborted fruits are easily identified due to their green color, early drop months prior to the fall of mature fruits, and the presence of a shriveled embryo. The low density of reproductive adults (Murawski and Hamrick 1991) and the distinctive samara morphology of each adult (Augspurger 1983) aid in the identification of seeds and seedlings specific to each maternal tree. A single tree may produce several thousand mature seeds in a good year.

Causes of early mortality include fruit abortion, predation, and fungal infection. Predation of immature fruit is not apparent until they reach the forest floor, where agoutis (*Dasyprocta punctata*) open aborted fruit cases and eat the embryos. Mature seeds are vulnerable to fungal pathogens and are heavily predated by bruchid beetle larvae. Seedlings suffer from several sources of mortality. However the greatest threat is fungal damping-off syndrome, which may reduce seedling survival by as much as 70–90% in the first two months after germination (Augspurger 1983; Augspurger 1984).

Sampling

Sampling began in late August 1997 when approximately 60 *P. elegans* adults were surveyed across BCI. Twelve adults had sufficient numbers of aborted fruits to allow collection of 50 or more aborted fruits per tree. Five of these adults were located on the FDP. Aborted fruits were rinsed in a 5% bleach solution to prevent rotting and transported to the University of Georgia, where embryos were extracted and snap-frozen in liquid nitrogen. Adult leaves were also collected to obtain maternal genotypes.

Subsequent sampling took place in March (for mature

fruits) and May 1998 (for germinated seedlings). Fifty each of mature fruits and seedlings were collected from seed shadows of the twelve trees with high levels of fruit abortion. Mature seeds were extracted from samara fruit cases and preserved in silica gel. Seedlings were collected at two intervals approximately four weeks apart in late May and June of 1998, and also preserved in silica gel prior to genetic analyses. Adults sampled included the twelve maternal trees as well as 125 additional trees either located on the FDP (23 trees) or distributed near the FDP or at random on the island (102 trees).

Additional field collections were completed in September 1998 and in March and October 1999. Due to relatively low rates of fruit abortion and mature seed set in 1998, collections of each life stage could only be made from three of the original twelve trees and sample sizes were variable. The results from these three trees allow a comparison of cohorts from two reproductive years (1997–1998 and 1998–1999). A total of 1814 progeny were sampled the first year and 409 the second year. These data are consistent with observed variation in seed production in this population. Collections made in 1997–1998 are characteristic of fruit production (and fruit abortion) among adult trees in a good year.

Microsatellite Analysis

Microsatellites are highly variable regions of tandem repeats found in the genomes of plants and animals (Tautz and Renz 1984; Wang et al. 1994). Microsatellite loci are amplified via PCR with minute quantities of DNA, and alleles consist of bands that vary in repeat length. Isolation of microsatellite markers in *P. elegans* was previously described in Hufford et al. (2000). Four of the five loci described were used for analyses in this study, specifically loci PE2-2, PE5-4, PE6-1, and PE14-1. These loci were eventually scored for 13, 17, 22, and 8 alleles respectively, including some alleles found in progeny that were not seen in the adult population.

DNA extractions of samples of each of the three life stages were accomplished with either the Qiagen DNeasy Plant Mini Kit or by the method of Edwards et al. (1991). Typical yield varied between life stages but the average concentration was approximately 30 ng/μl. Yields of adult leaf tissue were substantially greater (50–100 ng/μl). DNA samples were PCR-amplified with radio-labeled microsatellite primers and run on 6% acrylamide gels (Hufford et al. 2000).

Genotypes of maternal parents and those of their open-pollinated progeny arrays were compared at all loci for evidence of normal Mendelian segregation, including linkage equilibrium. All but one locus had patterns of variation consistent with Hardy-Weinberg expectations. Two alleles in locus PE14-1 were difficult to distinguish when present in the same individual. This resulted in an apparent ''null allele'' specific to that genotypic combination. As a result, we pooled these two alleles and conducted genetic analyses of seven representative alleles for that locus. In addition, we pooled two alleles in PE5-4 and PE6-1 to ensure scoring accuracy.

Data Analysis

Data were analyzed for the adult population and progeny sets of each of the twelve maternal trees. Measures of genetic variation include the mean number of alleles (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) averaged for all loci. In addition, the coefficient of inbreeding (F_{IS}) was calculated as $F_{IS} = 1 - (H_o/H_e)$ (Hedrick 1983).

Estimates of multilocus outcrossing rates (t) were calculated directly from each dataset. Specifically, progeny were scored as products of outcrossing or self-fertilization based on whether they shared all alleles with the maternal tree. The probability (α) of nonidentification of an outcross in this population was estimated to range between $0.01 \leq \alpha \leq 0.047$ (Shaw et al. 1981). To compare observed estimates with estimates of outcrossing that account for cryptic outcrossing events, we also calculated a conservative measure ($\alpha = 0.047$) of the multilocus outcrossing rate (t_m) described in Shaw et al. (1981). Outcrossing rates calculated for aborted fruit represent only the inviable component of the immature fruit life stage, in contrast to outcrossing rates calculated for the full datasets of mature seeds and seedlings.

Chi-square tests of heterogeneity were calculated for counts of selfed and outcrossed progeny between life stages within families and at the population level by pooling over all families. Additional tests were calculated to discern differences in the outcrossing rate between seedlings collected early and late in 1998, as well as to detect differences in outcrossing between the mature seed class and the early seedling subset. Separate collections of early and late germinated seedlings distinguished between selection at the point of germination (mature seeds vs. early seedlings) and selection during seedling growth and development (early vs. late seedlings).

Seed weight was measured to the nearest 0.01 g for embryos extracted from samara fruit cases and with seed coats removed. Each weight was assigned a specific seed number and later linked to data regarding the outcross status. At the same time, categorical observations of fungal infection (present or absent) were made for each mature seed. Fungal infection was apparent due to necrosis of mature seed tissue. Chi-square contingency tables were calculated to test the independence of outcross status and infection class. The effects of outcross status and infection class on seed weight were analyzed using a two-way analysis of variance (ANOVA).

Viability was measured between mature seeds and seedlings using the equation $v_{ij} = a_{ij}/f_{ij}$, where f_{ij} and a_{ij} are relative genotypic frequencies among mature seeds and seedlings respectively (Clegg and Allard 1973; Clegg et al. 1978). Given the large number of alleles at these loci, genotypes at each locus were pooled into two universal categories of homozygotes and heterozygotes (Clegg et al. 1978). Selection components and corresponding variances were then estimated for the two categories. Viability estimates could not be calculated for the transition between aborted seeds and mature seeds because aborted seeds do not give rise to mature seeds. For viability selection to be calculated between these two stages, genotype frequencies of the zygote pool before abortion would need to be known.

RESULTS

Diversity and Outcrossing

Pooled diversity measures are reported in Table 1 and individual estimates for the three life stages of the twelve trees

TABLE 1. Sample size (N), number of discernible outcrossed progeny (n), mean number of alleles (A), observed (H_o) and expected (H_e) heterozygosities, coefficient of inbreeding (F_{IS}), observed outcrossing rate (t) and estimated multilocus outcrossing rate (t_m) for the adult population and samples of aborted fruit, mature seeds, and seedlings pooled over all twelve trees and four loci for 1997–1998. Standard errors of t_m are in parentheses.

Life stage	N	n	A	H_o	H_e	F_{IS}	t	t_m
Aborted fruit	605	454	13.25	0.750	0.802	0.0654	0.75	0.79 (0.018)
Mature seeds	609	475	12.75	0.761	0.799	0.0482	0.78	0.82 (0.018)
Seedlings	600	522	13.75	0.768	0.801	0.0413	0.87	0.91 (0.015)
Adults	137	—	13.50	0.797	0.808	0.0137	—	—

are listed in Table 2. The four microsatellite loci were highly polymorphic, including high observed heterozygosity values (75–80%) and high allele counts (mean of 13.25). Observed heterozygosity was less than expected heterozygosity for adults and for each life stage pooled across the twelve trees (Table 1), indicating a slight heterozygote deficit at all stages in this population. Inbreeding coefficients decreased across successive life stages, but were not significantly greater than zero (Li and Horvitz 1953). Levels of inbreeding are lowest in the adult population.

Estimates of outcrossing were pooled for each life stage (Table 1; Fig. 1) and calculated separately for each tree (Table 2), revealing a trend of increasing outcrossing rate across life stages. Results of the chi-square test for pooled data were highly significant for the transition between the mature seed stage and the seedling stage ($P < 0.0001$), and aborted fruit and the seedling stage ($P < 0.0001$). No significant difference was observed between aborted fruit and mature seeds ($P = 0.2245$). Further testing showed no apparent difference between early and late seedling collections for outcross rate, but the chi-square test was significant for the comparison of mature seeds and the early seedling subset ($P < 0.0006$). These data indicate that aborted fruit and mature seeds are not significantly different despite the trend of increased out-

TABLE 2. Sample size (N), mean number of alleles (A), observed heterozygosity (H_o), and observed (t) and estimated (t_m) multilocus outcrossing rates for progeny from three life stages (A, aborted fruit; M, mature seeds; and S, seedlings) of twelve maternal trees. Where applicable, the sample size and estimated outcrossing rates for the 1998–1999 cohort are included. Standard errors of t_m are in parentheses.

Tree ID	Life stage	N	A	H_o	t 1997–1998	t_m 1997–1998	t_m 1998–1999
1	A	52, 51	8.75	0.699	0.67	0.69 (0.07)	0.80 (0.07)
	M	50, 52	9.25	0.775	0.82	0.86 (0.06)	0.88 (0.06)
	S	49, 31	8.75	0.673	0.84	0.88 (0.06)	0.89 (0.07)
2	A	51	7.75	0.784	0.84	0.88 (0.05)	—
	M	51	8.25	0.731	0.86	0.91 (0.05)	—
	S	51	8.00	0.755	0.90	0.95 (0.04)	—
3	A	50	7.50	0.721	0.80	0.84 (0.06)	—
	M	50	9.75	0.775	0.88	0.92 (0.05)	—
	S	51	9.50	0.735	0.94	0.99 (0.03)	—
4	A	52	9.75	0.759	0.90	0.94 (0.05)	—
	M	50	8.75	0.750	0.92	0.97 (0.04)	—
	S	51	9.00	0.750	0.92	0.97 (0.04)	—
5	A	50	6.25	0.580	0.54	0.57 (0.07)	—
	M	49	7.00	0.658	0.47	0.49 (0.07)	—
	S	45	7.75	0.739	0.71	0.75 (0.07)	—
6	A	51, 51	9.50	0.765	0.73	0.76 (0.07)	0.82 (0.06)
	M	52, 50	9.25	0.736	0.62	0.65 (0.07)	0.96 (0.05)
	S	50, 46	9.25	0.795	0.84	0.88 (0.05)	0.96 (0.05)
7	A	52	9.25	0.678	0.54	0.57 (0.07)	—
	M	50	8.50	0.765	0.64	0.67 (0.07)	—
	S	50	7.75	0.735	0.72	0.76 (0.07)	—
8	A	50	9.25	0.820	0.96	1.00 (0.03)	—
	M	53	9.75	0.849	0.94	0.99 (0.03)	—
	S	51	8.75	0.848	0.96	1.00 (0.03)	—
9	A	51	11.25	0.784	0.78	0.82 (0.06)	—
	M	51	9.50	0.750	0.80	0.84 (0.06)	—
	S	51	10.00	0.833	0.92	0.97 (0.04)	—
10	A	44	9.25	0.773	0.75	0.79 (0.07)	—
	M	51	10.75	0.790	0.76	0.80 (0.06)	—
	S	50	11.00	0.820	0.82	0.97 (0.04)	—
11	A	50	8.50	0.790	0.62	0.65 (0.07)	—
	M	50	7.50	0.715	0.70	0.73 (0.07)	—
	S	51	7.75	0.706	0.86	0.91 (0.05)	—
12	A	52, 36	9.00	0.851	0.88	0.93 (0.05)	0.93 (0.06)
	M	52, 50	9.25	0.827	0.92	0.97 (0.04)	0.90 (0.06)
	S	50, 42	9.00	0.820	0.98	1.00 (0.03)	0.97 (0.05)

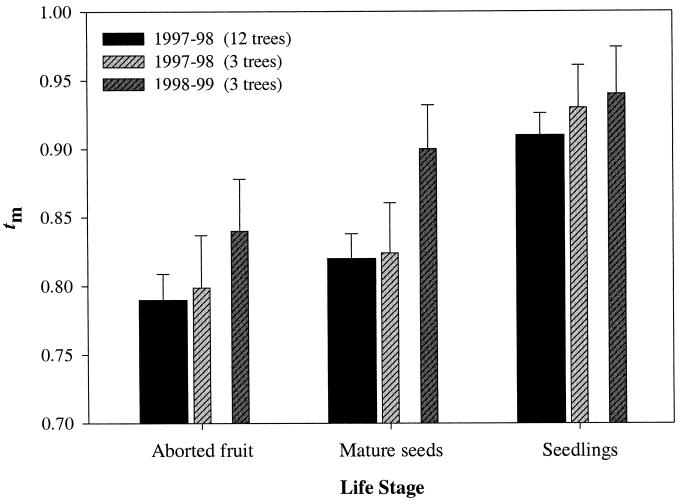

FIG. 1. Pooled outcross rates (t_m) and standard errors for the three life stages of the twelve adult trees in 1997–1998. For comparison, pooled outcross rates of the three adult trees collected in both 1997–1998 and 1998–1999 are also included.

crossing observed in mature seeds. Instead, the greatest change in outcross rate occurred at germination. Separate testing of the aborted fruit and mature seed stages for individual trees revealed no significant difference in outcross rate, and corresponded to results for pooled data.

Viability Components

Viability components of selection for the pooled categories of homozygotes and heterozygotes showed a heterozygote fitness advantage for two of the four loci (Table 3). Selective values for the other two loci suggest a slight homozygote fitness advantage. Selective components for the two loci in which heterozygotes are favored are proportionally higher than components for loci in which homozygotes are favored. These results, combined with the overall increase in heterozygosity from aborted fruit through adults (Table 1), suggest a slight fitness advantage of heterozygotes in this population.

Seed Weight

Mean seed weight for the mature seed stage of all twelve trees was 0.337 ± 0.005 grams. The results of the ANOVA (Table 4) showed that there were significant effects of out-

TABLE 3. Viability components and standard errors (in parentheses) for the transition between mature seeds and seedlings pooled for twelve adults. Genotypes were divided into two categories of homozygotes (v_{ii}) and heterozygotes (v_{ij}). Relative selective values are listed below viability estimates.

Locus	v_{ii}	v_{ij}
PE2-2	0.933 (0.08)	1.03 (0.04)
	1.00	1.104
PE5-4	1.08 (0.15)	0.987 (0.02)
	1.00	0.915
PE6-1	0.77 (0.10)	1.06 (0.03)
	1.00	1.364
PE14-1	1.09 (0.09)	0.96 (0.04)
	1.00	0.880

TABLE 4. Analysis of variance for the effect of infection class and outcross status on seed weight in the mature seed dataset for *Platypodium elegans* (1997–1998).

Effect	df	*F*	*P*
Infection class	1	34.569	<0.0001
Outcross status	1	6.622	0.0103
Interaction	1	1.881	0.1707

cross status on seed weight ($P = 0.0103$). Namely, outcrossed seeds weighed on average 0.030 g more than self-fertilized seeds. There was also a significant decrease in the weight of infected seeds ($P < 0.0001$). However, the lack of a significant interaction ($P = 0.1707$) between outcross status and infection class indicated that the lower weight of self-fertilized seeds was not confounded by weight differences due to fungal infection. Chi-square tests revealed no significant difference between the two categories of outcross status and fungal infection (healthy or diseased); self-fertilized seeds were no more likely to be infected than outcrossed seeds ($P = 0.6943$).

When seed weight data were further tested by omission of all but apparently healthy seeds, the significant difference in mean weight between selfed and outcrossed seeds increased (0.043 g, $P = 0.0032$) despite the smaller sample size (599 seeds total, 320 in the reduced dataset). The mean weight of healthy seeds was also larger than that of the full dataset, but with little corresponding increase in standard error (0.371 ± 0.006 g). This is strong evidence that the average seed weight of mature seeds resulting from self-pollination is significantly lower than seeds resulting from outcrossing events (Fig. 2).

Second-Year Data

Mating system results from the second year of collections for three of the original twelve trees are included in Figure 1 and Table 2. The overall increase in outcrossing from the aborted fruit to the seedling life stage was approximately

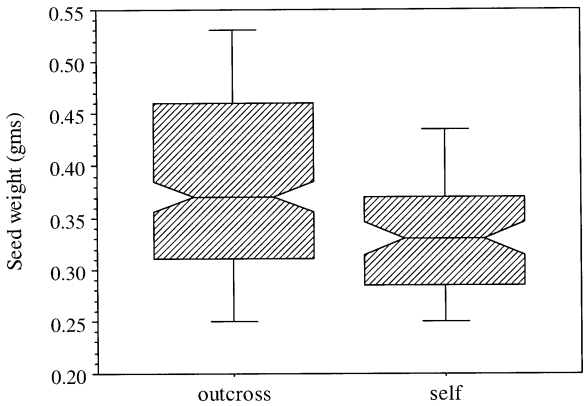

FIG. 2. Boxplot of the seed weights of outcrossed and self-fertilized progeny in the mature, healthy seed data. The length of each box is the interquartile range and the whiskers show the 10th and 90th percentiles. The line within each box represents the median seed weight, and notches around the median indicate 95% confidence intervals.

10% and chi-square analyses of aborted fruit and seedlings pooled for the three trees were significant ($P = 0.0175$). However, neither the comparison of aborted fruit to mature seeds nor the transition from mature seeds to seedlings showed a significant change in the number of self-fertilized progeny. The lack of a sharp decline in self-fertilized seeds at the transition from mature seeds to seedlings for this reproductive year (1998–1999) may be due to the reduced power of the smaller sample size (both in the total number of trees and the total number of progeny sampled; Table 2). Outcrossed seeds weighed more (0.01–0.06 g) on average than self-fertilized seeds for second-year samples, but this difference was not significant ($P = 0.6443$). The power of this test may also be low due to the small and uneven sample size ($N = 152$, of which 19 seeds were apparently self-fertilized).

Discussion

Fruit Abortion

The results document viability selection at early life stages of *P. elegans*. Earlier estimates of the outcrossing rate of seedlings of 92% (Murawski and Hamrick 1991) are accurate, but represent postselection estimates rather than the outcrossing rate at fertilization. Outcrossing estimates for individual trees in this study are as much as 35% lower, resulting from high rates of self-fertilization that are not apparent at later life stages. An unexpected result was the timing of selection between the mature seed and seedling life stages for the first year, indicating that the outcrossing rates of aborted fruits and mature seeds are similar. Because we compared aborted fruit (the progeny that did not survive) to mature seeds instead of the complete pool of immature fruit, the similar outcrossing rates reinforce the conclusion that viability selection is strongest between the mature seed and seedling stages. Given the lack of selection against self-fertilized ovules early in the life cycle, there is no evidence for late-acting self-incompatibility in this species. Inbreeding depression is evident as the basis for viability selection, but occurs after seed maturation. What then is the cause of the fruit abortion that occurs in *P. elegans* each year?

A likely explanation for fruit abortion in this species is resource limitation. Research examining flower and fruit abortion in tropical trees indicates that the number of flowers is often significantly greater than the number of fruits maintained through seed maturation (Bawa and Webb 1984). In the absence of pollen limitation, trees may initiate development of many more fruits than can be successfully matured (Lloyd 1980; Stephenson 1981). Competition for resources within a tree could then result in the episodes of fruit drop seen in *P. elegans*. It is noteworthy that trees with the highest fruit abortion in fall 1997 also produced some of the largest numbers of mature seeds in March 1998. This suggests that abortion did not lower the fecundity of individuals relative to other conspecifics in the population but instead may have resulted from the overproduction of fruits in an exceptionally good year.

An alternative explanation for fruit abortion is sexual selection, resulting in selective abortion of fruits by the maternal tree based on their paternity (Bawa and Webb 1984). This hypothesis can be tested with comparisons of paternity among maternal progeny arrays (K. M. Hufford, J. L. Hamrick, and S. R. Rathbun, unpubl. ms.). However, even if paternity differences are found among life stages, it is less likely that abortion is the result of maternal rejection of inferior fruits given that self-fertilized seeds (already proven inferior) are not selectively aborted at this life stage.

It is important to note that outcrossing estimates for the aborted fruit stage may not accurately estimate the outcrossing rate at fertilization in *P. elegans*. Sampling of aborted fruit late in fruit development may miss earlier episodes of postfertilization selection prior to embryo growth and development. In the absence of data for immature fruit (viable as well as aborted embryos) and given the similarity of outcrossing rates for aborted fruit and mature seeds, genetic marker analyses of aborted fruit provided the best possible estimate of the outcrossing rate in this population.

Viability Selection

Evidence for inbreeding depression and not late-acting SI is consistent with the trends observed in inbreeding coefficients, outcrossing rates, and heterozygosity for pooled data of the three life stages. In each case, inbreeding decreases and heterozygosity increases over time. Selection against selfed progeny is supported by the observation that in no case is the outcrossing rate greater in the aborted fruit stage than in the seedling stage. There is evidence for less heterozygosity and outcrossing between aborted fruits and mature seeds for some maternal individuals (Table 2). It is likely that variation in these estimates results from the inclusion in the aborted fruit samples of viable immature fruits that fell due to branch fall, wind disturbance, or animal activity.

Variation in selection components among loci in *P. elegans* is similar to variation seen in allozyme analyses of barley (Clegg et al. 1978), although standard errors for selection estimates of heterozygotes were considerably lower for microsatellites when compared to allozymes. Advantages of homozygotes may represent opposing selection among loci. However, there is also evidence for heterozygote fitness advantage due to the greater selective values of heterozygotes compared to homozygotes, and due to the overall increase in heterozygosity over time in this population. Viability components are best interpreted in combination with mating system estimates for each life stage.

Chi-square comparisons of outcrossed and self-fertilized progeny identified the point of greatest selection as the transition between mature seeds and newly established seedlings. Comparisons of early to late seedling collections did not reveal significant differences in outcrossing rate. In contrast, the significant differences in outcrossing rates seen between the mature seeds and newly established seedlings indicated that this was the period of the most intense selection. Significant differences in seed weight for outcrossed and selfed progeny further support this finding. Numerous examples in the literature have demonstrated that seed weight is an important factor in germination, growth rate, disease resistance, and competitive ability (see review in Roach and Wulff 1987). In addition, Augspurger and Kelly (1984) found a significant positive correlation between seed size and seed-

ling height in *P. elegans*, suggesting a growth advantage for seedlings resulting from larger seeds. Seed weight differences in *P. elegans* may be due to maternal or nuclear genetic effects (Roach and Wulff 1987). In either case, the lower survival and reduced weight of selfed seeds is common for trees sampled in this population.

Selection favoring outcrossed progeny also occurred during the second year (Table 2; Fig. 1). Namely, outcrossing increased significantly from the aborted fruit stage to the seedling stage for the three trees sampled. However, the strong episode of selection observed for the transition between mature seeds and seedlings was not as apparent in 1998–1999. This may be due to reduced sample sizes for that year. Given that the 1998–1999 sampling occurred during a year of low fruit production for all twelve trees, it is also possible that selection pressures were not as strong. *Platypodium elegans* was originally reported to flower only in alternate years (Croat 1978). It is now apparent that trees can flower annually but often experience considerable variation in seed production.

Outcrossing estimates varied among adult trees for each life stage in both years, although standard errors suggest that some estimates overlapped (Table 2). Variation in outcrossing among trees may be explained by variation in SI among adults (Lee et al. 2000). It is more likely, however, that differences in outcrossing among trees were the result of differences in pollinator behavior and floral display size. *Platypodium elegans* is pollinated by trap-lining bees that forage long distances and promote outcrossing (Janzen 1971). In contrast, large floral displays promote self-fertilization via geitonogamous pollination (e.g., Harder and Barrett 1995). Outcrossing differences among trees within a single year may result from a combination of long-distance pollinator foraging and geitonogamy. This hypothesis might also explain the higher outcrossing rate observed in the second year (Fig. 1), a period of poor fruit production and smaller floral displays. In 1998–1999, we predict that bees traveled long distances between trees but were less likely to remain within a single canopy (but see Franceschinelli and Bawa 2000). The height of reproductive adults prevented us from testing either of these hypotheses (variation in SI or pollinator behavior).

Models of Inbreeding Depression

In contrast to previous results, *P. elegans* experiences mixed mating with outcrossing rates at the earliest life stage as low as 50–60% for individual trees (mean = 79%). Strong episodes of inbreeding depression select against self-fertilized progeny to raise the mean value of outcrossing to 91% in seedlings. These values do not fit models of inbreeding depression described in Lande and Schemske (1985). Namely, populations with intermediate values of self-fertilization should purge high loads of recessive lethal mutations and avoid early inbreeding depression.

Lande et al. (1994) described an alternative model for plant populations with intermediate rates of selfing and strong early components of inbreeding depression. In this model, high rates of mutation to recessive lethal alleles combined with low survival of selfed seeds could maintain high levels of genetic load in mixed-mating species. In effect, selection cannot act on the selfing rate if self-fertilized seeds are purged from the population prior to reaching reproductive maturity. This model explains the intermediate rates of outcrossing seen in many conifers despite initially high rates of seed abortion (Lande et al. 1994; Koelewijn et al. 1999). Somatic cell lines in plants are not separate from germ lines and, as a result, somatic mutations can be transmitted to gametes. This may be particularly important in long-lived woody species (Klekowski 1988; Lande et al. 1994). The strong selection in *P. elegans* during germination when many genes are expressed fits this pattern, suggesting that *P. elegans* experiences a high genetic load that is purged early in development. In this case, only a selfing rate high enough to exceed a threshold that results in some survival of selfed progeny to reproductive maturity would result in purging of deleterious alleles that cause early inbreeding depression (Lande et al. 1994).

If inbreeding equilibrium is assumed in the adult population, the equation $F_{IS} = (1 - t)/(1 + t)$ can be solved for t to calculate a realized outcrossing rate of 97% for *P. elegans* adults on Barro Colorado Island (Wright 1922). This estimate suggests that selection against self-fertilized progeny continues to act on seedlings, saplings, and adults prior to reproductive maturity (see also Hamrick et al. 1993). Thus, virtually no self-fertilized progeny reach reproductive age. In essence, the adult population is equivalent to random mating and fits Hardy-Weinburg expectations. Similar evidence of decreasing inbreeding for the transition from seeds to adults has been observed in the tropical tree *Cecropia obtusifolia* (Alvarez-Buylla and Garay 1994) but the trend is not consistent among all plants studied (e.g., Tonsor et al. 1993). Variation among species in population inbreeding levels at each life-history stage may depend on the intensity of selection as well as the extent of population thinning.

Implications for Tropical Tree Population Dynamics

Numerous predictions have been made regarding the mating system and population genetic dynamics of tropical trees. Early theories (Baker 1959; Federov 1966) suggested that the low density of tropical tree species should result in high levels of self-fertilization and low levels of gene flow in tropical rainforests. Research has since shown that tropical trees are commonly self-incompatible, ensuring outcrossing (Bawa 1974; Seavey and Bawa 1986). In addition, a large body of research describing the breeding structure of tropical tree populations indicates that gene flow is extensive and may result in breeding units as large as hundreds of hectares (Hamrick and Loveless 1989; Hamrick and Murawski 1990; Nason et al. 1996; Nason et al. 1998; Konuma et al. 2000). Self-fertilization and mixed mating have also been documented in several tropical tree species, although less frequently (e.g., Hamrick and Murawski 1990).

Mixed mating in *P. elegans* combined with early selection for outcrossed progeny suggests that gene-flow events are particularly important for this species. The high rates of fruit abortion and seed mortality observed in *P. elegans* and other tropical forest trees (Bawa and Webb 1984) also suggest that selection at early life stages may maintain high levels of genetic variation in self-compatible species. Overall, viability

selection may play a significant role in altering the population genetic composition of not only self-compatible tropical trees, but also all angiosperms with mixed-mating systems.

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Literature Cited

Allard, R. W., A. L. Kahler, and M. T. Clegg. 1977. Estimation of mating cycle components of selection in plants. Pp. 1–19 *in* F. B. Christiansen and T. Fenchel, eds. Measuring selection in natural populations. Lecture notes in biomathematics. Vol. 19. Springer, Heidelberg, Germany.

Alvarez-Buylla, E. R., and A. A. Garay. 1994. Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. Evolution 48:437–453.

Augspurger, C. K. 1983. Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of seedlings from fungal pathogens. J. Ecol. 71:759–771.

———. 1984. Seedling survival among tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. Ecology 65:1705–1712.

Augspurger, C. K., and C. K. Kelly. 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. Oecologia 61:211–217.

Baker, H. G. 1959. Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol. 24: 177–191.

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28:85–92.

Bawa, K. S., and C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. Am. J. Bot. 71:736–751.

Bush, R. M., and P. E. Smouse. 1992. Evidence for the adaptive significance of allozymes in forest trees. New Forests 6:179–196.

Charlesworth, D. 1988. A method for estimating outcrossing rates in natural populations. Heredity 61:469–471.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18:237–268.

Clegg, M. T., and R. W. Allard. 1973. Viability versus fecundity selection in the slender wild oat, *Avena barbata* L. Science 181: 667–668.

Clegg, M. T., A. L. Kahler, and R. W. Allard. 1978. Estimation of life cycle components of selection in an experimental plant population. Genetics 89:765–792.

Croat, T. B. 1978. Flora of Barro Colorado Island. Stanford Univ. Press, Stanford, CA.

Darwin, C. R. 1859. On the origin of species by means of natural selection. John Murray, London.

———. 1876. The effects of cross and self fertilization in the vegetable kingdom. John Murray, London.

Edwards, K., C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Federov, A. A. 1966. The structure of the tropical rain forest and speciation in the humid tropics. J. Ecol. 54:1–11.

Franceschinelli, E. V., and K. S. Bawa. 2000. The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). Heredity 84:116–123.

Hamrick, J. L., and M. D. Loveless. 1989. The genetic structure of tropical tree populations: associations with reproductive biology. Pp. 129–146 *in* J. H. Bock and Y. B. Linhart, eds. The evolutionary ecology of plants. Westview Press, Boulder, CO.

Hamrick, J. L., and D. A. Murawski. 1990. The breeding structure of tropical tree populations. Plant Species Biol. 5:157–165.

Hamrick, J. L., D. A. Murawski, and J. D. Nason. 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107/108:281–297.

Harder, L. D., and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515.

Hardner, C. M., and B. M. Potts. 1997. Postdispersal selection following mixed mating in *Eucalyptus regnans*. Evolution 51: 103–111.

Hedrick, P. W. 1983. Genetics of populations. Science Books International, Boston, MA.

Hubbell, S. P., and R. B. Foster. 1983. Diversity of canopy trees in a neotropical forest and implications for conservation. Pp. 25–41 *in* L. C. Sutton, T. C. Whitmore, and A. C. Chadwick, eds. Tropical rain forest: ecology and management. Blackwell Scientific Pub., Oxford, U.K.

Hufford, K. M., G. Kochert, and J. L. Hamrick. 2000. Microsatellite primers and amplification of aborted embryos in *Platypodium elegans* J. Vogel (Fabaceae, Papilionoideae). Mol. Ecol. 9: 1174–1176.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70.

Jackson, D. P., J. D. Hayden, and P. Quirke. 1994. Extraction of nucleic acid from fresh and archival material. Pp. 29–50 *in* M. J. McPherson, P. Quirke, and G. R. Taylor, eds. PCR: A practical approach. Vol. 1. Oxford Univ. Press, Oxford, U.K.

Janzen, D. H. 1971. Euglossine bees as long-distance pollinators of tropical plants. Science 171:203–205.

Klekowski, E. J., Jr. 1988. Mutation, developmental selection and plant evolution. Columbia Univ. Press, New York.

Koelewijn, H. P., V. Koski, and O. Savolainen. 1999. Magnitude and timing of inbreeding depression in Scots pine (*Pinus sylvestris* L.). Evolution 53:758–768.

Konuma, A., Y. Tsumura, C. T. Lee, S. L. Lee, and T. Okuda. 2000. Estimation of gene flow in the tropical-rainforest tree *Neobalanocarpus heimii* (Dipterocarpaceae) inferred from paternity analysis. Mol. Ecol. 9:1843–1852.

Koski, V. 1973. On self-pollination, genetic load, and subsequent inbreeding in some conifers. Commun. Inst. For. Fenn. 7810: 1–42.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40.

Lande, R., D. W. Schemske, and S. T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. Evolution 48:965–978.

Lee, S. L., R. Wickneswari, M. C. Mahani, and A. H. Zakri. 2000. Mating system parameters in a tropical tree species, *Shorea leprosu* Miq. (Dipterocarpaceae), from Malaysian lowland dipterocarp forest. Biotropica 32:693–702.

Li, C. C., and D. G. Horvitz. 1953. Some methods of estimating the inbreeding coefficient. Am. J. Hum. Genet. 5:107–117.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86:69–79.

Mont, J., M. Iwanaga, G. Orjeda, and K. Watanabe. 1993. Abortion and determination of stages for embryo rescue in crosses between sweet-potato, *Ipomoea batatas* Lam. (2n = 6x = 90) and its wild relative, *I. trifida* (H.B.K) G.Don. (2n = 2x = 30). Sex. Plant Reprod. 6:176–182.

Murawski, D. A., and J. L. Hamrick. 1991. The effect of the density

of flowering individuals on the mating systems of nine tropical tree species. Heredity 67:167–174.
Nason, J. D., E. A. Herre, and J. L. Hamrick. 1996. Paternity analysis of the breeding structure of strangler fig populations: Evidence for substantial long-distance wasp dispersal. J. Biogeogr. 23:501–520.
———. 1998. The breeding structure of a tropical keystone plant resource. Nature 391:685–687.
Ritland, K. 1986. Inferences about inbreeding depression based on changes of the inbreeding coefficient. Evolution 44:1230–1241.
Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. Annu. Rev. Ecol. Syst. 18:209–235.
Seavey, S. R., and K. S. Bawa. 1986. Late-acting self-incompatibility in angiosperms. Bot. Rev. 52:195–219.
Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution 39:41–52.
Shaw, D. V., A. L. Kahler, and R. W. Allard. 1981. A multilocus estimator of mating system parameters in plant populations. Proc. Natl. Acad. Sci. USA 78:1298–1302.
Sorensen, F. C. 1969. Embryonic genetic load in coastal Douglas-fir, *Pseudotsuga menziesii* var. *menziesii*. Am. Nat. 103:389–398.
———. 1982. The role of polyembryonal vitality in the genetic system of conifers. Evolution 36:725–733.
Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. 12:253–279.
Stephenson, A. G., and J. A. Winsor. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. Evolution 40:453–458.
Tautz, D., and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. 12:4127–4138.
Tonsor, S. J., S. Kalisz, J. Fisher, and T. P. Holtsford. 1993. A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. Evolution 47:833–843.
Wang, Z., J. L. Weber, G. Zhong, and S. D. Tanksley. 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 88:1–6.
Weir, B. S. 1996. Genetic data analysis II. Sinauer Associates, Sunderland, MA.
Wright, S. 1921. Systems of mating. Genetics 6:111–178.
———. 1922. Coefficients of inbreeding and relationships. Am. Nat. 56:330–338.

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