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GENETIC CONSEQUENCES OF OUTCROSSING IN THE  
CLEISTOGAMOUS ANNUAL, *IMPATIENS CAPENSIS*. II.  
OUTCROSSING RATES AND GENOTYPIC  
CORRELATIONS

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*Abstract.*—The genetic consequences of a plant's mating system depend on both the degree of outcrossing and the genetic relationship between mates. We examined the electrophoretic genotypes of seeds derived from cleistogamous (CL) and chasmogamous (CH) flowers in six populations of the facultatively cleistogamous annual, *Impatiens capensis*. Multilocus estimates of the outcrossing rates for the strongly protandrous CH flowers ranged from 0.29 to 0.71 and were higher than estimates based on single-locus data. Such results suggest that the CH flowers experience variable levels of both geitonogamous self-fertilization and biparental inbreeding. A new and generally applicable technique based on the relative level of inbreeding within progeny groups provided direct estimates of the correlation between the genotypic values of outcrossed mates. These correlations varied widely among populations and contributed up to half of the inbreeding observed among the CH progeny. Such biparental inbreeding biases estimates of the outcrossing rate based on the mixed-mating model downward and influences mating-system evolution by decreasing the "cost of meiosis."

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Mating systems directly affect the expression of genetic variability in the next generation by determining how existing variation is transmitted. The resulting distribution of genotypes, in conjunction with patterns of dispersal, also affects the pattern of mating in the next generation. Such interactions are likely to be particularly complex in plants capable of self-fertilizing (Jain, 1976; Uyenoyama, 1986). Thus, our understanding of plant mating systems, their effects, and how selection might modify them, rests on our ability to characterize mating systems in relation to local genetic structure, even when these relationships are obscured by varying degrees of self-fertilization.

The analysis of plant mating systems requires both reliable genotypic data and an explicit model of how matings occur within the population. Progeny and maternal genotypes are then compared to infer the frequency of outcrossing. The simplest and most frequently used model is the "mixed mating model" introduced by Fyfe and Bailey (1951) and applied routinely to allozyme data for almost 20 years (Brown and Allard, 1970; Ritland, 1983; Brown et al., 1985). This model assumes that all mating events within a population involve either self-fertilization or random outcrossing and so can

be characterized by two parameters: the pollen allele frequency,  $p$ , and the outcrossing rate,  $t$ . This model further assumes that pollen genotypes are distributed randomly over all ovules and that outcrossing rates do not depend on maternal genotype. In natural plant populations, one or more of these assumptions will usually be violated. In particular, outcrossing is likely to involve related mates as the result of assortative mating between similar or adjacent plants. Such biparental inbreeding mimics self-fertilization and so leads to underestimates of the true outcrossing rate (Ennos and Clegg, 1982; Ritland, 1984).

Here, we present estimates of both the outcrossing rate and genotypic correlations in natural populations of the common woodland annual, *Impatiens capensis*. This plant regularly self-fertilizes via cleistogamous (CL) flowers, yet produces an increasing fraction of outcrossing chasmogamous (CH) flowers under favorable growing conditions (Carroll, 1919; Wood, 1975; Schemske, 1978; Waller, 1980). The CH flowers are mechanically prevented from direct self-fertilization by complete protandry and are visited primarily by bumblebees (*Bombus* spp.), honeybees, and humming-

birds. However, geitonogamy (cross-pollination between flowers on the same plant) can occur, producing selfed CH progeny.

Patterns of genetic variation in *Impatiens capensis* reveal considerable inbreeding in most populations and the widest range of inbreeding coefficients yet reported for a plant species (Knight and Waller, 1987). Most of the inbreeding results from the enforced self-fertilization of the abundant CL flowers. Similarly, most of the variation among populations results from the notoriously variable investment in CH and CL flowers in response to plant size and environmental conditions (Schemske, 1978; Waller, 1980). Nevertheless, some of both the inbreeding and the variation among populations could reflect variable levels of inbreeding among the ostensibly outcrossed CH flowers. Progeny from these flowers display a considerable excess of homozygotes (mean fixation index = 0.48 [Knight and Waller, 1987]). Such excess homozygosity must reflect the geitonogamous self-fertilization of CH flowers or positive genotypic correlations between truly outcrossed mates. The goal of this study was to measure the relative magnitude of these two sources of CH inbreeding. In other papers, we describe the overall genetic structure of these populations (Knight and Waller, 1987), patterns of linkage disequilibrium (Waller and Knight, 1989), and fine-scale geographic structure (Shoemaker and Waller, unpubl.).

#### MATERIALS AND METHODS

We conducted an electrophoretic survey of the genetic structure of 11 *Impatiens* populations distributed across Wisconsin, New Jersey, Rhode Island, and Massachusetts (Knight and Waller, 1987). Within each population, we collected four seeds from CL capsules on 60–71 randomly selected maternal parent plants (except one population [SF in Table 1] with 16 parents). In addition, all ripe CH capsules were collected from plants large enough to produce them. Four CH seeds (representing from one to many capsules) were then randomly selected for analysis. Six of the 11 populations had enough CH progeny (64–148) to allow us to estimate outcrossing rates and genotypic correlations. To minimize the likelihood of population substructuring (the Wahlund ef-

fect), we sampled plants within a small (2 × 5 m) grid in three of these populations (HF, GI, and AT in Table 1). Reduced densities required that a somewhat larger area be sampled in the other three populations (MZ, UWM, and SF in Table 1). The embryos from these seeds were assayed and scored for nine polymorphic loci (O'Malley et al., 1980). Only 1–3 loci varied appreciably within any given population, and gene frequencies were usually asymmetric (Knight and Waller, 1987; Table 1). The four CL progeny provide eight alleles and so allow accurate determination of the maternal genotype (i.e., the probability of misidentifying an *Aa* heterozygote by sampling either four *AA* or four *aa* CL progeny is  $(\frac{1}{2})^8 + (\frac{1}{2})^8 = 0.0078$ ). They also provide an estimate of inbreeding parameters for the CL group. The CH seeds provide progeny arrays for estimating outcrossing rates and the degree of inbreeding in the CH group. For further details concerning the sites, electrophoretic methods, and genetic results, see Knight and Waller (1987).

We initially estimate outcrossing rates using the traditional mixed-mating model, ignoring the effects of genotypic correlations. We then use these outcrossing rates, in conjunction with the fixation indexes estimated for the CL and CH progeny, to infer the genotypic correlations using a new method. While it would clearly be preferable to estimate the outcrossing rate, pollen allele frequencies, and genotypic correlation simultaneously, this is impossible given the limited information available (K. Ritland, pers. comm.).

We estimated fixation indexes ( $f$ ) (Wright, 1965; Crow and Kimura, 1970) for each variable locus and population using a bootstrapping technique (Efron, 1982) that randomly resampled observed individual genotypes 1,000 times. Values were then combined across loci using Weir and Cockerham's (1984) procedure that weights loci according to the variance in gene frequency ( $pq$ ). These values were in close agreement with those already reported for the CL, CH, and parent groups (Knight and Waller, 1987).

*Outcrossing Rates.*—Outcrossing rates were calculated individually for the variable loci in each population using a single-locus

maximum-likelihood procedure (Clegg et al., 1978) based on the mixed-mating model. We also calculated two multilocus estimates of the outcrossing rate: Shaw et al.'s (1981) method-of-moments estimator and the estimate based on Ritland and Jain's (1981) multilocus maximum-likelihood procedure. The two procedures involved in calculating these estimates are both capable of detecting an outcross at any of several loci, making them more resistant to the bias introduced by biparental inbreeding than single-locus measures (Ritland, 1984). They do, however, differ somewhat in their assumptions.

Shaw et al.'s (1981) method requires that maternal genotypes be known so that progeny can be assigned to one of two classes: those that contain a novel allele (and hence must be outcrossed) versus those whose genotype is ambiguous. The multilocus probability of not identifying an outcrossing event that actually occurred ( $\alpha$ ) is then estimated as the product of the proportion of ambiguous matings at each locus. This leads to an estimate of the multilocus outcrossing rate,  $t_{ML}$ :

$$t_{ML} = \frac{n}{N(1 - \alpha)} \quad (1)$$

where  $n$  is the number of discernable outcrossed progeny in a sample of size  $N$ . To reduce bias, maternal genotypic frequencies are weighted by the number of progeny each mother contributes to the total sample. However, because the probability of detecting an outcross can vary with maternal genotype when only a few marker loci are used, some bias may still occur (Brown et al., 1989). The expected variance of this estimate is:

$$\text{Var}(t_{ML}) = \frac{t[1 - t(1 - \alpha)]}{N(1 - \alpha)} \quad (2)$$

We wrote a Pascal program to compute these estimates and error variances (available from D.M.W. upon request).

Ritland and Jain's (1981) estimator generalizes Clegg et al.'s (1978) single-locus maximum-likelihood estimator to many loci. In this procedure, the allele frequencies and the outcrossing rate are estimated simultaneously via iterations that maximize a log-likelihood equation. Rather than as-

suming pollen allele frequencies to be a weighted average of maternal allele frequencies, pollen and maternal allele frequencies are estimated separately. By comparing these, we can check the assumption that there is an even distribution of pollen over maternal plants. This procedure also allows one to infer maternal genotypes, but the CL progeny provided these genotypes directly. K. Ritland provided a FORTRAN program for calculating estimates of these allele frequencies and the multilocus outcrossing rate.

*Genotypic Correlations.*—Estimates of the outcrossing rates were then combined with estimates of CL and CH fixation indexes to estimate the correlation that exists between the genotypic values of outcrossed mates in these populations. In the absence of selection, the fixation index expected among a group of self-fertilized progeny ( $f_{CL}$ ) can be expressed as a simple function of the fixation index of their parent ( $f_p$ ) (Wright, 1921):

$$\text{expected } f_{CL} = \frac{(1 + f_p)}{2} \quad (3)$$

In contrast, the expected fixation index among a group of randomly outbred progeny is 0. If, instead, a correlation exists between the genotypic values of mates within the local population, the fixation index will be positive with an expected value of

$$\text{expected } f_{CH} = \frac{(1 + f_p)m_t}{2} \quad (4)$$

(Wright, 1921), where  $m_t$  is the total genotypic correlation between pollen and ovule parents. Thus, an estimate of  $m_t$  may be obtained from the ratio of the observed CH and CL fixation indexes:

$$m_t = \frac{f_{CH}}{f_{CL}} \quad (5)$$

Because some CL heterozygotes may have been misclassified (Knight and Waller, 1987), we used parental genotypic data and Equation (3) above to estimate  $f_{CL}$  and  $m_t$ .

We calculated these overall genotypic correlations both directly (to obtain a point estimate) and indirectly using a bootstrapping procedure similar to that described above to estimate the  $f$ 's. The bootstrapping procedure also provided estimates of the

error associated with this estimate (a standard error). Because the bootstrapping procedure provided independent estimates of  $m_i$  and its error for each variable locus, these were averaged (as before) to obtain overall estimates for each population.

The expected CH fixation index,  $E(f_{CH})$ , should be the weighted average of two components, one due to geitonogamous selfing and the other due to outcrossing:

$$E(f_{CH}) = \frac{g(1 + f_P)}{2} + \frac{(1 - g)(1 + f_P)m_b}{2} \quad (6)$$

where  $g$  represents the fraction of geitonogamous self-fertilizations among the CH flowers and  $m_b$  is the biparental genotypic correlation between truly outcrossed mates. This formula gives an estimate of the latter as

$$m_b = \frac{(m_i - g)}{(1 - g)} \quad (7)$$

provided  $g$  and  $m_i$  are known. We estimated  $g$  as  $(1 - t)$  where  $t$  is the outcrossing rate estimated by the two multilocus methods described above. Unfortunately, it was not feasible to obtain bootstrapped estimates of the error in  $m_b$  because the outcrossing rates upon which this formula is based already combine data over several loci. While error estimates might be obtainable by combining error statistics associated with  $m_i$  and  $g$ , their inevitable covariance makes this difficult.

## RESULTS

**Outcrossing Rates.**—The three methods used to estimate outcrossing rates yielded somewhat different results. Most obviously, the single-locus estimates were generally lower than the multilocus estimates (Tables 1, 2). This was expected, since multilocus estimators better resist the bias introduced by correlated mating events (Ritland and Jain, 1981). The single-locus estimates of outcrossing in the CH flowers ranged from a minimum of 0.13 to a maximum of 0.66 among the variable loci of the six populations (Table 1). Three of the six populations had at least two reliable and variable loci, which allowed comparison of outcrossing

TABLE 1. Sampled populations, estimated fixation indexes, variable loci, and associated single-locus estimates of the outcrossing rate based on Clegg et al.'s (1978) method. The fixation rates and their errors are estimated using a bootstrapping method. Values in brackets are unreliable due to observed heterogeneity in gene frequencies between maternal parents and CH progeny and data from these loci are therefore not used in subsequent analyses.

Population	State	Estimated fixation index (SE)	Variable loci	Estimated outcrossing rate
HF	RI	0.48 (0.13)	<i>Idh-1</i>	0.466
UWM	WI	0.42 (0.14)	<i>Idh-2</i> <i>Fea</i>	0.604 [1.00]
GI	WI	0.63 (0.12)	<i>Idh-2</i> <i>Gpt-1</i>	0.417 0.407
MZ	WI	0.62 (0.13)	<i>Idh-2</i> <i>Fea</i> <i>Gpt-1</i>	0.657 0.395 0.130
SF	NJ	0.41 (0.26)	<i>Idh-1</i> <i>Idh-2</i> <i>Pgi-2</i>	0.289 0.392 0.308
AT	WI	0.40 (0.12)	<i>Idh-2</i> <i>Mdh</i> <i>Gpt-1</i>	[0.063] 0.449 [0.616]

estimates among the loci. In populations GI and SF, the estimates are similar among loci, but in MZ, loci *Gpt-1*, *Fea*, and *Idh-2* give estimates of 0.13, 0.40, and 0.66, respectively, spanning the entire range of outcrossing rates observed among the populations.

The multilocus estimates of the CH outcrossing rate based on the Shaw et al. (1981) method vary between 0.40 (population AT) and 0.71 (population UWM) (Table 2) and are consistently, but not significantly, higher than the estimates provided by the Ritland and Jain (1981) procedure (range: 0.30 [AT] to 0.68 [UWM]). The two estimates were closely correlated with each other ( $r = 0.78$ ) but do not appear to depend on either the number of variable loci or the population fixation index (Table 4).

**Genotypic Correlations.**—A high correlation exists between the genotypic values of "outcrossed" mates in all the populations, ranging from 0.48 (population GI) to 0.64 (HF) as indicated by the direct-estimation technique (mean  $m_i = 0.59$ ) or from 0.43 (AT) to 0.72 (SF) as indicated by the bootstrapped estimates (mean = 0.57) (Table 3). All of these values are significant in

TABLE 2. Estimated rates of outcrossing ( $t$ ) and associated standard errors based on the multilocus estimation procedures of Shaw et al. (1981) and Ritland and Jain (1981). Due to mechanical self-incompatibility, the rate of geitonogamous CH selfing is estimated to be  $g = 1 - t$ .

Popula- tion	Shaw et al.		Ritland and Jain	
	$t$	SE	$t$	SE
HF	0.526	0.126	0.467	0.106
UWM	0.712	0.142	0.678	0.130
GI	0.610	0.097	0.432	0.082
MZ	0.553	0.067	0.615	0.083
SF	0.481	0.083	0.489	0.086
AT	0.405	0.102	0.295	0.084
Means:	0.548	0.106	0.496	0.136

the sense that they are more than twice as large as their estimated standard errors. Surprisingly, the largest standard error (0.25) was not associated with the population with the smallest sample size (SF) but rather with the population with the highest estimated outcrossing rate (UWM). For those populations with more than one reliable variable locus (i.e., GI, MZ, and SF), these estimates of error are conservative (i.e., are overestimates), as they represent simple weighted averages of the presumably independent error associated with each locus.

The total correlation results from two components: geitonogamous selfing (also reflected in the intermediate outcrossing rates) and the residual genotypic correlation between truly outcrossed mates ( $m_b$ ). The estimated residual genotypic correlations

TABLE 3. The total genotypic correlations between uniting gametes ( $m_t$ ), calculated directly and via a bootstrapping procedure, and genotypic correlations between truly outcrossed mates ( $m_b$ ), based on the bootstrapped values. Populations HF, GI, and AT were sampled at the finest spatial scale ( $2 \times 5$  m).

Population	Genetic correlations			
	$m_t$		$m_b$	
	Direct	Bootstrap (SE)	Shaw et al.	Ritland & Jain
HF	0.64	0.58 (0.16)	0.20	0.10
UWM	0.56	0.60 (0.25)	0.44	0.41
GI	0.48	0.48 (0.17)	0.16	-0.19
MZ	0.62	0.61 (0.13)	0.30	0.37
SF	0.64	0.72 (0.22)	0.41	0.42
AT	0.60	0.43 (0.18)	-0.40	-0.92
Means:	0.59	0.57	0.18	0.03

TABLE 4. Simple correlations between various estimated genetic parameters of  $f$ , the population fixation index. Multilocus estimates of the outcrossing rates were calculated based on the techniques of Shaw et al. (1981) and Ritland and Jain (1981).

Estimated parameter	Correlation coefficient with $f$
Multilocus outcrossing rate ( $t_{ML}$ )	
Shaw et al. method	-0.03
Ritland and Jain method	-0.16
Genotypic correlation	
Total ( $m_t$ )	-0.18
Between outcrossed mates ( $m_b$ )	
Shaw et al. $t$	0.16
Ritland and Jain $t$	0.13

varied considerably among populations and somewhat with which outcrossing estimator was used (Table 3). Using the Shaw et al. (1981) outcrossing estimates,  $m_b$  varies from one negative value, -0.40 for population AT, to a high of 0.44 for population UWM (mean = 0.24); for the Ritland and Jain outcrossing estimates  $m_b$  is more variable, with two negative values (-0.92 in AT and -0.19 in GI) and a maximum value of 0.42 (SF) (mean = 0.03). If these values are used to estimate the proportion of inbreeding observed among the CH progeny attributable to biparental inbreeding [Eq. (6)], they range from (ignoring negative values) 0.20 to 0.52 (Shaw et al. [1981] estimator), or from 0.08 to 0.46 (Ritland and Jain [1981] procedure). Thus, up to half of the CH inbreeding in some populations may be due to biparental inbreeding, rather than geitonogamous selfing. As with the outcrossing rates, the genetic correlations appear to be independent of the population fixation index (Table 4).

## DISCUSSION

Progeny in many species result from a mixture of self-fertilization, matings with related individuals, and true (i.e., random) outcrossing. The sources of inbreeding observed within such species are difficult to disentangle because both self-fertilization and biparental inbreeding result in similarly inbred progeny. The methods presented here provide at least preliminary estimates of the magnitude of both sources of inbreeding. They are also general in the sense that they

can be applied to other species whenever sufficient data on multilocus genotypes in progeny arrays are available (see below).

For simplicity and ease of comparison with other studies, we used the mixed-mating model to estimate outcrossing rates. This model, however, ignores biparental inbreeding, causing estimates based on it to often underestimate the actual (although not the "effective") outcrossing rate (Ritland, 1984, 1986). Unfortunately, it was not possible to estimate all parameters simultaneously, forcing us to adopt the "trick" of initially assuming that no genotypic correlations existed in order to estimate the outcrossing rate and then estimating the magnitude of the residual correlation using this outcrossing estimate. Perhaps a recursive iterative technique could be developed to make use of this correlation to reestimate the outcrossing rate and correlation, but this was not attempted here.

*Outcrossing Rates.*—The estimated outcrossing rates vary considerably among populations but are never above 0.72 nor below 0.30. Because CH flowers in *Impatiens* are strongly protandrous and are usually mechanically prevented from fertilizing themselves, these values imply the existence of considerable geitonogamous self-fertilization. As expected, the single-locus estimates were generally lower than the multilocus estimates, presumably because multilocus estimators better resist the bias introduced by correlated mating events (Ritland and Jain, 1981; see below). Single-locus estimates of  $t$  are also subject to greater errors of estimation, which might account for part of the variation observed among loci in population MZ. Nevertheless, multilocus estimates are not immune from inaccuracy and bias, especially in cases where only two or a few loci with limited variability are available. Ritland and Jain (1981) suggest that 4–5 unlinked loci are necessary to provide most of the information concerning outcrossing, but in this study, only an average of slightly more than two variable loci were expressed per population, and these contained limited allelic variability. Thus, the multilocus estimates of outcrossing reported here should be considered minimum estimates of the true outcrossing rate, and the estimated rates of geitonogamy (30–

70%) should be considered maximum estimates.

Although the mechanical self-incompatibility system and strong dichogamy appear to prevent direct self-fertilization within flowers, these results suggest surprisingly high rates of self-fertilization among the CH flowers through geitonogamy. The lack of genetic incompatibility, combined with the well-known tendency for localized foraging by the predominant bumblebee pollinators, apparently makes this species vulnerable to self-fertilization even among the ostensibly outcrossed CH flowers. Rates of geitonogamy are likely to vary among plants as well as among populations. Larger plants predictably have more CH flowers (Schemske, 1978; Waller, 1980) making them more likely to experience geitonogamy, and chance factors involving specific patterns of pollinator visitation could obviously contribute to this variation. The considerable variation in geitonogamy observed among populations could be due to any of several external factors, including average plant size, the foraging patterns of local pollinators, site-specific differences in pollinator availability, or perhaps subtle differences among populations in floral characteristics that promote or retard geitonogamous pollinator flights or direct CH selfing. Differences among populations in geitonogamy do not appear to be an artifact of the number of variable loci or to depend on the extent to which a population is already inbred.

The multilocus estimates of outcrossing obtained by the Ritland and Jain (1981) procedure were consistently greater than those obtained by the Shaw et al. (1981) method. The most likely explanation for this discrepancy is that the Ritland and Jain procedure, rather than inferring pollen gene frequencies from the maternal genotypes, estimates these directly from the progeny genotypes. Thus, when the pollen and ovule groups diverge in allelic frequencies, the Ritland and Jain method will adjust for this while the Shaw et al. method could respond by overestimating the outcrossing rate (Ritland, 1986).

Both the single-locus and multilocus estimates of the outcrossing rate presented here are based on the mixed-mating model and therefore rest on its assumptions. For ex-

ample, the model assumes that the probability of an outcrossing event is independent of maternal genotype. This assumption could be violated if larger plants are predictably more likely to experience geitonogamy and there is a genetic component to variation in size. However, most of the phenotypic variation in these populations appears to be environmental (Waller, 1984, 1985; Mitchell-Olds and Waller, 1985). The mixed-mating model also assumes that no selection affects the marker loci between the time of mating and the time when progeny genotypes are assayed. This assumption appears to be reasonably well supported, since seeds were assayed directly and selection has only occasionally been shown to be operating directly on electrophoretic alleles in other species (Mitton and Grant, 1984). Multilocus techniques also assume that there is no linkage (or linkage disequilibrium) among the loci. In this study, the number of loci used is small relative to the number of chromosomes ( $2n = 20$  [Wood, 1975]), and linkage disequilibrium does not appear to be extensive (Waller and Knight, 1989).

Perhaps more onerously, the mixed-mating model assumes that allele frequencies in the outcross-pollen pool are distributed uniformly over the population of maternal plants. Assortative mating violates this assumption and produces positive genotypic correlations like those found in most of these populations. Such correlations arise easily in plant populations when mating occurs among neighbors within locally structured populations (Ellstrand et al., 1978; Linhart et al., 1981). In *Impatiens*, both seed dispersal (Schmitt et al., 1985) and pollen movement (Zimmerman and Cook, 1985) appear to be local. Locally structured populations may be the rule in plant populations subject to restricted pollen and seed movement (Ehrlich and Raven, 1969; Levin and Kerster, 1974; Levin, 1981). Thus, biparental inbreeding such as that reported here probably typifies many natural populations, biasing estimates of the outcrossing rate based on the mixed-mating model (Ennos and Clegg, 1982).

Alternatives to the mixed-mating model exist but still retain some simplifying assumptions (see reviews by Ritland [1983] and Brown et al. [1985]). For example, the

one-pollen-parent model (Schoen and Clegg, 1984; Schoen, 1988) does not assume an even distribution of pollen alleles over maternal parents and is thus well-suited for analyzing progeny arrays from single fruits when these are likely to have been pollinated by pollen from a single source. Chasmogamous progeny from single capsules in *Impatiens* could share the same pollen parent, suggesting that the one-pollen-parent model might be appropriate. However, the single-pollen-parent model has not yet been extended to incorporate multilocus data, and unfortunately, seeds were not separated by capsule in this study. A more complete view of the correlation structure within the CH progeny might emerge from a nested analysis of the components of variance among multiple CH capsules (cf. Schoen, 1985).

*Genotypic Correlations.*—The estimated genotypic correlations between uniting gametes ( $m_c$ ) were sizable (0.43–0.72, mean = 0.57; Table 2) and at least partly attributable to the frequency of self-fertilization. All these values were more than twice as large as the (conservative) standard errors estimated using the bootstrap technique and, thus, are likely to be statistically significant. The estimated errors varied over a twofold range (0.13–0.25) and depended more on the patterns of genetic variability expressed in these populations than on the number of individuals analyzed.

Outcrossing rates estimated by the multilocus methods usually exceeded those estimated from single-locus data, suggesting that biparental inbreeding was occurring in these populations. Such proved to be the case in most instances, with the genotypic correlations between outcrossed mates ( $m_b$ ) exceeding 0.30 in half the populations. This value is greater than that expected between half-sibs or double first cousins in a panmictic population (0.25). Thus, the inbreeding observed among the CH progeny can result not only from geitonogamy but also from "outcrossed" matings involving related plants. The fraction of CH inbreeding attributable to mating with relatives ranges up to 50% in population UWM, yet population AT and possibly population GI exhibited negative genotypic correlations, suggesting the presence of disassortative mating. Such heterogeneity among popu-

lations suggests that details of population structure, floral presentation, and pollinator visitation could have important effects on the genetic consequences of outcrossing.

Interestingly, the three populations with the lowest  $m_t$  and  $m_b$  values (HF, GI, and AT) were the three dense populations sampled on the finest spatial scale ( $2 \times 5$  m), chosen to minimize the likelihood of local population structure. Preliminary analyses of the geographic distribution of genotypes in these populations also revealed little substructure (Knight and Waller, 1987). Nevertheless, some correlation was evident in population HF, and further analyses revealed the presence of subtle fine-scale structure in some populations (Shoemaker and Waller, unpubl.). These results suggest that population density and the scale of sampling can affect the size of the correlations observed. The negative genotypic correlations resulted numerically from having an outcrossing rate and  $m_t$  that sum to less than 1. While we expected  $m_t$  and  $t$  to vary inversely (as increasing CH outcrossing should reduce  $f_{CH}$  and so  $m_t$ ), they were positively correlated among these populations ( $r = 0.61$ ), possibly because outcrossing simultaneously reduces  $f_{CL}$ .

What is the source of the positive correlations between CH mates? The obvious possibility is that mating is occurring between adjacent or nearby plants, which are more related to each other than two random plants from the sampled population. Another possibility is that assortative mating is occurring based on genetically based differences in floral appearance or phenology. These possibilities were not investigated.

Relative to the Shaw et al. (1981) method, the Ritland and Jain (1981) procedure generally gave lower  $t$  estimates and consequently gave lower estimates of  $m_b$  (Tables 2, 3). As discussed above, the Ritland and Jain estimates should better handle heterogeneous allele frequencies but could still represent an underestimate of the true outcrossing rate. Hence, the estimates of  $m_b$  based on the Ritland and Jain procedure should be considered minimum estimates. To estimate the genotypic correlations, we also assumed that the fixation index of pollen parents was the same as that observed

in the maternal parents. Genetic analyses of individual capsules or pollen could perhaps allow this assumption to be tested.

*Related Studies and Evolutionary Implications.*—Biparental inbreeding has been detected in several other studies. For example, detasseled (hence, obligately outcrossed) maize still displayed “apparent selfing” at two of six loci which Bijlsma et al. (1986) attributed to nonrandom mating. Ennos and Clegg (1982) noted heterogeneous gene frequencies across subpopulations, which they also interpreted in this way. Ellstrand et al. (1978) suggested that the difference between single-locus and multilocus estimates of the outcrossing rate itself could be interpreted as evidence for biparental inbreeding. Finally, Ritland and Ganders (1985) regressed pollen gene frequency on ovule genotype in the Hawaiian endemic species *Bidens menziesii* and interpreted the slope as a quantitative measure of biparental inbreeding. We are not aware of any studies in which evidence for biparental inbreeding in plant populations has been sought but not found.

The genetic consequences of selfing and outcrossing clearly depend on local population genetic structure and patterns of pollen and seed movement. The CH flowers of *Impatiens capensis*, although ostensibly outcrossed, often produce inbred seed. While much of this inbreeding appears to result from geitonogamous self-fertilization, biparental inbreeding also occurs as the result of mating with related individuals. Despite these sources of inbreeding, the CH flowers are successful in reducing fixation substantially from what it would be in the absence of outcrossing and maintaining some genetic variation in these populations. This limited, but real, increase in heterozygosity may provide the mechanism to account for the higher fitnesses often observed among CH progeny (Waller, 1984, 1985; Mitchell-Olds and Waller, 1985).

Biparental inbreeding also affects the evolutionary dynamics of plant mating systems by simultaneously decreasing the relatedness between a maternal parent and its outcrossed progeny (the “cost of meiosis” [Williams, 1975]) and decreasing the genetic difference between selfed and outcrossed progeny (Uyenoyama, 1986). The former

tends to favor the evolutionary retention of outcrossing, while the latter, by decreasing the relative inbreeding depression of selfed progeny, tends to favor selfing. The combined effects are complex but can allow the retention of mixed mating systems in models that otherwise predict the evolution of exclusive self- or cross-fertilization (Uyenoyama, 1986).

Although the CL seeds facilitated the computation of outcrossing rates and genetic correlations in this study, the techniques presented here are general and extend to other species where arrays of progeny and their maternal parents can be genotyped. In fact, since maternal genotypes can usually be inferred from a moderate number of naturally pollinated progeny (Ritland, 1983; Schoen and Clegg, 1984), sufficiently large outcrossed progeny arrays alone can be used to estimate  $t$ ,  $f_{CH}$ , and  $f_P$  and, hence, to estimate the expected  $f$  of selfed progeny and the genotypic correlations. Thus, it should be straightforward to collect information on biparental inbreeding for other populations and species. Such information will be of considerable value for interpreting the evolutionary statics and dynamics of breeding systems.

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