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GENDER-SPECIFIC INBREEDING DEPRESSION IN A GYNODIOECIOUS PLANT, *GERANIUM MACULATUM* (GERANIACEAE)[1]

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In gynodioecious species, females coexist with hermaphrodites in natural populations even though hermaphrodites attract more pollinators, are capable of reproducing through pollen, and can self-fertilize. This study tests the hypothesis that inbreeding depression helps to maintain females in natural populations. It also examines whether gender lineages that differ in selfing rates might experience different levels of inbreeding depression. Female and hermaphroditic lineages of the gynodioecious species *Geranium maculatum* were used in self, sib-cross and outcross experiments to examine inbreeding depression levels and to determine whether these levels differ between hermaphroditic and female lineages. Six fitness correlates were measured in the greenhouse and compared among pollination types and between genders. Severe inbreeding depression was found for both individual fitness traits and cumulative fitness in early life history stages. Inbreeding depression levels were slightly higher in hermaphroditic than in female lineages, but this difference was not statistically significant. Because females are unable to self-pollinate and are less likely to experience inbreeding than hermaphrodites under natural conditions, these results suggest that severe inbreeding depression could confer a selective advantage for females that could help to maintain females in natural populations.

Key words: gender; Geraniaceae; *Geranium maculatum*; gynodioecy; inbreeding depression; selfing; sib-crossing; spotty geranium.

A major trend in angiosperm evolution is the transition from perfect, hermaphroditic flowers to single-sex flowers. One step in this transition is thought to be the development of gynodioecy. In gynodioecious species, some individuals carry mutations that cause them to produce only female flowers while the rest of the population produce hermaphroditic flowers (Darwin, 1877; Schnable and Wise, 1998; Hanson et al., 1999; Budar et al., 2003). Establishment of such genetic polymorphism in natural populations has received a good deal of attention because female plants are expected to be at a disadvantage relative to hermaphrodites in three major aspects of reproduction (Lewis, 1941; Lloyd, 1975, 1976; Charlesworth and Charlesworth, 1978; Charlesworth, 1981, 1992; de Haan et al., 1997).

First, hermaphrodites often attract more pollinators than females because hermaphrodites generally have larger flowers (e.g., Darwin, 1877; Delph, 1996; Williams et al., 2001; Asikainen and Mutikainen, 2005b). Hence, females may be subject to pollen limitation that could reduce the fertilization rate of ovules, i.e., seed production (Graff, 1999; Asikainen and Mutikainen, 2005a; but see López-Villavicencio et al., 2003), especially when outcross pollen is rare because of either small population size or low pollinator activity. Second, while hermaphrodites can pass their genes through both pollen and ovules, females can only reproduce through ovules. Finally, self-compatible hermaphrodites can produce selfed seeds that contain two copies of their genomes, giving them a transmission advantage over females, which are restricted to producing only outcrossed progeny and pass on only one copy of their genome in their seeds (Fisher, 1941; Maynard Smith, 1978).

The degree to which these factors are detrimental to the establishment of female plants depends, in part, on how male sterility (or female) is genetically determined. If male sterile genes are located in the nucleus, all three factors may be important because these genes give up potential transmission through pollen (affected by the latter two factors in the previous paragraph) and rely on transmission through ovules (reduced due to the first factor). If male sterility is caused by cytoplasmic male sterility genes (often located in mitochondria), with or without nuclear genes that restore pollen production, the latter two factors involving pollen fitness would be irrelevant because cytoplasmic organelles in flowering plants are transmitted only through ovules (but see McCauley et al., 2005). In either case, these disadvantages are expected to keep females from becoming established or persisting in hermaphroditic populations. However, a substantial number of the angiosperm species (~7%) are gynodioecious (Dem'yanova, 1985; Richards, 1997), suggesting that mechanisms exist that counteract these disadvantages and support the presence of females in gynodioecious systems.

One major mechanism proposed by many theoretical and empirical studies to be critical for female establishment is the production of higher seed fitness (more and/or better quality) by female plants. This phenomenon, referred to as "female compensation" (Darwin, 1877) has been observed in most gynodioecious species (e.g., Kohn, 1989; Ramsey and Vaughton, 2002; Schultz, 2003). It is generally considered to be due to reallocation of resources from pollen to seeds in females and/or to inbreeding depression in hermaphrodites (e.g., Lloyd, 1975; Charlesworth and Charlesworth, 1978; Schultz, 2002; but see Schultz, 1999).

Inbreeding depression is the reduction in fitness of inbred vs. outcrossed progeny caused by the expression of deleterious

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recessive alleles or the loss of heterozygote advantage (Crow, 1948; Charlesworth and Charlesworth, 1987). Most studies of inbreeding depression in gynodioecious species propose that, if hermaphrodites reproduce at least partially through self-pollination (e.g., Kohn and Biardi, 1995; Schultz and Gander, 1996; Sakai et al., 1997; Collin and Shykoff, 2003), they might produce seeds with a lower average fitness than seeds produced by females, which are obligate outcrossers. This would counterbalance the advantages possessed by hermaphrodites and enable females to become established in a population. However, for inbreeding depression alone to have such an effect, hermaphrodites would have to experience a very high level of inbreeding depression, considering the multiple fitness advantages they have over the females. In addition, inbreeding depression is not restricted to hermaphrodites. Female plants can also experience inbreeding depression if the pollen grains that sire their ovules come from related hermaphroditic individuals. Such "biparental" inbreeding frequently happens in natural populations when both seed dispersal and pollen travel distance are limited (e.g., Godt and Hamrick, 1991; Herlihy and Eckert, 2004; Zárate et al., 2005).

The possibility that high inbreeding depression levels in hermaphrodites could enable females to become established in gynodioecious populations is further complicated by theoretical studies that show how repeated high selfing rates over generations could actually lead to lower levels of inbreeding depression through a process called genetic purging (Lande and Schemske, 1985; Uyenoyama and Waller, 1991a). Genetic purging occurs when families with high selfing/inbreeding rates produce a high proportion of homozygous progeny exposing deleterious alleles (genetic load) to selection. Over time, selection reduces (purges) the frequency of these alleles as well as inbreeding depression levels (Byers and Waller, 1999). Lineages that have low selfing histories, in contrast, would continue to suffer severe inbreeding depression when selfed because purging would not have occurred.

Traditionally, hermaphrodites have been thought to experience selfing more frequently than obligate outcrossing females and would therefore have a lower genetic load (e.g., Charlesworth et al., 1990; Uyenoyama and Waller, 1991a, b). This is likely to be the case when male sterility is controlled only by cytoplasmic genes because females would be produced only by female maternal plants through outcrossing and, therefore, would experience a low selfing history. However, when male sterility is controlled by nuclear genes only, theories have made contrasting predictions. For example, when male sterility is controlled by a dominant allele, females, which are always heterozygous, are only produced through outcrossing of female maternal plants and would have very little selfing history. Therefore, females may harbor high levels of deleterious alleles in the heterozygous state in their genomes. Depending on the selfing rates in hermaphrodites, they may have similar or lower genetic load and level of inbreeding depression compared to females (Schultz, 1999). In contrast, when male sterility is recessive, females are often the product of selfing by heterozygous hermaphrodites, which carry a high genetic load. As a result, the homozygous females would be associated with homozygous deleterious alleles and, therefore, would be purged along with these alleles. In this case, though the surviving females may have high selfing history and could eventually purge their genetic load, the low viability of females actually makes the initial establishment of females very difficult (Schultz, 1999). Finally, the association between level of inbreeding depression and gender when the latter is controlled by cyto-nuclear interactions is still not clear even though this is likely the most common form of genetic determination for gynodioecious species (Budar and Pelletier, 2001). Nevertheless, these theoretical predictions suggest that genders are likely to differ in their genetic load and inbreeding depression levels. Such gender-specific inbreeding depression, combined with the difference in inbreeding (and selfing) rates seen in females and hermaphrodites, will determine the contribution of inbreeding depression to female compensation.

Given that the genetic determinations have not been worked out in most gynodioecious species, the pattern of gender-specific inbreeding depression becomes, to a large extent, an empirical question. Several studies have found significant inbreeding depression in gynodioecious populations (e.g., Mutikainen and Delph, 1998; Emery and McCauley, 2002; Thompson et al., 2004; Koelewijn and van Damme, 2005; Keller and Schwaegerle, 2006). However, results from studies that have examined or provided data for calculating inbreeding depression separately in female and hermaphroditic plants are somewhat equivocal. For example, inbreeding depression in *Sidalcea oregana*, *Phacelia dubia*, and *Silene acaulis* were higher in females than in hermaphrodites (Ashman, 1992; del Castillo, 1993; Keller and Schwaegerle, 2006). These studies, however, used both female and hermaphroditic maternal plants in the same comparisons, which did not allow separation of the short-term maternal effects mediated through maternal gender and the effect of long-term selfing history on progeny fitness (del Castillo, 1993; Muktikainen and Delph, 1998). In contrast, Mutikainen and Delph (1998) controlled for the effects of maternal gender and selfing history of plants in their experiment and specifically tested for a difference in inbreeding depression between female and hermaphroditic lineages in *Silene acaulis*. This study found no difference in the level of inbreeding depression experienced by female and hermaphroditic lineages.

Additional studies are needed before conclusions can be made as to whether lineage-specific inbreeding depression is a general and important phenomenon in gynodioecy. This paper describes greenhouse experiments that empirically test whether gender-specific inbreeding depression is present in the self-compatible gynodioecious species *Geranium maculatum* and whether the pattern of lineage-specific inbreeding depression contributes to maintaining polymorphism in floral sexual expression in this species. Population genetic studies showed the hermaphroditic plants in natural population self to a moderate level (15∼40% selfing rare; C. Deen, University of Georgia, and S.-M. Chang, unpublished data), and previous studies have shown that female compensation does occur (Ågren and Willson, 1991; Chang, 2006). Two questions are addressed in this study. First, do progeny derived from selfing or sib-crossing have a lower average fitness due to inbreeding depression than progeny derived from outcrossing? Second, do female and hermaphroditic lineages experience different levels of inbreeding depression?

MATERIALS AND METHODS

Study organism and natural populations—*Geranium maculatum* is an early spring perennial commonly found in moist woods and meadows in eastern North America (Radford et al., 1968). Ramet leaves usually emerge in February and the inflorescences emerge soon after. Flowering usually starts in

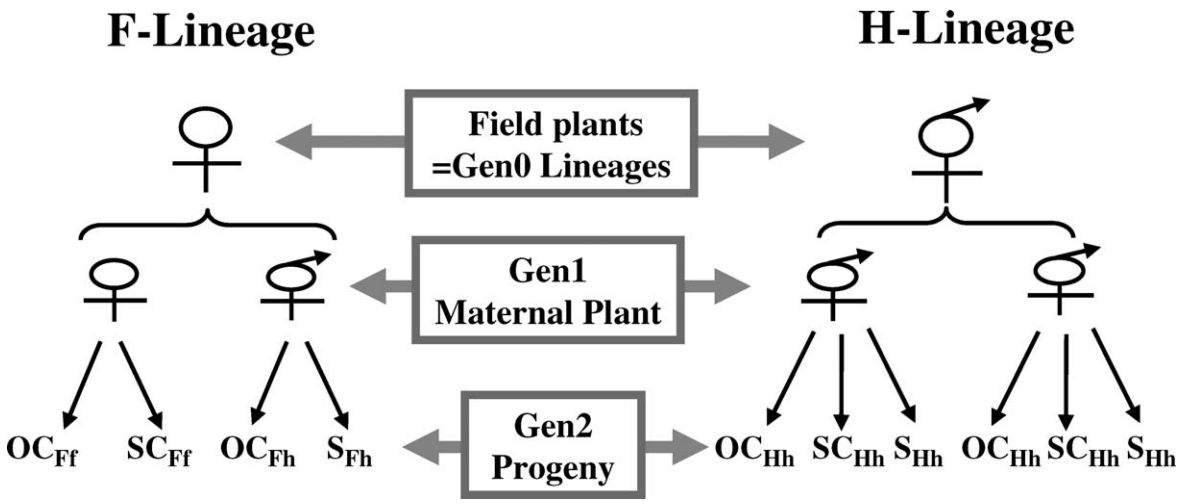

Fig. 1. Two representative crossing schemes for one lineage from each of the two genders (Female : F and Hermaphrodite : H) in *Geranium maculatum*. Gen0, the field plants that started each lineage; Gen1, the maternal plants derived from the field plants and that received the pollination treatments; Gen2, the progeny that were the product of the different crosses (OC, outcrossing; SC, sib-crossing; S, self-pollination). The first subscript (H or F) indicates the family lineage gender. The second subscript (h or f) indicates the maternal gender.

early to mid March, peaks in April, and ends in June. Seeds mature ca. 4 wk after fertilization. Ramets are annual structures that die back in early fall (August–September). Individuals remain underground as dormant rhizomes until the following spring, and populations appear to be stable from year to year. Local extinction has not been reported and is likely to be very low.

Natural populations of *G. maculatum* have been reported to consist of from 0 to 50% female plants (Ågren and Willson, 1991; Chang, 2006). Gender determination in this species is not fully understood. Preliminary data have ruled out the possibility that only cytoplasmic factors are responsible (S.-M. Chang, unpublished data), but whether male sterility is controlled by nuclear genes only or by interactions between nuclear and cytoplasmic genes still needs to be resolved. Both genders have similar flower color, ranging from pink to lavender, but females have petals that are significantly shorter (ca. 37%) than hermaphrodites (Chang, 2006). Ten anthers are present on both genders, but hermaphrodites have fertile anthers ca. 3 mm long with ca. 380 pollen grains each while females have sterile anthers <1 mm long, with no pollen. Some individuals produce both fertile and sterile anthers within the same or in separate flowers (Ågren and Willson, 1991) but such plants are relatively rare (<3%) in the study populations and were not included in this study. In north Georgia the insect visitors most often observed included bumblebees (*Bombus* sp.), honey-bees (*Apis mellifera*), helictid bees (*Halictidae* spp.), hoverflies (*Syrphidae* spp.), and occasional butterflies.

Seeds used as the maternal plants in this study were collected in 2003 from two natural populations, designated MP and OT, located about 3.2 km apart in Clarke County, Georgia, USA (Chang, 2006). Both populations are located in the understory of deciduous forests. In 2003 when the seeds were collected, the MP and OT populations had ca. 1800 and 1200 flowering individuals, among which ca. 35% and 50% were females. These percentages were calculated using flowering ramets as units. Ramets generally produced only one inflorescence, with a small proportion producing two. Though this species has been observed to clonally propagate under optimal conditions in greenhouses and experimental gardens (Martin, 1965), nearly all rhizomes examined in the OT population in another study were linear without branches, suggesting that cloning is not prevalent in this population. More rhizome branching has been observed in the MP population, but the level of clonal propagation is currently not known. Greenhouse experiments indicate that cloning occurs with equal frequency in females and hermaphrodites (M. Van Etten, University of Georgia, and S.-M. Chang, unpublished data), suggesting that even if cloning does occur in MP, it probably has not affected the relative frequency of these genders in that population. Nevertheless, cloning could increase the likelihood of geitonogamous selfing between flowers on two separate ramets of the same genet.

Choice of the family lineages and the maternal plants—Multiple seeds were collected from female (F) and hermaphroditic (H) field plants (generation 0, Gen0 hereafter) that were at least 2 m apart in the study populations. These field-collected seeds were planted in the greenhouse (see details on growing conditions in Chang, 2006) to produce the generation 1 (Gen1) plants. Gen1 plants were grown for three seasons in a greenhouse before this experiment. Plants that started producing flowers were scored for their gender in the early seasons. All of the plants that derived from the same Gen0 plant are considered to be in the same Gen0 lineage, and the gender of the Gen0 plant that started a family lineage is referred to as Gen0 lineage gender (written with a capital letter). Each Gen1 plant grown from seeds collected from a Gen0 plant, and which later served as a maternal plant to produce selfed, sib-crossed, and outcrossed seeds for this experiment, is referred to as the Gen1 maternal plant and its gender as the Gen1 maternal gender (with a lowercase letter). Crosses were carried out with the maternal plants to generate the progeny (Gen2) whose fitness characteristics were measured in this study (Fig. 1).

A major goal of this study was to compare the fitness of two types of inbred progeny, those from selfing and sib-crossing, with those produced from outcrossing. To achieve this, each Gen0 lineage needed to have at least one hermaphroditic plant to provide pollen for sib-crossing. For this reason, only lineages that had either (1) two or more hermaphroditic Gen1 plants or (2) at least one hermaphrodite and one female Gen1 plant blooming at the same time could be used for this study. These requirements were difficult to meet in the Gen0 Female lineages because of the production of only female Gen1 plants, leaving 10 Gen0 Female lineages that were usable for this study.

An additional factor affecting the number of usable families was the skewed sex ratio of the seeds produced in the hermaphroditic lineages. Gen0 Hermaphroditic lineages can produce both female and hermaphroditic Gen1 plants, but the sex ratios in these lineages are highly hermaphrodite biased. Overall, 93% (in MP) and 86% (in OT) of the Gen1 plants from Gen0 Hermaphroditic lineages were hermaphroditic (S.-M. Chang, unpublished data). Less skewed progeny sex ratios were observed in Female lineages with about 37% (in MP) and 68% (in OT) of the Gen1 plants being female. As a result of the skewed sex ratios, Gen0 Hermaphroditic lineages contain families that produce only hermaphroditic Gen1 plants (Fig. 1). Gen0 Female lineages, on the other hand, contain families that produce both female and hermaphroditic Gen1 plants.

In summary, I was able to utilize 16 Gen0 lineages from the MP population (six Female and 10 Hermaphroditic) and 13 in OT (four Female and nine Hermaphroditic). Most of these families had 2–3 Gen1 maternal plants, except two families contained four Gen1 maternal plants.

Crossing designs—Three crossing treatments were included in this study depending on the source of pollen. In treatment (1), pollen grains were obtained from a different flower on the same plant (selfing or S). In treatment (2), pollen was obtained from a sibling that belonged to the same family (sib-crossing or SC). In treatment (3), pollen was obtained from a randomly chosen plant from the same population (outcrossing or OC) (Fig. 1).

Because of the biased sex ratios in some families and asynchronous flowering among siblings, not all crosses were possible for all Gen1 maternal plants. This resulted in an insufficient number of seeds in the sib-crossing treatment for the hermaphroditic Gen1 maternal plants of the female lineages, and this category was excluded from the analysis. Thus in the female lineages, hermaphroditic maternal plants produced only selfed and outcrossed seeds and female maternal plants produced only sib-mated and outcrossed seeds. Overall, only plants that received at least one inbreeding treatment (S or SC) and the outcrossing treatment were included in the analyses. A total of 598 pollinations were included in the analyses, among which 165 were selfing, 93 were sib-crossing, and 340 were outcrossing.

In the following, the gender of lineages and maternal plants are identified using two subscripts for the three crossing treatments (S, SC, or OC) with the first (capital) subscript identifying the lineage gender—H for hermaphrodites and F for females, and the second (lowercase) subscript identifying the maternal gender—h and f. For example, SC_{Fh} refers to sib-crossed progeny derived from a hermaphroditic Gen1 maternal plant from a Female Gen0 lineage.

Fitness traits measured—A total of six fitness correlates were measured. The first two were fruit production per pollination and the number of seeds produced in each successful fruit. For each hand-pollinated flower, I recorded whether a fruit formed and, if so, the number of seeds produced. Fruits were collected individually, and mature seeds were extracted from the fruits within 2 wk of collection.

The third and fourth fitness traits, individual seed mass and germination, were recorded for each seed produced. Individual seeds were weighed to within 0.01 mg and stored separately in 96-well plates in a cold room (5°C). The seed coat was nicked with a razor blade and placed in water for 14 d at 5°C to break seed dormancy. Seeds were then planted individually and randomly assigned to positions on a mist bench. Seeds whose cotyledons successfully emerged and expanded were recorded as having successfully germinated. Germination was observed as early as 2 d after planting and continued until day 32, with the peak on day 6.

The fifth fitness trait measured was seedling survival. Seedlings were moved to a regular greenhouse bench after 6 wk and allowed to grow for five additional months, roughly one growing season for natural populations. Seedlings survival (yes/no) to this stage was recorded. By the end of this period, seedlings began to show signs of senescence: yellow and/or red leaves.

The last fitness trait measured was the cumulative growth of surviving seedlings in the first season. Belowground biomass, a trait generally considered to be a good measure of plant growth in perennial species, was used as an index for the first season's growth. To excavate belowground structures, the soil was carefully washed away from the roots and the rhizome. The excavated roots and rhizomes were air dried on a bench for ca. 16 h (overnight) before their biomasses were measured. Wet, instead of dry, biomass was measured because these rhizomes were needed for a continuing study.

Statistical analyses—*Maternal gender effects*—To determine whether progeny from the two maternal genders in the Female lineages (in particular the OC_{Fh} and OC_{Ff}, Fig. 1) could be combined in the comparison between Female and Hermaphroditic lineages, it was necessary to determine whether maternal gender alone, independent of lineage gender or pollination type, affected progeny fitness. To answer this question, the fitness traits of OC progeny derived from female and hermaphroditic maternal plants in the Female lineage (i.e., OC_{Ff} and OC_{Fh}) were compared.

In this analysis, a mixed linear model (using PROC MIXED and PROC GLIMMIX in SAS, 1999) including individual fitness traits for OC_{Ff} and OC_{Fh} as response variables was used to evaluate the fixed effect of maternal gender, population, their interaction, and the random effect due to maternal plant (nested under population) on each of the six fitness traits measured. The variance components were estimated using an iterative, restricted maximum-likelihood method (REML) (Littell et al., 1996). The significance of the fixed effects was tested using *F* statistics. Type III sums of squares were used for these tests. The degrees of freedom for the denominators were obtained using the Satterthwaite approximation (SAS, 1999). The significance of the random effects in this and all other analyses was tested using likelihood ratio χ^2 Test as described in Littell et al. (1996).

Detecting inbreeding depression—Individual traits were first analyzed separately to characterize the expression of inbreeding depression in the experimental plants. Three variables (the number of seeds produced per fruit, seed mass, and belowground biomass) were analyzed using a linear mixed-model (using PROC MIXED in SAS). The independent variables included fixed effects (population, lineage gender, pollination type, and their interactions) and random effects (family [nested under lineage gender × population] and family × pollination type). Because only two populations were included in this study, population was considered as a fixed effect.

The three remaining traits (fruit formation, germination, and survival) had the values of 0 or 1 and were analyzed using a logistic regression approach (Proc GLIMMIX in SAS). The predicting variables were the same as the ones in previous paragraph. Because a significant interaction effect was found between population and pollination type in the belowground biomass and germination, populations were also analyzed separately for these two traits.

Separate analyses were performed to evaluate the levels of inbreeding depression due to selfing and sib-crossing. For inbreeding depression associated with selfing, treatments S and OC from the hermaphroditic maternal plants of both hermaphroditic and female lineages (S_{Hh}, S_{Fh}, OC_{Hh}, and OC_{Fh}) were used. The OC treatment of female maternal plants (OC_{Ff}) was not used in this analysis because maternal gender had a significant influence on some of the traits measured (see Results). By using only hermaphroditic maternal plants, this analysis eliminated the effect from maternal gender and focused on the effect from lineage gender. For inbreeding depression associated with sib-crossing, SC and OC from the female maternal plants in Female lineages and hermaphroditic maternal plants in Hermaphroditic lineages (designated as SC_{Hh}, SC_{Ff}, OC_{Hh}, and OC_{Ff}) were used. Note that the genders of the maternal plants for hermaphrodite and female lineages are different because the Female lineages had produced an insufficient number of hermaphroditic progenies. However, because I did not find maternal gender effects on five of the six traits measured, this design is unlikely to lead to significantly different results even if it were possible to use hermaphroditic maternal plants from female lineages. Average inbreeding depression (δ) for each trait was calculated as the proportional reduction in the least square mean trait values of S and SC relative to OC, using the following equations: Selfing-$\delta_H = (OC_{Hh} - S_{Hh})/OC_{Hh}$, Selfing-$\delta_F = (OC_{Fh} - S_{Fh})/OC_{Fh}$, SC-$\delta_H = (OC_{Hh} - SC_{Hh})/OC_{Hh}$ and SC-$\delta_F = (OC_{Ff} - SC_{Ff})/OC_{Ff}$.

Difference in the magnitude of inbreeding depression between lineage genders—To evaluate whether the magnitude of inbreeding depression differed between the female and hermaphroditic lineages, similar analyses were carried out as the ones described in the section *Detecting inbreeding depression* except that log-transformed data of seeds per fruit, individual seed mass, and belowground biomass were used as the dependent variables (Johnston and Schoen, 1994). The independent variables for these analyses were the same as the analyses for the untransformed data. The log-transformation of the data makes the interaction between lineage gender and pollination type an estimate of the difference in magnitudes of inbreeding depression between female and hermaphroditic lineages (Johnston and Schoen, 1994).

The binary traits, including fruit formation, germination, and survival, could not be analyzed directly using the log-transformation method described previously. Instead, the proportions of (1) pollinations that resulted in a fruit, (2) seeds that germinated, and (3) seedlings that survived the first growing season were calculated for each pollination type in each lineage family, and their log-transformed values were used as the dependent variables in similar analyses as ones described in section *Detecting inbreeding depression*. This calculation generated one value for each family–pollination combination. As a result, the family effect could not be evaluated and was removed from these analyses.

Cumulative inbreeding depression—Fruit formation and seed number per fruit are expressed prior to seed dispersal and, therefore, are more likely to experience a higher degree of maternal influence than germination, survival, and growth. For this reason, two cumulative inbreeding depression levels were analyzed separately for the pre- and post-dispersal traits.

To determine cumulative inbreeding depression for fruit formation and seed number per fruit, a composite fitness for each pollination type in each family was calculated by multiplying the proportion of pollinations resulting in a fruit with mean seed production per successful fruit. These fitness values were then log-transformed and analyzed using Proc GLM (SAS, 1999) with population, lineage, pollination type, and their interaction terms as the fixed main effects. A significant interaction between pollination type and lineage would suggest a significant difference in the cumulative inbreeding depression between the female and hermaphroditic lineages.

For the postdispersal stages, a fitness value of 0 was assigned for each seed

TABLE 1. Analyses for fitness traits of *Geranium maculatum* receiving selfing and outcrossing treatments. (A) Fixed effects, (B) Random effects.

	Traits																			
	Fruits/pollination			Seeds/fruit			Individual seed mass			Germination			Survival			Belowground biomass			
A) Fixed effects	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N,D)	*F*	*P*	
Pop	**1, 18.6**	**9.82**	**0.006**	**1, 32.3**	**11.98**	**0.0015**	1, 19.3	1.81	0.19	1, 12.4	0.38	0.55	1, 33.7	0.06	0.81	1, 17.5	0.03	0.87		
LinGen	1, 18.6	0.07	0.79	1, 32.3	3.13	0.09	1, 19.3	0.40	0.53	**1, 12.4**	**4.82**	**0.05**[c]	1, 33.7	1.08	0.31	1, 17.5	0.38	0.55		
P-type	**1, 26.8**	**7**	**0.01**	**1, 32.3**	**16.42**	**0.0003**	**1, 16.4**	**28.48**	**0.0001**	1, 482	2.41	0.12	**1, 33.7**	**3.73**	**0.06**	**1, 12.9**	**51.51**	**0.0001**		
Pop × LinGen	1, 18.6	0.11	0.74	1, 32.3	0	0.99	1, 19.3	1.45	0.24	**1, 12.4**	**6.06**	**0.03**[c]	1, 33.7	0.28	0.60	**1, 17.5**	**6.81**	**0.02**[c]		
Pop × P-type	1, 26.8	0.24	0.63	1, 32.3	0.51	0.48	1, 16.4	0	0.95	**1, 482**	**3.79**	**0.05**[d]	1, 33.7	1.13	0.30	1, 12.9	0.23	0.64		
LinGen × P-type	1, 26.8	1.83	0.19	1, 32.3	0.54	0.47	1, 16.4	0	0.98	1, 482	0.12	0.73	1, 33.7	0.03	0.86	1, 12.9	0.13	0.73		
Pop × LinGen × P-type	1, 26.8	0.19	0.66	1, 32.3	0.37	0.55	1, 16.4	0	0.98	1, 482	0	0.95	1, 33.7	0.51	0.48	1, 12.9	0.02	0.88		
B) Random effects	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	P	Estimate (SE)	χ^2	*P*		
Lineage[a]	0.14 (0.27)	1.02	>0.1	0[b] (–)	0	>0.1	**0.72 (0.25)**	**22**	**<0.001**	0.02 (0.08)	0.22	>0.1	3.2×10^{-18}(–)	0	>0.1	0.12 (0.10)	1.6	>0.1		
Lin × P-type[a]	0.05 (0.28)	1.03	>0.1	0.08 (0.10)	0.6	>0.1	0.04 (0.03)	2.4	>0.1	4.8×10^{-18}(–)	0	>0.1	0.42 (0.33)	0.32	>0.1	0.06 (0.09)	0.6	>0.1		

Note: Lin = lineage; LinGen = lineage gender; Pop = population; P-type = pollination type; df(N, D) = degrees of freedom for the numerator and denominator obtained by Satterthwaite approximation; $F = F$ statistics; P = significance level for the F tests.

[a] Lineage and Lin × P-type are both nested under the interaction of Pop and LinGen.

[b] Value was set to be 0 during the restricted maximum-likelihood process.

[c] Analyses on populations separately showed a significant LinGen effect in MP but not in OT; see the Discussion for more details.

[d] Analyses on populations separately showed a significant P-type effect in OT but not in MP.

that did not germinate or did not survive the first season. For seeds that germinated and survived to the end of the first season, the belowground biomass was used as the fitness value. The two study populations were analyzed separately because a preliminary analysis revealed a significant interaction between lineage and population. mixed model analyses were carried out with lineage, pollination type, and their interaction terms as the fixed effects and family and family × pollination type as random effects. A significant interaction between pollination type and lineage gender would suggest a difference in the magnitude of cumulative inbreeding depression between female and hermaphroditic lineages. Analyses were carried out on both the original data and the log-transformed data [= $\log_e$(original value + 0.01)] to determine the presence and the magnitude of inbreeding depression in the two gender lineages, respectively.

RESULTS

Effects of maternal gender—Within the Female lineages, female and hermaphroditic maternal plants produced outcross progeny with similar fitness measurements with one exception, belowground biomass. There was a significant interaction between maternal gender and population ($F_{1,60.5} = 5.31$, $P = 0.02$) on the belowground biomass produced by the progeny. Specifically, progeny produced by the two maternal genders were similar in the MP population (2.1 ± 0.21 mg in female and 2.3 ± 0.25 mg in hermaphroditic maternal groups, $t = -0.76$, $P = 0.45$) but significantly different in the OT population, with progeny of female maternal plants gaining 41% more belowground biomass the first season than progeny of hermaphroditic maternal plants (2.4 ± 0.16 mg in female and 1.7 ± 0.26 mg in hermaphroditic maternal groups, $t = 2.59$, $P = 0.01$). There was no evidence for any interaction between maternal gender and other factors included in this study.

Detecting inbreeding depression in individual traits—Analyses for individual traits revealed that pollination type significantly affected all fitness traits measured (Tables 1 and 2). Self pollination resulted in lower fruiting probability, average seed production per fruit, seed mass, survival of the seedlings, and belowground biomass (Fig. 2). The magnitude of inbreeding depression ranged from 0.1 for germination and seed mass to 0.59 for the belowground biomass. Sib-crossing had a similar trend but to a lesser degree than selfing (Table 2, Fig. 2). Inbreeding depression due to sib-crossing ranged from 0 in germination to 0.33 in belowground biomass.

A significant lineage gender effect was found only in the germination of selfing progeny, though the effect of lineage gender × population was significant for germination and belowground biomass in selfing (Table 1) and for fruiting, seed mass, and germination in sib-crossing (Table 2). Analyzing the two study populations separately revealed that the emale lineages tended to perform better than Hermaphroditic lineages in the traits mentioned in the MP population but not in the OT population.

The two study populations were generally similar to each other except for fruiting probability and seed production per fruit (Table 1). The OT population appeared to have a higher fruiting probability and produced more seeds per fruit than the MP population (Fig. 3A). In addition, effect of family was significant for individual seed mass in both selfing and sib-crossing analyses (Tables 1 and 2).

Differences in the magnitude of inbreeding depression between lineage genders—*Individual traits*—Analysis of log-

transformed and untransformed data had very similar patterns. Most importantly, these analyses did not show any effect of interaction between pollination type and lineage gender on seed number, size, or belowground biomass, suggesting that, even though inbreeding depression was detected in most of the traits examined, its magnitude was similar in both female and hermaphroditic lineages. Similarly, when the logarithmic transformed germination rates and survival rates were used to test for lineage specific inbreeding depression, no significant effects were found. Overall, there was no evidence that female and hermaphroditic lineages differed in the degree of inbreeding depression due to either selfing or sib-crossing.

Cumulative inbreeding depression—Traits measured before seed dispersal had significant cumulative inbreeding depression in the selfing treatment (Table 3). Average inbreeding depression was generally higher in the Hermaphroditic lineages (0.70 for MP and 0.40 for OT, Fig. 3A) than in Female lineages (0.52 for MP and 0.29 for OT, Fig. 3A) in both populations, though not significantly (Table 3) as indicated by the nonsignificant lineage gender × pollination type effect. The difference between fitness of OC and SC treatments was not statistically significant (Fig. 3A, Table 3), though SC exhibited inbreeding depression in all four comparisons and the level of inbreeding depression was as high as 0.42 in female lineages in the MP population.

The initial analysis for the postdispersal traits revealed a strong pollination type effect but the effect of lineages gender varied between populations (Table 4). In the MP population, both lineage gender and pollination type significantly affected the cumulative fitness of the seedlings. Specifically, female lineages had a higher cumulative fitness than hermaphroditic lineages, and selfing resulted in significantly lower cumulative fitness compared to outcrossing (Fig. 3B). Sib-mating had a lower fitness than outcrossing, but the difference was not statistically significant (Fig. 3B), though SC again exhibited inbreeding depression in all four comparisons, and the level of inbreeding depression was as high as 0.50 in Female lineages in the OT population. The interaction between lineage gender and pollination type was not statistically significant (Table 4). On the basis of these results, therefore, we could not reject the null hypothesis that female and hermaphroditic lineages had similar levels of inbreeding depression in the MP population. In the OT population, both selfed and sib-crossed progeny had lower fitness than the outcrossed progeny (Fig. 3B). However, the effect of lineage gender was not significant (Fig. 3B) even though overall fitness was higher in the Hermaphroditic than in Female lineages.

Similar to the analyses for individual traits, log-transformed cumulative fitness did not reveal any significant interaction between pollination type and gender lineage, suggesting that the magnitude of inbreeding depression was similar for both gender lineages.

DISCUSSION

Using controlled greenhouse experiments, I found that (1) *Geranium maculatum* expresses severe inbreeding depression for both individual traits and cumulative fitness, (2) female and hermaphroditic lineages express similar levels of inbreeding depression, and (3) maternal gender does not affect the number of seeds produced but female maternal plants produced

TABLE 2. Analyses for fitness traits of *Geranium maculatum* receiving sib-crossing and outcrossing treatments. (A) Fixed effects, (B) Random effects.

	Traits																			
	Fruits/pollination			Seeds/fruit			Individual seed mass			Germination			Survival			Belowground biomass			
A) Fixed effects	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*	
Pop	1, 137.3	2.19	0.14	1, 17.2	2.32	0.15	1, 23.2	2.22	0.15	1, 33.1	0.36	0.55	1, 19.4	0.19	0.67	1, 22.3	2.36	0.14	
LinGen	1, 134.4	3.29	0.07	1, 17.2	0.04	0.85	1, 23.2	0.77	0.39	1, 33.1	0.93	0.34	1, 19.4	3.02	0.1	1, 22.3	0.12	0.74	
P-type	1, 35.6	3.27	0.08	**1, 13.8**	**7.30**	**0.02**	1, 14.2	1.53	0.24	1, 33.1	0.18	0.67	1, 17.7	0.56	0.46	**1, 284**	**21.73**	**0.0001**	
Pop × LinGen	**1, 137.3**	**4.50**	**0.04**[c]	1, 17.2	0.09	0.77	**1, 23.2**	**5.00**	**0.04**[c]	**1, 33.1**	**6.97**	**0.01**[c]	1, 19.4	0.52	0.48	1, 22.3	2.85	0.11	
Pop × P-type	–	–	–	1, 13.8	2.17	0.16	1, 14.2	0.13	0.72	1, 33.1	0.92	0.34	1, 17.7	0.24	0.63	1, 284	2.4	0.12	
LinGen × P-type	1, 35.6	0.49	0.48	1, 13.8	0.13	0.73	1, 14.2	0.00	0.97	1, 33.1	0.23	0.64	1, 17.7	0.34	0.57	1, 284	0.25	0.62	
Pop × LinGen × P-type	–	–	–	1, 13.8	0.21	0.66	1, 14.2	1.84	0.20	1, 33.1	0.37	0.54	1, 17.7	0.13	0.73	1, 284	1.07	0.30	
B) Random effects	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*	
Lineage[a]	1.3×10^{-17} (–)	0	>0.1	0.16 (0.15)	1.3	>0.1	**0.61 (0.22)**	**12**	**<0.001**	7.8×10^{-20} (–)	0	>0.1	0.004 (0.34)	0.01	>0.1	0.09 (0.06)	2.4	>0.1	
Lin × P-type[a]	**0.85 (0.52)**	**30.4**	**<0.001**	0.03 (0.13)	0.1	>0.1	**0.11 (0.06)**	**14.3**	**<0.001**	0.17 (0.12)	2.06	>0.1	0.34 (0.49)	(−5.8)	>0.9	0[b] (–)	–	–	

Note: See Table 1 for descriptions of the values listed. Sib = sibling.

[a] Lineage and Lin × P-type are both nested under the interaction of Pop and LinGen.

[b] Value was set to be 0 during the restricted maximum-likelihood process.

[c] Analyses on populations separately showed a significant LinGen effect in MP but not in OT; see the Discussion section for more details.

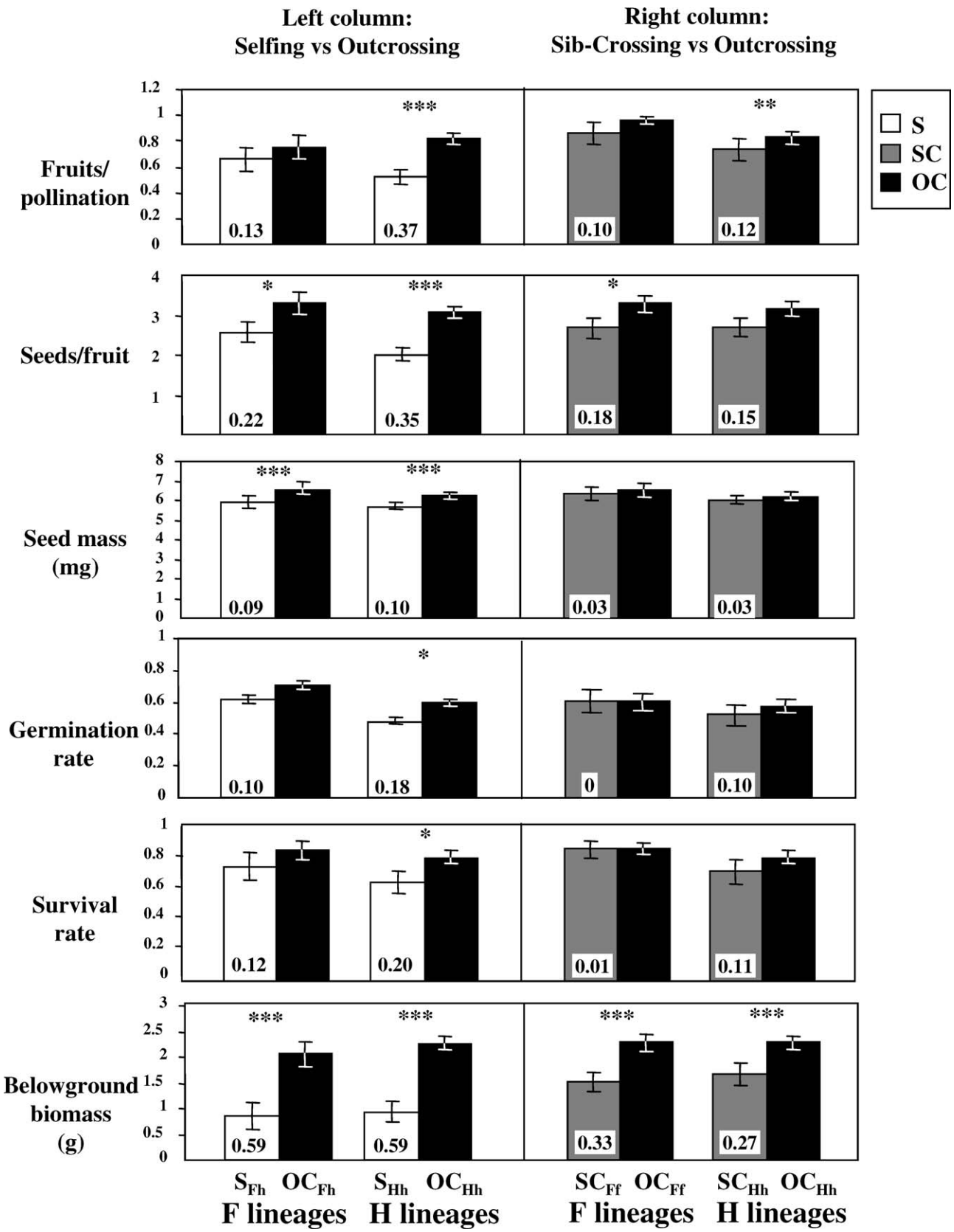

Fig. 2. Comparisons of fitness traits between selfing and outcrossing (left column) and sib-crossing and outcrossing (right column) treatments in *Geranium maculatum*. Cross and subscript designations are the same as in Fig. 1. Values on the inbred (S or SC) bars indicate the corresponding inbreeding depression: δ_F and δ_H. Asterisks indicate significant differences between inbred (S or SC) and outcross (OC) treatments within lineages after a Tukey adjustment. The error bars represent ±1 SE. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. See Results for more details on the significance of the main effects.

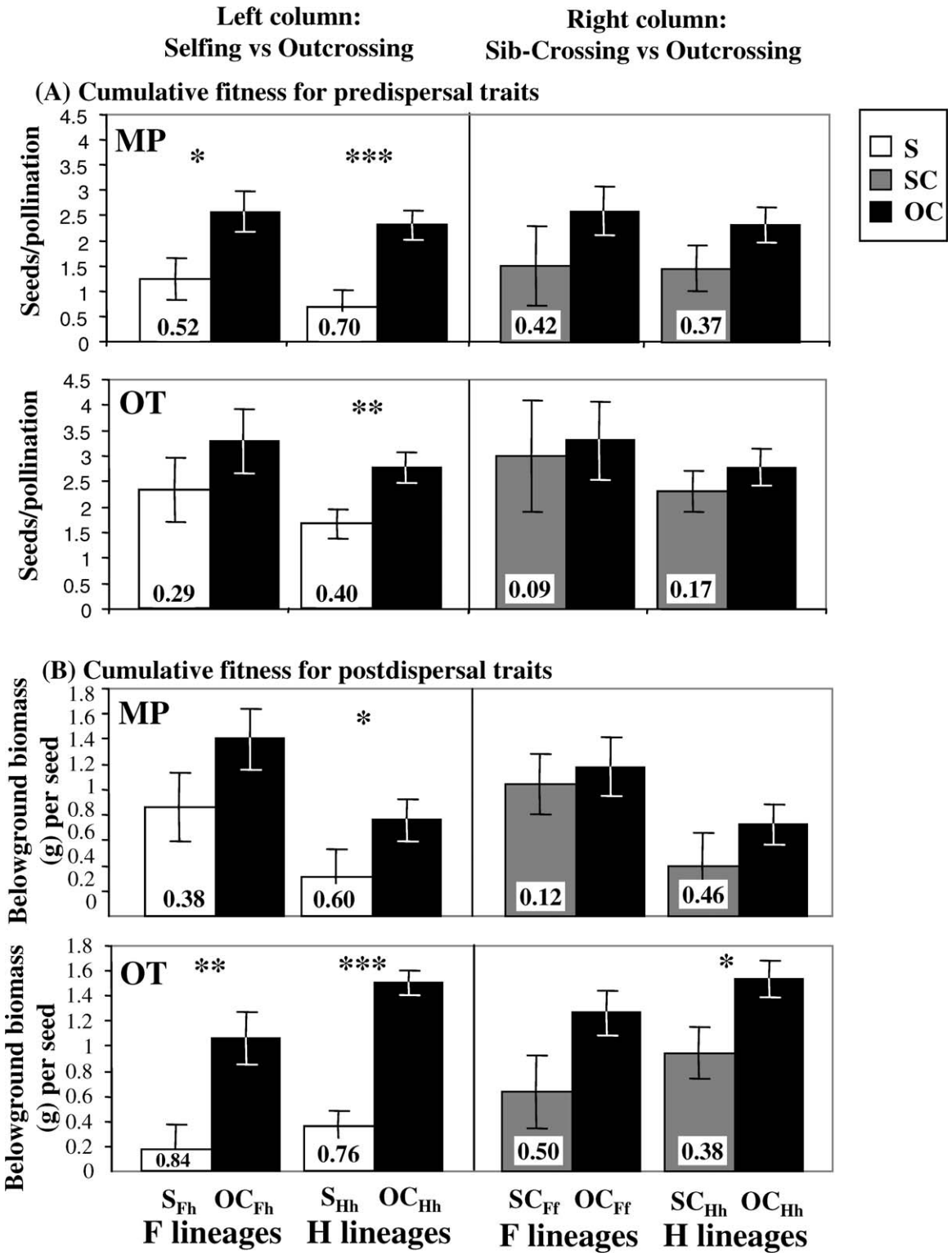

Fig. 3. Comparisons of the cumulative fitness for pre- and postdispersal traits between selfing and outcrossing (left column) and sib-crossing and outcrossing (right column) treatments in *Geranium maculatum*. Cross and subscript designations are the same as in Fig. 1. Values on the inbred (S or SC) bars indicate the corresponding inbreeding depression: δ_F and δ_H. Asterisks indicate significant differences between inbred (S or SC) and outcross (OC) treatments within lineages after a Tukey adjustment. The error bars represent ± 1 SE. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. See Results for more details on the significance of the main effects.

TABLE 3. Analyses of variance for the predispersal cumulative fitness in *Geranium maculatum*.

	Selfing vs. outcrossing			Sib-crossing vs. outcrossing		
Fixed effects	df	*F*	*P*	df	*F*	*P*
Pop	**1**	**6.00**	**0.01**	**1**	**4.67**	**0.05**
LinGen	1	2.02	0.11	1	0.87	0.40
P-type	**1**	**26.79**	**0.0002**	1	2.72	0.14
Pop × LinGen	1	0.23	0.74	1	0.30	0.62
Pop × P-type	1	0.70	0.48	1	0.49	0.52
LinGen × P-type	1	0.16	0.73	1	0.001	0.97
Pop × LinGen × P-type	1	0.01	0.91	1	0.05	0.84

Note: See Table 1 for descriptions of the values listed. Sib = sibling.

progeny that grew faster in the first growing season than progeny from hermaphroditic maternal plants. The potential mechanisms explaining these results and their implications are discussed in the next section

Inbreeding depression in G. maculatum—Inbred progeny consistently showed signs of inbreeding depression in almost all fitness traits measured in this study (Fig. 2). The magnitude of inbreeding depression ranged from <10% for seed mass to ca. 60% in belowground biomass for selfed progeny. With regard to seed fitness, selfing resulted in a 50% reduction in seed production compared to outcrossing and a >75% reduction when seed production and seedling fitness were combined. Though these measurements are within the range of inbreeding depression reported in outcrossing angiosperms, they are much higher than the average inbreeding depression reported in the literature (0.32 ± 0.053 [mean ± s.e.] for seed production and 0.49 ± 0.038 for cumulative fitness [Husband and Schemske, 1996]). These estimates are also higher than most values reported for gynodioecious species (Mutikainen and Delph, 1998), indicating that *G. maculatum* has an overall high degree of inbreeding depression. In addition, this study was carried out in the greenhouse, an environment often considered to lower the expression of inbreeding depression (Dudash, 1990), and it is possible that inbreeding depression levels experienced by plants under natural conditions may be even higher.

The high levels of inbreeding depression observed in *G. maculatum* may be due to low rates of selfing (i.e., high rates of outcrossing), which would prevent purging of deleterious alleles within a population. Preliminary results from a genetic study using seeds have shown that females do indeed have a selfing rate close to 0% in both the MP and OT populations (C. Deen, University of Georgia, and S.-M. Chang, unpublished data), similar to what is seen in many other gynodioecious species (e.g., Collin and Shykoff, 1993; Kohn and Biardi, 1995; Gibson and Wheelwright, 1996). Hermaphrodites also had relatively low selfing rates, ranging from 9.8% (OT) to 16.5% (MP). These values are on the low end of the selfing rate distribution even among outcrossing species (Vogler and Kalisz, 2001). However, use of mature seeds would underestimate the primary selfing rates (i.e., sefling rates for all fertilized ovules) if inbreeding depression is observed at stages prior to seed maturation, as shown in this study (Lande et al., 1994; Husband and Schemske, 1996). Using the value of the predispersal inbreeding depression estimated in this study (listed on Fig. 3A) and the selfing rates estimated using mature seeds, primary selfing rates are estimated to be 15.3% and 39.7% for the OT and MP populations, respectively. The low selfing rate for the OT population is consistent with the hypothesis that low selfing (high outcrossing) rate makes purging more difficult. However, the higher primary selfing rate for the MP population (39.7%) seems too high to prevent potential purging.

Alternatively, the high inbreeding depression estimated in this study could be due to a high level of recessive or partial dominant lethal mutations maintained by high mutation rates. Lande et al. (1994) showed theoretically that, when selfing rate is low, high rates of lethal mutations may kill nearly all selfed progeny and leave the population with only outcrossed progeny, a phenomenon called selective interference (Ganders, 1972). Under this situation, populations are practically 100% outcrossing, and purging would not occur until selfing rates reached a critical threshold below which a combination of a moderate selfing rate and high inbreeding depression levels could exist. Though this prediction is consistent with the high early inbreeding depression levels associated with substantial selfing rates in other gynodioecious species (Kohn, 1988; Sakai et al., 1989; Schultz and Ganders, 1996;) and in the MP

TABLE 4. Analyses of variance for the postdispersal cumulative fitness in *Geranium maculatum*. (A) Fixed effects, (B) Random effects.

	Selfing vs. outcrossing			Sib-crossing vs. outcrossing		
A) Fixed effects	df(N, D)	*F*	*P*	df(N, D)	*F*	*P*
Pop	1, 9.01	0.05	0.82	1, 39	2.69	0.11
LinGen	1, 9.01	0.76	0.40	1, 39	0.7	0.41
P-type	**1, 9.92**	**36.87**	**0.0001**	**1, 39**	**7.45**	**0.01**
Pop × LinGen	**1, 9.01**	**9.6**	**0.01**[c]	**1, 39**	**7.16**	**0.01**[c]
Pop × P-type	1, 9.92	3.90	0.08	1, 39	1.43	0.24
LinGen × P-type	1, 9.92	0.18	0.68	1, 39	0.06	0.81
Pop × LinGen × P-type	1, 9.92	0.37	0.55	1, 39	0.15	0.70
B) Random effects	Estimate (SE)	χ^2	*P*	Estimate (SE)	χ^2	*P*
Lineage[a]	0.03 (0.06)	0.3	>0.1	0 [b] (.)	–	–
Lin × P-type[a]	0.006 (0.05)	0	>0.1	0.08 (0.04)	1.1	>0.1

Note: See Table 1 for descriptions of the values listed. Sib = sibling.

[a] Lineage and Lin × P-type are both nested under the interaction of Pop and LinGen.

[b] Value was set to be 0 during the restricted maximum-likelihood process.

[c] Analyses on populations separately showed a significant LinGen effect in MP but not in OT; see the Results and Discussion sections for more details.

population, more studies are needed to evaluate whether and how frequently lethal mutations are involved in early inbreeding depression of these species.

In conclusion, observations in this study are consistent with predictions from at least two hypotheses. First, high outcrossing rates maintain a high genetic load and, hence, high inbreeding depression levels. Second, high lethal mutation rates may help to maintain severe inbreeding depression with moderate selfing rates. Further studies on the nature of genetic causes for inbreeding depression in this species would be needed to fully evaluate these hypotheses. In addition, this study has mainly focused on the early inbreeding depression up to seedling establishment. Examining other aspects of the reproductive processes, such as reproductive success in later life history stages, floral morphology (e.g., herkogamy and dichogamy), pollinator abundance and activity, the size of floral display each day, seed and pollen dispersal distance, and the spatial arrangement of the two genders in natural populations, would help to further define the interaction between inbreeding depression and selfing rates as well as identify other potential factors contributing to the pattern found in this species.

Gender-specific inbreeding depression—One of the main goals of this study was to examine whether hermaphrodites and females in *G. maculatum* expressed significantly different levels of inbreeding depression. Although results here show that Hermaphroditic lineages had greater inbreeding depression than Female lineages (Fig. 3), analyses using both log-transformed and untransformed data revealed that these differences were not statistically significant. This result held true for both individual traits and cumulative inbreeding depression levels.

As discussed in the introduction, theoretical studies predict that the pattern of gender-specific inbreeding depression depends greatly on how gender is genetically determined. Data from the sex ratios of progeny in *G. maculatum* (M. Van Etten, University of Georgia, and S.-M. Chang, unpublished data) rule out the possibility that gender in this species is determined by cytoplasmic genes only. Hence, hermaphrodites would not be expected to have lower inbreeding depression than females. Results from the current study are consistent with this prediction. Beyond this, the available genetics data do not allow us to distinguish between complete nuclear and cyto-nuclear control of male sterility genes in *G. maculatum*. Nonetheless, the similar levels of inbreeding depression seen in females and hermaphrodites are in line with predictions by models that assume females are caused by nuclear dominant rather than recessive alleles (Schultz, 1999). This, however, does not eliminate the possibility that gender in *G. maculatum* is in fact determined by cyto-nuclear interactions, a system for which little is known regarding gender-specific inbreeding depression.

Alternatively, factors other than gender determination might be equally or more important in determining the level of inbreeding depression in natural populations. Schultz and Willis (1995) argue that stochastic factors might lead to substantial variation in inbreeding depression that does not necessarily correlate with selfing rates within families. This is consistent with the observation by Mutikainen and Delph (1998) that variation among families in *Lobelia siphilitica* overwhelmed differences between genders. In the current study, however, no interaction was seen between family and pollination type for selfing or for cumulative fitness, providing little evidence that variation among families outweighs differences between lineage gender (but see Fox, 2005). In addition, differences in the selfing history of female and hermaphroditic lineages might not be large enough for the association suggested by theoretical studies to have formed. As discussed earlier, selfing rates were relatively low, at least in one population, which may have lead to signals that are too weak to detect in these populations.

Regardless of which mechanism might be responsible, gender-specific inbreeding depression does not appear to be a critical factor in helping to establish female plants in natural populations of *G. maculatum*. In contrast, the finding that both genders have high inbreeding depression levels is consistent with the possibility that inbreeding depression helps to counteract the advantage hermaphrodites experience through selfing, giving females an advantage through seed production and progeny quality (Chang, 2006).

Effect of maternal gender on progeny fitness—Because females produce smaller petals and no pollen, it is generally assumed that the resources that would have been used to produce these structures could be allocated to produce more and larger seeds (Darwin, 1877; Shykoff, 1988; Ashman, 1992; Poot, 1997). Interestingly, results from this study do not support this. Female and hermaphroditic maternal plants derived from female lineages were very similar in the predispersal traits measured, including seed number and size. Perhaps because field plants of *G. maculatum* produce a small number of flowers (6–8 flowers on average), the resources saved by producing female (diameter $\sim$ 2.2 cm) vs. hermaphroditic (diameter $\sim$ 3.2 cm) flowers is rather small. This slight difference in resources might not be sufficient to substantially affect seed quantity or quality.

The lack of difference between female and hermaphroditic maternal plants in seed number per fruit and seed mass contrasts with an earlier study using the same natural populations (Chang, 2006) in which female plants produced heavier seeds and more seeds per fruit after open pollination than hermaphroditic plants. One likely reason for these different findings is that the current study used only cross-pollination (outcrossing-near) to control for pollen source and inbreeding levels while in the previous study plants were open pollinated by natural pollinators. Open pollination is likely to include selfing (hermaphrodites only) in addition to biparental inbreeding and outcrossing. Given the severe inbreeding depression at seed production stage, it is not surprising to find fewer seeds per fruit in hermaphroditic relative to female plants in natural populations (Ågren and Willson, 1991; Chang, 2006). In addition, the current study grew plants under greenhouse conditions, whereas the previous studies examined plants grown in their native environment. Differences between genders might be more pronounced under harsher field conditions, though in a study that manipulated the resource availability in another gynodioecious plant, *Gypsophila repens*, López-Villanicencio et al. (2005) found that the difference between genders did not increase when resources were limited. More studies on other gynodioecious systems using similar manipulative design would provide critical data to evaluate this possibility.

This study does reveal, however, that female maternal plants produced progeny that gained 84% more belowground biomass than hermaphroditic maternal plants but only for the OT

population. A similar trend was found in an earlier study (Chang, 2006) in which the progeny derived from female maternal plants gained 27% more belowground biomass than progeny from hermaphroditic maternal plants in the OT but not in the MP population. The consistent differences under different conditions suggest that the relative growth rates for progeny produced by the two genders in these populations might be genetically based.

Conclusion: the role of inbreeding depression in the maintenance of gynodioecy—The ultimate goal of this study was to understand the role of inbreeding depression in maintaining gynodioecy in *Geranium maculatum*. Three main findings of this study speak to this subject. First, severe inbreeding depression was seen in early life history stages, potentially providing a selective advantage to female plants. Second, biparental inbreeding reduced progeny fitness, suggesting that factors capable of influencing the degree of biparental inbreeding, such as fine scale genetic structure and pollen dispersal distance, could also affect the relative fitness of female and hermaphroditic lineages. Finally, the magnitude of inbreeding depression tended to be higher in hermaphroditic lineages than in female lineages but the difference was not statistically significant. Combined, this study shows that while there was no evidence for lineage-specific differences, the level of inbreeding depression in this population is extremely high, which will facilitate the maintenance of the obligate outcrossers (females). Future work examining the genetic basis of the male sterility, the nature of genes controlling inbreeding depression, selfing rates in natural population, and other ecological factors affecting relative fitness of females and hermaphrodites should broaden our understanding of gynodioecy in *G. maculatum* and other species.

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