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Density- and frequency-dependent inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius*

Namgay Lhamo, Mike Ramsey* and Glenda Vaughton

Botany, University of New England, Armidale, NSW 2351, Australia

ABSTRACT

Question: Does the magnitude of inbreeding depression fluctuate in response to the density and frequency of inbred and outbred plants in a neighbourhood?

Hypothesis: Inbreeding depression is greater when plant density is high and when selfed progeny compete with crossed progeny.

Organism: The self-pollinating annual plant *Hibiscus trionum* var. *vesicarius*.

Methods: We estimated inbreeding depression by comparing selfed and crossed progeny from eight maternal families. We grew plants in monocultures at two densities (1 or 4 plants per pot), and in a replacement series (4 plants per pot) in which the frequencies of the progeny types were manipulated.

Results: Contrary to expectations, in monocultures inbreeding depression was less at high than at low density. This occurred because the reduction in fitness between low and high densities was less for selfed progeny than for crossed progeny. In the replacement series, inbreeding depression increased with increasing frequency of crossed progeny. Thus, inbreeding depression cannot be characterized independently of the density and frequency of inbred and outbred plants in a population.

Keywords: asymmetric competition, density dependence, frequency dependence, mixed mating, self-fertilization, selfing rate, size hierarchy.

INTRODUCTION

It is widely recognized that inbreeding can result in progeny of lower fitness than outcrossing in plant populations (i.e. inbreeding depression) (Darwin, 1876; Charlesworth and Charlesworth, 1987; reviewed by Byers and Waller, 1999; Keller and Waller, 2002). Inbreeding depression is generally attributed to the expression of deleterious recessive alleles at homozygous loci (Charlesworth and Charlesworth, 1999). Because inbreeding exposes deleterious recessive alleles to selection, inbreeding depression is often lower in predominantly selfing species than outcrossing species, reflecting the increased opportunities for purging of deleterious alleles (Charlesworth *et al.*, 1990;

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* Author to whom all correspondence should be addressed. e-mail: mramsey@une.edu.au

Husband and Schemske, 1996; Byers and Waller, 1999). However, the selfing rate is only one factor potentially affecting the magnitude of inbreeding depression. Inbreeding depression can vary depending on the physical and biotic environment in which relative performance is estimated. In general, relative performance of selfed progeny is reduced and inbreeding depression increases under more stressful environmental conditions (Dudash, 1990; Eckert and Barrett, 1994; Ramsey and Vaughton, 1998; Hayes *et al.*, 2005).

Intraspecific competition is an important component of the biotic environment and can magnify fitness differences between selfed and crossed progeny (Antonovics, 1968; Waller, 1984; Schmitt and Ehrhardt, 1990; Wolfe, 1993; Cheptou *et al.*, 2001; Cheptou and Schoen, 2003; Koelewijn, 2004). Because plants are sessile, competitive interactions are highly localized and generally occur with nearest neighbours. Interactions are frequently asymmetric, with larger plants exerting a much greater effect on smaller plants than vice versa [i.e. dominance and suppression (Weiner, 1985, 1990)]. The extent to which competitively dominant plants are able to suppress the growth of smaller plants is expected to increase with density (Uyenoyama *et al.*, 1993). Thus, intraspecific competition could potentially affect inbreeding depression in two ways (Cheptou *et al.*, 2001; Koelewijn, 2004). First, the magnitude of inbreeding depression may increase with density, if selfed plants simply become stressed due to a lack of space or resources (density dependence). Second, if crossed plants are better competitors than selfed plants, then dominance and suppression could occur, such that inbreeding depression increases with the frequency of crossed plants in the neighbourhood (frequency dependence). As a consequence of these density- and frequency-dependent effects, inbreeding depression estimates may show considerable spatial and temporal variation depending on the biotic neighbourhood (Schmitt and Ehrhardt, 1990; Cheptou *et al.*, 2000; Koelewijn, 2004).

Variation in inbreeding depression may be evolutionarily significant because fitness differences between selfed and crossed progeny are a key factor in the evolution of selfing rates in plant populations (Charlesworth and Charlesworth, 1987). If inbreeding depression is considered as a fixed characteristic of a population, theory predicts only two stable evolutionary states – complete selfing or complete outcrossing (Lloyd, 1979; Lande and Schemske, 1985; Charlesworth *et al.*, 1990; Uyenoyama *et al.*, 1993). Selfing is favoured when the population level of inbreeding depression is <0.5, whereas outcrossing evolves when inbreeding depression is >0.5. However, intermediate selfing rates may occur if inbreeding depression is assumed to vary. For example, models that assume density or frequency dependence in inbreeding depression give rise to stable intermediate selfing rates under a range of conditions, depending on the nature of the competitive interactions (Cheptou and Dieckmann, 2002; Cheptou and Schoen, 2003). Thus, intraspecific competition may play a role in the evolution and maintenance of mixed mating systems by influencing the relative fitness of selfed and crossed progeny.

Here we investigate the effects of intraspecific competition on inbreeding depression in the annual *Hibiscus trionum* var. *vesicarius* (Malvaceae). We grow selfed and crossed progeny from eight maternal plants singly and in mixtures in a glasshouse to determine the effect of different competition regimes on inbreeding depression. First, we test whether increased competitive stress results in higher inbreeding depression by assessing the relative performance of selfed progeny when plants of the same cross type are grown together compared to when they are grown singularly. Next, we test whether dominance and suppression occurs by assessing how the frequency of selfed and outcrossed individuals in mixtures affects relative performance of selfed progeny, and whether inbreeding depression increases with the frequency of crossed plants.

MATERIALS AND METHODS

Study species

Hibiscus trionum var. *vesicarius* Hochr. (Malvaceae) is a summer-growing annual native to northern and inland areas of Australia (Harden, 2000). Seedling numbers in excess of 150 per square metre can occur in suitable habitat and intraspecific competition can cause high mortality (Johnson, 2003). Plants take about 8 weeks to flower and substantial phenotypic variation in size at flowering and reproductive output occurs, depending on the growing conditions (Johnson, 2003; M. Ramsey, personal observation). Individual plants probably produce a mixture of selfed and crossed seeds, the frequency of each depending on pollinator abundance and visitation. Plants produce large showy flowers that open in the morning and close by mid-afternoon. Pollinators include butterflies, introduced and native bees, and flies. In the absence of pollination, delayed autonomous self-pollination occurs. The styles progressively curve so that the stigmas contact the anthers at the end of floral life. Self- and cross-pollinated flowers yield similar numbers of seeds but selfed progeny are less fit, especially at later life-cycle stages (Seed *et al.*, 2006).

Field sampling and generation of F1 progeny

We established an experimental population by harvesting seeds from plants growing near Narrabri in western NSW, Australia (30°12′S, 149°39′E, 214 m above sea level). Plants were growing at least 5 m apart and were from a 100 m^2 area. One seed from each plant was hand-scarified to break the seed coat-imposed dormancy and placed on moist filter paper in Petri dishes. Dishes were randomly arranged in a growth cabinet set at 20°C and a 12 h light/12 h dark photoperiod (Ramsey *et al.*, 2003). Seedlings were planted in 1.5-litre pots containing sand, loam, and peat (1 : 1 : 1) and grown to flowering in a glasshouse.

To generate F1 progeny, we either self- or cross-pollinated four newly opened flowers on each of eight maternal plants. Crossed flowers were emasculated and their stigmas covered with pollen from another plant. Selfed flowers were pollinated with pollen from the same flower, and the anthers were removed. After 5 weeks, we dissected the fruits and counted the number of seeds. Because replicate cross-pollinated fruits potentially had different fathers, we mixed the seeds of the four crossed fruits from each maternal plant; we also mixed the seeds of the selfed fruits.

We individually weighed ten selfed and crossed seeds from each maternal family to the nearest 0.1 mg (total $N = 160$ seeds). Seeds were germinated as described above, using 54 selfed and 54 crossed seeds from each family (total $N = 864$ seeds). The number of seeds that germinated was recorded. Most seeds germinated within 3 days (>98%). Using 10-day-old seedlings, we assessed initial seedling mass using four selfed and crossed seedlings from each maternal plant. Seedlings were dried at 80°C for 48 h and weighed to the nearest 0.1 mg.

We assessed the effects of cross type on seed set, seed mass, and seedling mass with two-way analyses of variance (ANOVA; JMP 5.0.1; SAS Institute Inc., Cary, NC). Cross type and maternal family were fixed and random factors, respectively. Seed set and seed mass did not require transformation to meet assumptions of ANOVA, but seedling mass was transformed using natural logarithms. In preliminary analyses, the cross type × family interactions were not significant for seed set and seedling mass (both $P > 0.12$), and the interactions were pooled with the error terms to provide a more powerful test of the effect of cross type. The interaction term was retained in the final analysis for seed mass. We compared percentage seed germination following arc-sine square-root transformation, with a two-way ANOVA without replication.

For each trait, we calculated inbreeding depression as $\delta = 1 - w_s/w_c$, where w_s and w_c are the performances of selfed and crossed progeny, respectively (Charlesworth and Charlesworth, 1987). We calculated inbreeding depression for each family and then calculated an overall mean (± standard error). We tested whether the inbreeding depression estimates differed from zero, using one-sample *t*-tests (Sokal and Rohlf, 1995).

Density- and frequency-dependent effects on performance

The experiment consisted of a density series and a replacement series that generally followed the design presented in Koelewijn (2004). For each of the eight maternal families, 7-day-old seedlings were planted into 1.5-litre pots containing sand, loam, and peat (1 : 1 : 1). We used 168 pots, representing three replicates of seven different density and frequency treatments for each of the eight families. Replicate pots were randomized with respect to family and treatment within each of three blocks. The density and frequency treatments were:

1 one selfed or one crossed seedling (two pots);
2 four selfed or four crossed seedlings (two pots);
3 one selfed and three crossed seedlings (one pot);
4 two selfed and two crossed seedlings (one pot); and
5 three selfed and one crossed seedlings (one pot).

Treatments 1 and 2 represent a density series for testing the effect of competition between progeny of the same cross type (self or cross) on the magnitude of inbreeding depression (i.e. density-dependent inbreeding depression). Plant densities in treatments 1 and 2 were 64 and 256 plants per m^2, respectively. Treatments 2, 3, 4, and 5 represent a replacement series. These test the effect of competition between progeny of different cross types on inbreeding depression while keeping density constant (i.e. frequency-dependent inbreeding depression). We achieved this by examining the performance of selfed and crossed progeny in response to the number of competitors of the other cross type. The frequencies of cross types used in treatment 2 reflect selfing rates of 1.0 and 0, and treatments 3, 4, and 5 reflect selfing rates of 0.25, 0.50, and 0.75, respectively.

Plants were watered every 2–3 days and fertilized every 2 weeks with 50 ml of Aquasol®. Seedlings that died within 5 days of transplanting (< 1%) were replaced with seedlings from the same cross type and family. This mortality did not vary across cross types or other treatments and was probably due to damage during transplanting. No subsequent mortality occurred. After 11 weeks, we counted the number of flowers and autonomously pollinated fruits that each plant had produced. We also counted the number of seeds in one autonomous fruit per plant. Autonomous seed production per plant was estimated as the product of the numbers of seeds per fruit and fruits per plant (seeds × fruits). Plants were harvested by cutting them at soil level. The vegetative (stalk and leaves) and reproductive (all flowers, fruits, and seeds) parts of each plant were separated, dried at 80°C for 72 h and weighed to the nearest 0.1 mg. Root mass was not assessed because we could not separate the roots of different plants in the same pot. We calculated total plant mass as the sum of the reproductive and vegetative masses.

Data analyses

To initially examine the effects of density, we compared selfed and crossed progeny grown at low and high densities with one-way analyses of variance, using data pooled over blocks. For high and low densities, we calculated inbreeding depression for each trait as described above, and calculated cumulative inbreeding depression as the product of seeds × fruits and vegetative dry mass. Vegetative mass was included as an indicator of plant size to reflect lifetime reproductive success. For the frequency-dependent experiment, we calculated cumulative inbreeding depression for each of the treatments.

To examine the combined effects of cross type and either density (low or high) or the number of competitors of the other cross type (0, 1, 2, or 3 competitors), we used factorial mixed-model analyses of variance. The main treatments of cross type and density, or number of competitors, were considered as fixed factors and maternal families and blocks as random factors. In these analyses, we were particularly interested in the density × cross type and the number of competitors × cross type interactions. If significant, these interactions indicate that differences between selfed and crossed progeny depend on the density or the number of competitors, respectively. In all analyses, the density (or number of competitors) × cross type × family interactions were not significant (all $P > 0.220$), and they were pooled with the error for the final analyses. Data were either transformed using natural logarithms (numbers of flowers and fruits, vegetative, reproductive, and total dry masses) or square roots (numbers of seeds, seeds × fruits) before analysis.

As outlined by Koelewijn (2004), density experiments can violate the assumptions of independence of data, and hence are difficult to analyse statistically. We overcome this problem by using the mean of the four plants in each pot from the high-density treatment in analyses rather than individual plants. Replacement series can also be problematic because there are unequal numbers of cross types in the competing mixtures, rendering the design unbalanced. Furthermore, in the monoculture treatments (i.e. all selfed or all crossed plants) no observations are made of the other cross type. To overcome these problems, we adopt the view that monocultures compete with zero individuals of the other cross type. The other treatments can then be considered a replacement of either selfed or crossed progeny with the other cross type, while keeping density constant. These designs can be analysed using ANOVA, enabling a powerful test to detect interactions between the two types of progeny (Koelewijn, 2004).

RESULTS

F1 progeny

Seed set, seed mass, and seed germination following self- and cross-pollination did not differ significantly, but crossed seedlings weighed 8% more than selfed seedlings (Table 1). Families varied significantly for all traits, except seed germination. The cross type × family interaction was marginally significant for seed mass; crossed seeds weighed more than selfed seeds in four families and vice versa in the other families. Inbreeding depression was low for all traits (<0.07; Table 1), and was only significantly greater than zero for seedling mass (one-sample t-test, $n = 8$, $t = 2.83$, $P = 0.025$).

Table 1. Performance of selfed and crossed progeny and inbreeding depression (δ) at early life-cycle stages in *Hibiscus trionum* var. *vesicarius*

(a) Means	Seeds/fruit	Seed mass (mg)	Seed germination (%)	Seedling mass at 10 days (mg)
Self	42.8 ± 1.1 (32)	9.0 ± 0.1 (80)	98.2 ± 1.1 (432)	3.7 ± 0.1 (32)
Cross	41.5 ± 1.3 (32)	9.1 ± 0.1 (80)	99.5 ± 0.3 (432)	4.0 ± 0.1 (32)
δ	0.035 ± 0.023	0.007 ± 0.014	0.013 ± 0.012	0.069 ± 0.024

(b) ANOVA Source	d.f.	*F*	d.f.	*F*	d.f.	*F*	d.f.	*F*
Cross type	1	0.92	1	0.21	1	1.37	1	5.34*
Family	7	4.51***	7	24.25***	7	0.81	7	5.59***
Cross type × family	—	—	7	1.92$^{\#}$	N.A.	N.A.	—	—
Error	55		144		7		55	

Note: (a) Means (± standard errors) are pooled over eight maternal families. Sample sizes are given in parentheses. (b) Results of two-way analyses of variance examining the effects of cross type and maternal family. Non-significant cross type × family interactions (all $P > 0.12$) for seeds/fruit and seedling mass were pooled with the error. For seed germination, a two-way ANOVA without replication was used, and values for the cross type × family interaction are not available (N.A.). Significance levels are: $^{\#}P < 0.10$; $^{*}P < 0.05$; $^{***}P < 0.001$.

Effects of density

All trait values except seeds/fruit declined when plants were grown at high density compared with low density, indicating that plants competed at high density (Table 2). At low density, crossed progeny significantly outperformed selfed progeny for six of the seven traits (Table 2). For these traits, inbreeding depression ranged between 0.12 and 0.30. At high density, crossed progeny significantly outperformed selfed progeny for only two traits, seeds/fruit and seeds × fruits. Inbreeding depression for these traits was 0.14 and 0.18, respectively. For both densities, inbreeding depression was highest for seeds × fruits. Cumulative inbreeding depression was significantly greater at low density than at high density (Table 2; one-way ANOVA, $F_{1,14} = 4.77$, $P = 0.046$).

In the factorial analyses of variance, the density × cross type interactions were significant for flowers, fruits, seeds × fruits, and reproductive dry mass, reflecting larger differences between selfed and crossed progeny at low density than at high density (Tables 2, 3). The density × family interaction was significant for vegetative mass ($P = 0.05$) and marginally significant for fruits/plant ($P = 0.07$), indicating that density affected families slightly differently. None of the cross type × family interactions were significant.

The main effects of density and cross type were significant for all traits (Table 3). The density effect indicates that all traits were less under high density than under low density when pooled over cross type. An exception was seeds/fruit, for which values were less under low density. The cross type effect indicates that all trait values were greater for crossed progeny than for selfed progeny when pooled over density (Table 2). Significant variation among families was detected for flowers, fruits, and vegetative mass. The block effect was significant for flowers, fruits, reproductive mass, vegetative mass, and total mass, indicating that the position of pots in the glasshouse affected these traits.

Table 2. Performance of selfed and crossed progeny planted at different densities (1 vs. 4 plants per pot) and inbreeding depression (δ) in *Hibiscus trionum* var. *vesicarius*

Traits	Density	Self	Cross	P	δ
Flowers	1	7.8 ± 0.4	9.3 ± 0.3	**	0.16 ± 0.04
	4	2.4 ± 0.1	2.5 ± 0.1	N.S.	0.04 ± 0.03
Fruits	1	7.6 ± 0.3	9.2 ± 0.2	**	0.17 ± 0.04
	4	2.3 ± 0.1	2.5 ± 0.1	N.S.	0.08 ± 0.03
Seeds/fruit	1	16.6 ± 0.9	20.5 ± 0.8	*	0.19 ± 0.06
	4	20.3 ± 0.8	23.5 ± 0.6	*	0.14 ± 0.04
Seeds × fruits	1	127 ± 9	186 ± 13	**	0.30 ± 0.06
	4	47.4 ± 2.3	58.0 ± 1.2	***	0.18 ± 0.04
Reproductive mass (mg)	1	1720 ± 72	2058 ± 72	**	0.17 ± 0.03
	4	529 ± 14	557 ± 10	N.S.	0.05 ± 0.03
Vegetative mass (mg)	1	2130 ± 116	2292 ± 93	N.S.	0.07 ± 0.05
	4	405 ± 21	425 ± 15	N.S.	0.05 ± 0.05
Total mass (mg)	1	3855 ± 150	4350 ± 139	**	0.12 ± 0.03
	4	934 ± 21	981 ± 20	#	0.05 ± 0.02
Cumulative	1	—	—		0.36 ± 0.05
	4	—	—		0.23 ± 0.03

Note: Means (± standard errors) are pooled over eight maternal families. Also given are P-values for one-way analyses of variance (all d.f. = 1,14) testing for differences between selfed and crossed progeny. Cumulative inbreeding depression was estimated using the traits seeds × fruits and vegetative dry mass. Significance levels are: N.S. = not significant; $^{\#}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Effects of frequency of cross types

When the frequency (number) of competitors was manipulated by replacing selfed progeny with crossed progeny or vice versa, there were substantial differences in plant performance between the cross types (Fig. 1). For all the traits examined, increased competition with the other cross type resulted in increased performance by crossed progeny but decreased performance by selfed progeny. Accordingly, cumulative inbreeding depression was magnified significantly with an increasing number of cross competitors (Fig. 1H; one-way ANOVA, $F_{3,28} = 3.85$, $P = 0.020$). Inbreeding depression increased by 2.2-fold when selfed progeny competed with maximum number of crossed progeny compared with zero crossed competitors. To test whether the inbreeding depression estimates were an artifact of comparing the opposite responses of the two cross types within each treatment (e.g. Koelewijn 2004), we recalculated the estimates using the pure stand of crossed progeny as a control. This did not change the estimates overly, nor the pattern of increasing inbreeding depression with the number of crossed competitors ($\delta = 0.21$, 0.20, 0.38, and 0.46 for 0, 1, 2, and 3 competitors of the other type, respectively).

The contrasting effects of competition on selfed and crossed progeny are highlighted in the factorial analyses of variance. All of the number of competitors × cross type interactions were significant except for vegetative mass, which showed the same trend as the other traits (Table 4, Fig. 1). All families showed the same trend with respect to the number of competitors, as evidenced by the non-significant number of competitors × family interactions. The cross type × family interactions were significant for reproductive mass and total

Table 3. Results of ANOVA for traits examined in relation to density (1 or 4 plants per pot), cross type (self or cross), and family

Source of variation	d.f.	Flowers	Fruits	Seeds/fruit	Seeds × fruits	Reproductive mass	Vegetative mass	Total mass
Main effects								
Block	2	6.79**	4.08*	0.88	0.04	11.71***	12.19***	20.27***
Density	1	1166.09***	1033.85***	8.00*	160.58***	2249.69***	870.63***	3279.62***
Cross type	1	16.93**	28.20***	11.44*	21.54***	63.40***	6.56*	48.27***
Family	7	2.92**	2.58*	0.73	0.96	2.10#	2.45*	0.90
Interactions								
Density × cross type	1	7.62**	7.19**	0.04	5.25*	4.99*	0.10	2.30
Density × family	7	1.66	2.01#	1.63	1.43	0.81	2.19*	0.99
Cross type × family	7	0.90	0.74	0.90	0.95	0.27	0.54	0.25
Error	69							

Note: *F*-ratios are given. The denominators of the *F*-ratios for density and cross type were the mean squares for density × family and cross type × family, respectively. For all other effects, the mean square error was used. Significance levels are: $^{\#}P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

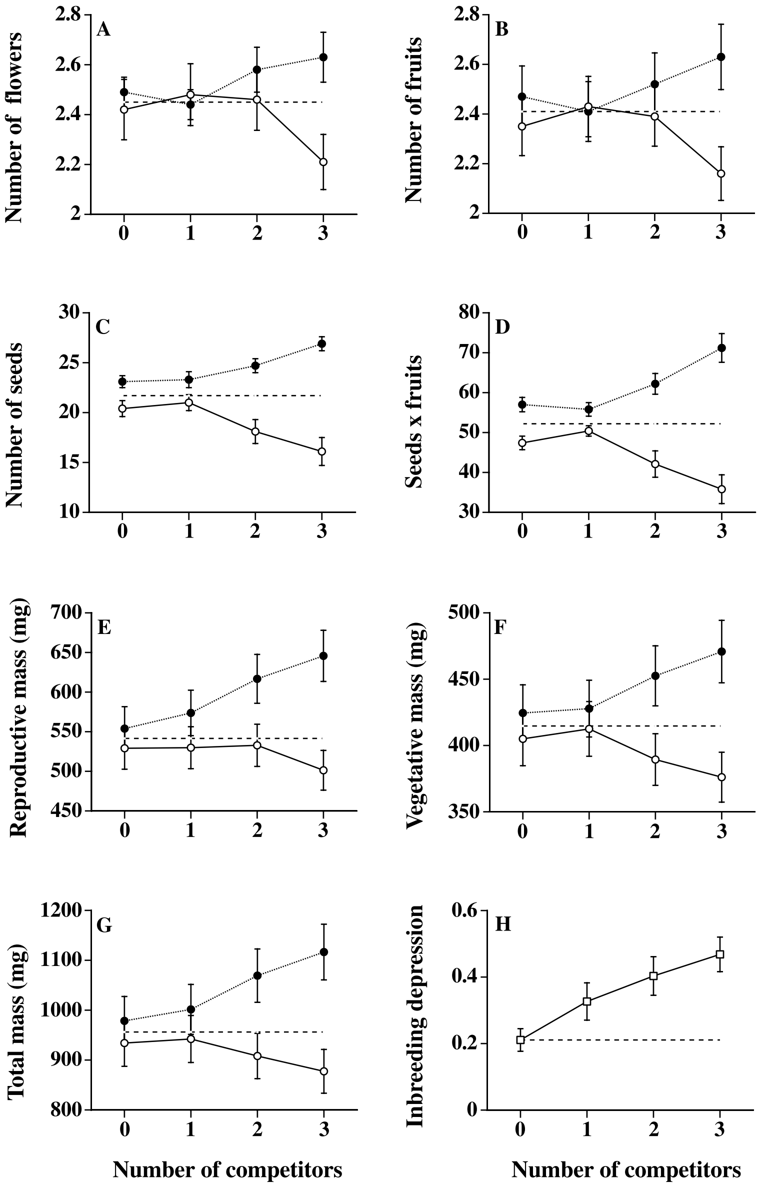

Fig. 1. Performance of selfed and crossed progeny in response to increasing numbers of plants of the other cross type in *H. trionum* var. *vesicarius* (A–G). ○, selfed progeny; ●, crossed progeny. Data are means (± standard errors) from eight maternal families. Also given is the cumulative inbreeding depression when selfed progeny compete with an increasing number of crossed competitors (H). For (H), numbers of crossed competitors reflect outcrossing rates of 0, 0.25, 0.50, and 0.75. The dashed lines are the mean value of selfed and crossed progeny in monoculture (0 competitors of the other type).

Table 4. Results of ANOVA for traits examined in relation to the number of competitors (0, 1, 2, or 3 competitors per pot), cross type (self or cross), and family

Source of variation	d.f.	Flowers	Fruits	Seeds/fruit	Seeds × fruits	Reproductive mass	Vegetative mass	Total mass
Main effects								
Block	2	5.03**	3.36*	5.77**	1.68	2.04	9.49***	9.63***
No. of competitors	3	0.71	0.29	1.11	0.54	0.29	0.17	0.09
Cross type	1	4.18$^{\#}$	10.12*	57.50***	86.97***	11.25*	5.74*	14.10**
Family	7	2.60*	4.75***	2.82**	3.08**	3.84***	6.51***	2.22*
Interactions								
Competitors × cross type	3	3.17*	3.72*	10.12***	12.78***	3.66*	1.28	3.98**
Competitors × family	21	0.98	1.19	0.78	0.77	1.19	0.74	1.18
Cross type × family	7	1.98	1.27	1.31	0.98	2.71*	1.61	2.21*
Error	147							

Note: *F*-values are given. The denominators of the *F*-ratios for competitors and cross type were the mean squares for competitors × family and cross type × family, respectively. For all other effects, the mean square error was used. Significance levels are: $^{\#}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

mass, indicating that the superior performance of crossed progeny compared with selfed progeny was greater in some families than others.

The main effect of cross type was significant for all traits, indicating that crossed progeny outperformed selfed progeny when pooled over the number of competitors (Table 4, Fig. 1). For the main effect of number of competitors, none of the traits were significant because increases in trait values by crossed progeny were closely reflected by decreases by selfed progeny owing to the strong competitor × cross type interactions. The family effect was significant for all traits, and the block effect was significant for flowers, fruits, seeds, and vegetative and total mass.

DISCUSSION

Intraspecific competition affected the expression of inbreeding depression in *H. trionum* var. *vesicarius*. We found that both the density and the frequency of competitors influenced the performance of progenies derived from selfing and outcrossing. However, contrary to predictions that selfed progeny should be more stressed at high densities, the magnitude of inbreeding depression was actually greater when plants were grown singularly than when they were grown in competition. When selfed and crossed progeny were grown in mixed stands, selfed progeny were less competitive, and inbreeding depression increased with the number of crossed competitors. Thus, inbreeding depression is a dynamic property of populations, the magnitude of which potentially varies with the density and relative frequency of selfed and crossed individuals (see also Cheptou *et al.*, 2000; Koelewijn, 2004).

We found no evidence that increased competitive stress, brought about by an increase in density, caused higher inbreeding depression in *H. trionum* var. *vesicarius*. Increased density had a pronounced negative effect on both cross types, indicating that competition reduced individual performance as space and resources became limiting. However, the adverse effect of density was more pronounced for crossed progeny than for selfed progeny, and as a result relative performance of selfed progeny actually increased at higher density. Thus, although inbreeding depression was density-dependent, inbreeding depression decreased with density, at least for the two levels of density used in our experiment. It is likely that crossed progeny experienced greater competitive stress at high density than did selfed progeny. Because crossed progeny grow more vigorously than selfed progeny, they may have been more resource-limited at high density. Interestingly, trait variability as measured by coefficients of variation (CV) decreased at high density compared with low density (e.g. CV for seeds × fruits: selfed progeny = 32% and 18%; crossed progeny = 38% and 15%). Because growth was restricted at high densities, opportunities for competitive suppression were reduced. Other studies have also failed to detect an increase in inbreeding depression with density. Similar to the present results, Koelewijn (2004) reported that inbreeding depression decreased with density in *Plantago coronopus*, while density did not affect inbreeding depression in *Crepis sancta* (Cheptou *et al.*, 2001) and *Arenaria uniflora* (Fishman, 2001). In a study of the effect of water limitation on inbreeding depression, Heschel *et al.* (2005) suggested that higher inbreeding depression in well-watered compared with water-limited *Impatiens capensis* was a consequence of drought stress hindering growth so that fitness differences between the cross types were not realized.

In our mixed stands, the frequencies of selfed progeny emulate population selfing rates of 0.25 (one selfed plant), 0.50 (two selfed plants), and 0.75 (three selfed plants), while the pure stands of four selfed or crossed plants relate to rates of 1.0 and 0, respectively. Crossed progeny outperformed selfed progeny in both the pure and mixed stands. However, while the fitness of crossed progeny increased, the fitness of selfed progeny decreased with the number of competitors of the other cross type. This negative relationship between fitness of selfed progeny and the frequency of crossed competitors was probably a consequence of the greater competitive ability of the crossed progeny. These findings indicate that the magnitude of inbreeding depression was frequency-dependent, and increased when population selfing rate decreased. Other studies have also found that inbreeding depression is magnified when selfed and crossed plants are grown in competition compared to when they are grown in pure stands (Antonovics, 1968; Schmitt and Ehrhardt, 1990; Cheptou *et al.*, 2001; Koelewijn, 2004; but see Mayer *et al.*, 1996). Together with the present results, these studies indicate that frequency-dependent inbreeding depression may be common in natural populations.

Asymmetric competition occurs between individuals that differ in competitive ability and can markedly increase size hierarchies in populations through pre-emption of resources (Weiner, 1990). Because plant size is correlated with survival and reproduction, asymmetric competition can greatly increase fitness differences between competing individuals. In this study, asymmetric competition in mixed stands probably accentuated small initial differences in performance between selfed and crossed seedlings, resulting in dominance and suppression of selfed progeny. Size and reproductive hierarchies of selfed progeny increased with the number of crossed competitors, as indicated by coefficients of variation (e.g. CV for seeds × fruit = 18%, 13%, 39%, and 50% for treatments with 0, 1, 2, and 3 crossed competitors, respectively). By contrast, variation for crossed progeny was slight (CV = 16%, 16%, 21%, 25%, respectively). Although seed mass did not differ between the cross types, the smaller size of selfed seedlings 10 days after germination (Table 1) may have contributed to the final inequalities in size and reproduction in our experiment. By growing more rapidly, crossed progeny would have been able to usurp resources, and thereby progressively increase their competitive advantage over time. In addition, inbreeding depression in *H. trionum* var. *vesicarius* is expressed primarily at later life-cycle stages (Seed *et al.*, 2006), rendering crossed progeny even more competitive as the experiment progressed. Compared with mixed stands, competition in pure stands was probably more symmetrical. In pure stands, the competitors were more genetically similar to each other, providing fewer opportunities for size and reproductive hierarchies to develop.

Further empirical work is required to verify the effects of the density and frequency of competitors on inbreeding depression in natural populations of *H. trionum* var. *vesicarius*. Nevertheless, it is likely that selfed and crossed plants would compete under natural conditions. Although outcrossing rates in populations have not been determined, individual plants may produce both selfed and crossed seeds (Seed *et al.*, 2006). Because seeds are not adapted for dispersal, neighbouring plants are likely to be related, resulting in sibling competition (Cheplick, 1992). Furthermore, plant densities used in this experiment are similar to those that can occur in natural populations. Indeed, populations are likely to be characterized by marked temporal changes in plant density. Plants occur in disturbed semi-arid habitats and growing conditions can vary dramatically, depending on disturbance and rainfall events. In annual plants such as *Hibiscus trionum* var. *vesicarius*, fitness is dependent entirely on one season's seed production. Consequently, the effects of inbreeding depression on individual fitness could be severe, if inbreeding impairs the ability of individuals to take advantage of favourable growing conditions and produce maximal numbers of seeds.

The balance between inbreeding depression and the cost of outcrossing drives the evolution of selfing in populations (Lloyd, 1979; Lande and Schemske, 1985; Uyenoyama *et al.*, 1993). The major finding of our glasshouse study is that the fitness of plants arising from selfing or outcrossing cannot be characterized independently of the density and frequency of inbred and outbred plants in the population. As a consequence, the population selfing rate potentially influences the expression of inbreeding depression, which in turn influences selection on the selfing rate. Theoretical models predict that temporal variation in ecological conditions that causes inbreeding depression to fluctuate should result in intermediate selfing rates (Cheptou and Schoen, 2001; Cheptou and Dieckmann, 2002). In *H. trionum* var. *vesicarius*, if inbreeding depression fluctuates among generations in response to changes in density and frequency of inbred and outbred plants, then intermediate selfing rates and mixed mating could be maintained.

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REFERENCES

Antonovics, J. 1968. Evolution of closely adjacent plant populations. V. Evolution of self-fertility. *Heredity*, **23**: 219–238.

Byers, D.L. and Waller, D.M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.*, **30**: 479–513.

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

Charlesworth, D. and Charlesworth, B. 1999. The genetic basis of inbreeding depression. *Genet. Res, Camb.*, **74**: 329–340.

Charlesworth, D., Morgan, M.T. and Charlesworth, B. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multi-locus system with no linkage. *Evolution*, **44**: 1469–1489.

Cheplick, G.P. 1992. Sibling competition in plants. *J. Ecol.*, **80**: 567–575.

Cheptou, P.-O. and Dieckmann, U. 2002. The evolution of self-fertilization in density-regulated populations. *Proc. R. Soc. Lond. B*, **269**: 1177–1186.

Cheptou, P.-O. and Schoen, D.J. 2001. The cost of fluctuating inbreeding depression. *Evolution*, **56**: 1059–1062.

Cheptou, P.-O. and Schoen, D.J. 2003. Frequency-dependent inbreeding depression in *Amsinckia. Am. Nat.*, **162**: 744–753.

Cheptou, P.-O., Imbert, E., Lepart, J. and Escarré, J. 2000. Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant *Crepis sancta* (Asteraceae). *J. Evol. Biol.*, **13**: 522–531.

Cheptou, P.-O., Lepart, J. and Escarré, J. 2001. Inbreeding depression under intraspecific competition in a highly outcrossing population of *Crepis sancta* (Asteraceae): evidence for frequency-dependent variation. *Am. J. Bot.*, **88**: 1424–1429.

Darwin, C.R. 1876. *The Effects of Cross and Self Fertilization in the Vegetable Kingdom*. London: Murray.

Dudash, M.R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution*, **44**: 1129–1139.

Eckert, C.G. and Barrett, S.C.H. 1994. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution*, **48**: 952–964.

Fishman, L. 2001. Inbreeding depression in two populations of *Arenaria uniflora* (Caryophyllaceae) with contrasting breeding systems. *Heredity*, **86**: 184–194.

Harden, G.J. 2000. *Flora of New South Wales*, Vol. 1. Sydney, NSW: University of New South Wales Press.

Hayes, C.N., Winsor, J.A. and Stephenson, A.G. 2005. Environmental variation influences the magnitude of inbreeding depression in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *J. Evol. Biol.*, **18**: 147–155.

Heschel, M.S., Hausmann, N. and Schmitt, J. 2005. Testing for stress-dependent inbreeding depression in *Impatiens capensis*. (Balsaminaceae). *Am. J. Bot.*, **92**: 1322–1329.

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

Johnson, S.B. (2003). Ecology and management of bladder ketmia (*Hibiscus trionum*) and other emerging problem Malvaceae weeds. Unpublished report for Cotton Research and Development Corporation, Narrabri, NSW, Australia.

Keller, L.F. and Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.*, **17**: 230–241.

Koelewijn, H.P. 2004. Sibling competition, size variation and frequency-dependent outcrossing advantage in *Plantago coronopus*. *Evol. Ecol.*, **18**: 51–74.

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

Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.*, **113**: 67–79.

Mayer, S., Charlesworth, D. and Meyers, B. 1996. Inbreeding depression in four populations of *Collinsia heterophylla* NUTT (Scrophulariaceae). *Evolution*, **50**: 879–891.

Ramsey, M. and Vaughton, G. 1998. Effect of environment on the magnitude of inbreeding depression in seed germination in a partially self-fertile perennial herb (*Blandifordia grandiflora*, Liliaceae). *Int. J. Plant Sci.*, **159**: 98–104.

Ramsey, M., Seed, L. and Vaughton, G. 2003. Delayed selfing and low levels of inbreeding depression in *Hibiscus trionum* (Malvaceae). *Aust. J. Bot.*, **51**: 275–281.

Schmitt, J. and Ehrhardt, D.W. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. *Evolution*, **44**: 269–278.

Seed, L., Vaughton, G. and Ramsey, M. 2006. Delayed autonomous selfing and inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius* (Malvaceae). *Aust. J. Bot.*, **54**: 27–34.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry*, 3rd edn. New York: W.H. Freeman.

Uyenoyama, M.K., Holsinger, K.E. and Waller, D.M. 1993. Ecological and genetic factors directing the evolution of self-fertilization. *Oxford Surv. Evol. Biol.*, **9**: 327–381.

Waller, D.M. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution*, **38**: 427–440.

Weiner, J. 1985. Size hierarchies in experimental populations of annual plants. *Ecology*, **66**: 743–752.

Weiner, J. 1990. Asymmetric competition in plant populations. *Trends Ecol. Evol.*, **5**: 360–364.

Wolfe, L.M. 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding and parental mating history. *Evolution*, **47**: 374–386.