
Inbreeding and Outbreeding Depression in Natural Populations of *Chamaecrista fasciculata* (Fabaceae)

CHARLES B. FENSTER* AND LAURA F. GALLOWAY†

*Department of Biology, H. J. Patterson Hall, University of Maryland, College Park, MD 20742-5815, U.S.A, and Botanisk Institutt, NTNU, Trondheim, N-7034, Norway, email cf25@umail.umd.edu

†Department of Biology, University of Virginia, Charlottesville, VA 22903-2477, U.S.A.

Abstract: *The deleterious consequences of inbreeding have been well documented. There are, however, few empirical studies that have examined the consequences of restoring heterozygosity and hence the fitness of inbred populations by conducting interpopulation crosses and measuring the performance of later-generation hybrids under field conditions. We conducted interpopulation crosses of 100 m to 2000 km, which spans the range of Chamaecrista fasciculata (Fabaceae) in eastern North America. We then contrasted the performance of the F1 and later-segregating F3 hybrids with the parental generation. We found almost universal F1 superiority over the parents. The F3 hybrids suffered a loss of fitness compared to the F1 hybrids. The drop off in fitness of the F3 reflects both the loss of heterozygosity and the disruption of coadapted gene complexes. The F3 performance, however, was still often equal to that of the parents, suggesting that heterosis can outweigh the loss of coadaptation except for the longest-distance crosses. In a subset of environments, the F3 performance of long-distance (≥ 1000 km) interpopulation crosses was less than that of both parents and indicated true outbreeding depression. For C. fasciculata, it appears that crossing populations of up to intermediate distances of hundreds of kilometers has a short-term beneficial effect on progeny performance through F1, and that longer-term effects are not necessarily disruptive of fitness, at least relative to parental performance. The degree of F1 heterosis and F3 outbreeding depression varied between site and year, however, indicating an important role for the environment in the expression of these effects.*

Depresión por Endogamia y Exogamia en Poblaciones Naturales de *Chamaecrista fasciculata* (Fabaceae)

Resumen: *Las consecuencias nocivas de la endogamia han sido bien documentadas. Sin embargo, hay pocos estudios empíricos que hayan examinado las consecuencias de la restauración de la heterocigosidad y por lo tanto la adaptabilidad de poblaciones endógamas mediante la conducción de cruzas interpopulacionales y mediante la medición del rendimiento de nuevas generaciones de híbridos bajo condiciones de campo. En este trabajo realizamos cruzas interpopulacionales de 100–2000 km, las cuales abarcan el rango de distribución de Chamaecrista fasciculata (Fabaceae) en Norteamérica occidental. Además contrastamos el rendimiento de la generación F1 y segregamos subsecuentemente a los híbridos F3 de la generación de padres. Encontramos una superioridad casi universal de los F1 sobre los padres. Los F3 sufrieron una pérdida de adaptabilidad comparados con los F1. La caída de la adaptabilidad de los F3 refleja tanto la pérdida de heterocigosidad y la disrupción de genes complejos coadaptados. Sin embargo, el rendimiento de los F3 fue aún frecuentemente igual al de los padres, sugiriendo que la heterosis puede tener más peso que la pérdida de coadaptación con la excepción de las cruzas de distancias más grandes. En un subconjunto de ambientes, el rendimiento de los F3 de cruzas interpopulacionales de larga distancia, ≥ 1000 km, fue menor que el de ambos padres, e indicó una verdadera depresión por cruza exogámica. Para C. fasciculata, aparentemente las cruzas de poblaciones de distancias de hasta distancias intermedias de cientos de kilómetros, tiene un efecto benéfico a corto plazo en el rendimiento de la progenie mediante los F1 y los efectos a largo plazo nos son necesariamente negativos para el rendimiento, al menos en lo relacionado con el rendimiento parental. El grado de heterosis F1 y de depresión por exogamia varía entre sitios y entre años, indicando la existencia de un papel importante del ambiente en la expresión de estos efectos.*

Paper submitted May 7, 1999; revised manuscript accepted December 28, 1999.

Introduction

Small populations may have reduced population mean fitness and suffer increased extinction rates because of increased expression of inbreeding depression, decreased levels of genetic diversity, and higher probabilities of fixing deleterious mutations, relative to larger populations (Barrett & Kohn 1991; Fenster & Dudash 1994; Frankham 1995; Dudash & Fenster 2000). Consequently, conservation managers often must consider the possibility of supplementing populations with individuals from other populations to increase population size and to restore genetic diversity. But bolstering population size with individuals from other populations may result in outbreeding depression and reduced fitness of population hybrids relative to either parent (Fenster & Dudash 1994; Dudash & Fenster 2000).

The low fitness of hybrid progeny relative to either parent might arise for the following reasons. First, there might be dilution of genes associated with local adaptation. Imagine that each parental population represents an ecotype in which a certain number of loci confer local adaptation. Where these loci are fixed for alternative alleles between populations, hybrids will have on average only half the genes of either parent and therefore may be less fit than either parent in the parental environment. Second, hybridization between two populations may result in the disruption of coadapted gene complexes (Fenster & Dudash 1994). Genetic coadaptation reflects epistatic gene action, the interaction among loci that enhances fitness (Falconer & Mackay 1996). Thus, if the selective advantage of a particular allele depends on alleles present at other loci, and if each population represents a unique mixture of alleles across loci, then mixing gene pools may lead to the disruption of well-integrated genotypes. Therefore the consequences of mixing distant gene pools will depend on whether gene interaction is prevalent and whether selection is responsible for adaptive differentiation (Fenster & Dudash 1994; Fenster & Galloway 2000a, 2000b; Galloway & Fenster 1999, 2000).

Hybrid vigor and hybrid breakdown can be expressed simultaneously in hybrid populations because of the simultaneous masking of recessive deleterious alleles, dilution of genes that confer local adaptation, and disruption of coadapted gene interactions (Lynch 1991). Unfortunately, the long-term consequences of mixing populations of endangered or threatened species has not been adequately documented (Whitlock et al. 1995; Fenster et al. 1997). Thus, the issue of whether heterosis can offset hybrid breakdown, and the relative magnitude of heterosis and hybrid breakdown compared to the performance of the original parental populations, remain important unanswered questions.

Elsewhere we provide evidence that natural populations of the native North American outcrossing annual legume *Chamaecrista fasciculata* are inbred (Fenster 1988; Fenster 1991a, 1999b; Fenster & Dudash 1994; Galloway et al., unpublished data) and have differentiated via co-

adapted gene complexes (Fenster & Galloway 2000a, 2000b; Galloway & Fenster 1999, 2000). We determined the consequences of population admixture in a conservation context by comparing the fitness of F1 and F3 hybrids directly to either parent under field conditions. Specifically, we asked the following questions: (1) Can crosses among populations lead to the expression of F1 heterosis such that F1 performance is greater than that of either parent? (2) To what extent does heterosis carry over to the segregating generations such that the F3 still outperforms the parents, even when coadapted gene complexes are disrupted or genes conferring local adaptation are diluted? (3) Is there a limit to the crossing distance between populations in which either F1 or F3 performance is enhanced relative to the parents; in other words, do the fitness consequences of recombining populations depend on the distance separating the populations?

Methods

Study Organism and Sites

Chamaecrista fasciculata Michx., partridge pea, is a self-compatible, mostly outcrossing (Fenster 1991a, 1995) annual legume native to disturbed sites in eastern North America. Because *C. fasciculata* is an annual, total lifetime fitness can be approximated well by assessment of germination, survivorship to flowering, and total fruit production (e.g., Fenster 1991b).

Five populations of *C. fasciculata* in each of three regions were selected to quantify the effects of mixing gene pools on both early- and later-generation hybrids. The three regions span the species range in eastern North America. In each region, we chose a target or home-site population. Target populations were located at the margin of a disturbed field in Maryland (U.S. Department of Agriculture Beltsville) and in tallgrass prairie preserves in Kansas (Konza Prairie) and Illinois (Gooselake Prairie Preserve). The remaining four populations in each region were located at increasing distances from the target population (0.1, 1, 10, 100 km; specific site locales and description are given by Galloway & Fenster 2000). Population selection was based only on distance. Environmental variables such as edaphic conditions and moisture regime were not taken into consideration and in some cases differed dramatically from those of the target population. Because we were able to quantify only limited genetic effects on fitness for the Illinois site (Fenster & Galloway 2000a, 2000b; Galloway & Fenster 1999, 2000) here we present only the results for Maryland and Kansas.

Crossing Design

Each of the target or home populations was crossed to populations in the same state and to the other target

populations, for a total of six interpopulation crossing distances. Within-population crosses were conducted between randomly selected individuals for each parental population. Each population was also reciprocally crossed with its local target population. The Maryland and Kansas target populations were crossed to Illinois and to each other to generate 1000- and 2000-km crossing distances, respectively. These crosses resulted in parental seed and F1 hybrids between populations 0.1, 1, 10, 100, 1000, and 2000 km apart. All crosses were conducted in the University of Maryland greenhouse.

Crosses were conducted for two more generations to produce F3 hybrids between populations and parental seed that had experienced cultural conditions equivalent to those of hybrids. The F1 plants within a seed type were randomly crossed to one another for two generations to produce F3 individuals. To hold the number of generations in the breeding program constant, F1 hybrid and parental seed were also created on parents grown two generations in the greenhouse. As a result of the recreation of F1 and parental seed, all generations of seed were produced in the same season (summer of 1994). This also helped minimize maternal environmental effects by creating all progeny (including the parental populations) in the greenhouse and under the same growing conditions.

Field Experiment to Quantify Performance

For 2 years, 1995 and 1996, we planted experimental seeds in natural vegetation in field plots located within the target population in each region. Experimental seeds for each seed type were weighed so that seed size effects could be statistically factored out of the assessment of performance. Seeds were hand-scarified to increase germination (Fenster & Galloway 2000b) and were planted in early spring so their germination would correspond to the natural emergence of seedlings.

The planting design differed slightly between the 2 years. In the first year, seeds were planted into 12 blocks at each site with like seeds grouped within a block, whereas in the second year the number of blocks per site was increased to 36 and seeds were randomly located within each block. Forty-three seed types were planted in each block (6 crossing distances \times 2 reciprocals/distance \times 3 hybrid generations = 36 hybrid seed types + 7 parental populations), and at least 10 maternal families were represented for each seed type. Each block in 1995 had 30 seeds for each parental and F1 seed type and 40 for each F2 and F3 crossing distance (10,440 seeds/site). In 1996, 10 seeds per target parental, 7 seeds per nontarget parental, and 10 F1, 10 F2, and 10 F3 seeds per crossing distance were planted in each block (8136 seeds/site). To present a simplified version of our work, we discuss only F1 and F3 progeny performance relative to that of the parents. Performance of F2

progeny is presented elsewhere (Fenster & Galloway 2000a, 2000b; Galloway & Fenster 2000).

Germination, survivorship to maturity, final biomass, and fruit production were quantified for experimental individuals. We present results for the number of fruit produced per seed planted, an estimate of cumulative fitness. In the two sites (Maryland and Kansas) across the 2 years, we quantified fruit production in approximately 37,000 genotypes. Fitness components such as germination and survivorship are discussed elsewhere (Fenster & Galloway 2000b). Individuals that either did not germinate or that died prior to reproduction were assigned a fruit production (and hence fitness) of zero. Plots were fenced with poultry wire to reduce mammalian herbivory.

Statistical Analysis

We used an analysis of covariance to evaluate the performance of the F1 and F3 progeny relative to the parents. We included year, planting location, crossing distance, generation (parent from the home site, parent from the away site, F1, F3), and block (nested within year and planting location) as main effects in the analysis and seed weight as a covariate (to control for maternal effects). With the exception of block, all effects were fixed. We combined the reciprocal crosses for each hybrid generation. To meet the assumptions of analysis of variance and to reduce heteroscedasticity, we conducted all analyses using block means as the replicates. There was significant three-way interaction among year, planting location, and generation ($F_{3,5266} = 6.91, p < 0.001$) in addition to significant two-way interaction among these variables; therefore, analyses were conducted separately for each year and planting location.

We evaluated the fitness consequences of mixing populations by constructing a priori contrasts of the early-generation F1 hybrids and the later generation F3 hybrids, with each of the parents contributing to the cross. We conducted the contrasts for each crossing distance for each site and year, resulting in 24 contrasts for each analysis of variance (6 distances \times 2 hybrid generations \times 2 parents per distance). The contrasts took the following form: F1 – home parent; F1 – away parent; F3 – home parent; F3 – away parent. We used sequential Bonferroni corrections (Rice 1989) to reduce Type I error for each analysis.

Results

The F1 hybrids generally outperformed the parents in both years and sites, although the magnitude of the superiority varied among crossing distances (Fig. 1). The F1 hybrids significantly outperformed the home parent in 3/6, 5/6, and 1/6 Bonferroni-adjusted contrasts in

Kansas in 1995, Maryland in 1996, and Kansas in 1996, respectively (Table 1; Fig. 1). The F1 hybrids significantly outperformed the away parent in 3/6, 1/6, 3/6, and 2/6 Bonferroni contrasts in Maryland in 1995, Kansas in 1995, Maryland in 1996, and Kansas in 1996, respectively (Table 1; Fig. 1).

The F3 hybrids did not demonstrate the same superiority to the parents as did the F1 (Table 1; Fig. 2). In general, they demonstrated performance roughly equal to that of either parent, with the exception of decreased performance for the longer-distance crosses in Kansas in 1996. The F3 hybrids also outperformed the away parent for the longest-distant cross (2000 km) in both Maryland and Kansas in 1996.

Discussion

We investigated a situation that conservation and restoration biologists are encountering more and more frequently (Bowles & Whelan 1994): the possible need to combine genetic material from different populations to increase population size or found new populations. Our experiment represents one of the first examinations of the consequences of mixing gene pools through the F3 generation to restore population vigor as measured under field conditions. In *C. fasciculata*, a surprising lack of consistent home-site advantage suggests that even long-distance transplants will fare reasonably well in

terms of restoration of extinct populations (Galloway & Fenster 1999, 2000).

First-generation hybrids typically out-performed both parents, regardless of the distance between populations; but the significance of F1 heterosis varied among the different crosses, whether the comparison was made between F1 hybrids and the home parent or F1 hybrids and the away parent (Table 1; Fig. 1). Because the performance of the F1 hybrids was often greater than that of either parent, one management response to supplementing a population is to introduce the F1 of an inter-population cross involving the home population, rather than to supplement the population with parental individuals from other populations. Thus, in addition to the F1 progeny expressing heterosis, F1 individuals will also have at least one-half their genes from the local population, which might confer some degree of adaptiveness to the local environment.

The F3 generation in our study provided evidence that population fitness is in part composed of gene interactions, and that crossing populations leads to a disruption of these gene interactions (Fenster & Galloway 2000a, 2000b; Galloway & Fenster 1999, 2000). Nevertheless, F3 fitness was often equal to and sometimes greater than that of one of the parents, suggesting that heterosis may offset the decrease in fitness associated with disrupting coadapted genes. Thus, mixing gene pools to restore the population vigor of small populations of *C. fasciculata* suggests that outbreeding depression is unlikely to be of significant conservation concern if the crosses are con-

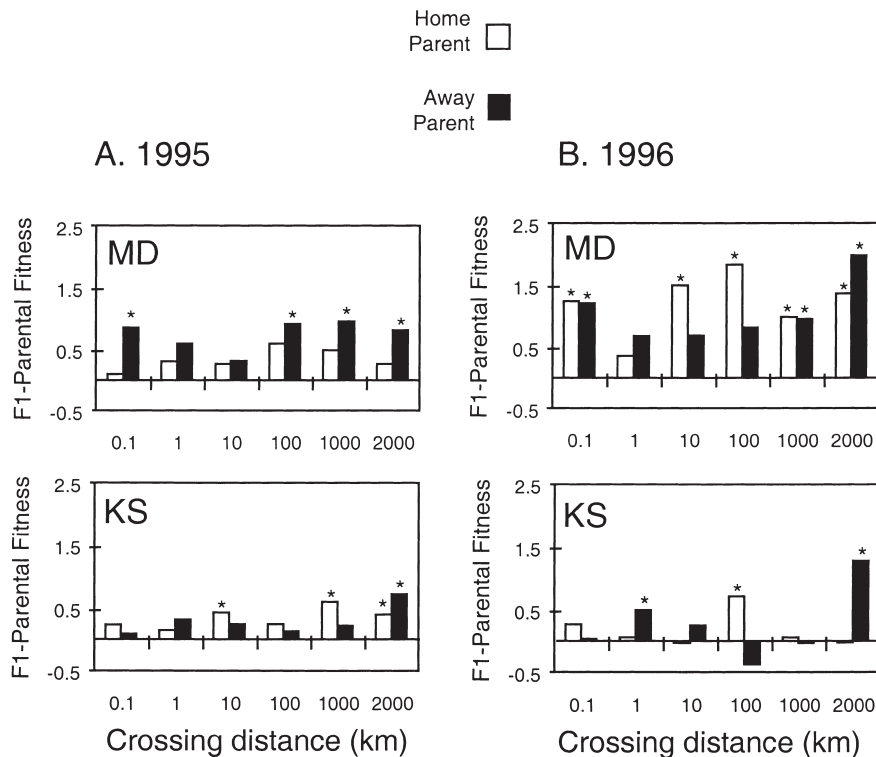


Figure 1. Fitness of the F1 hybrids between populations of *Chamaecrista fasciculata* 0.1–2000 km apart relative to each of the parental populations contributing to the cross. Results are presented for the replicate crosses conducted for Maryland (MD) and Kansas (KS). Square-root transformed means adjusted for initial seed size are presented. Comparisons that differ at $p < 0.05$ following sequential Bonferroni correction indicated by an asterisk. Open bar, F1 fitness–home parent fitness; solid bar, F1 fitness–away parent fitness. If bar is < 0 (horizontal line), then performance of F1 is below that of parent. If bar is > 0 , then performance of F1 is below that of parent.

Table 1. Analysis of variance to evaluate the performance of F1 and F3 hybrids derived from six different interpopulation crossing distances in *Chamaecrista fasciculata*.^a

Year and planting location	Contrast ^b	Crossing distance (km)					
		0.1	1	10	100	1000	2000
1995							
Maryland	F1 vs. HP	0.12	1.02	0.49	3.08	3.54	1.13
	F1 vs. AP	12.23 ^{**b}	3.65	1.02	10.91 ^{**b}	10.79 ^{**b}	7.21 ^{**}
	F3 vs. HP	0.90	0.08	0.21	0.74	1.25	5.24 [*]
Kansas	F3 vs. AP	5.40 [*]	0.26	0.40	5.03 [*]	0.27	0.00
	F1 vs. HP	2.75	1.38	11.98 ^{**b}	3.48	13.46 ^{**b}	11.19 ^{**b}
	F1 vs. AP	0.40	7.22 ^{**}	4.66 [*]	0.71	1.91	36.40 ^{**b}
	F3 vs. HP	1.70	0.33	1.49	0.12	1.13	0.24
	F3 vs. AP	5.07 [*]	0.66	0.06	0.19	1.11	4.12 [*]
1996							
Maryland	F1 vs. HP	36.26 ^{***}	1.21	18.70 ^{***}	31.83 ^{***}	15.82 ^{***}	32.64 ^{***}
	F1 vs. AP	29.63 ^{***}	2.62	4.27 [*]	0.90	15.20 ^{***}	78.88 ^{***}
	F3 vs. HP	3.75	0.12	0.79	3.62	0.01	1.45
Kansas	F3 vs. AP	2.97	0.27	2.18	0.07	0.00	10.46 ^{**}
	F1 vs. HP	2.35	0.70	0.06	10.89 ^{**}	0.14	0.04
	F1 vs. AP	0.05	15.32 ^{***}	2.97	7.64 ^{**}	0.05	65.19 ^{***}
	F3 vs. HP	1.52	0.93	0.82	0.09	11.30 ^{***}	14.83 ^{***}
	F3 vs. AP	10.11 ^{**}	0.54	0.77	43.38 ^{***}	9.33 ^{**}	18.83 ^{***}

^aThe *f* and *p* values of *a priori* contrasts (with 1 *df*) of the F1 and F3 generations with the parental populations are presented. Seed weight is included as a covariate. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

^bContrasts that are significant (*p* < 0.05) following sequential Bonferroni correction. Abbreviations: HP, home parent; AP, away parent.

fined to within 100 km. Even for the longer-distance crosses, the F3 generation sometimes outperformed the away parent, which suggests that hybrids will outperform long-distant transplants, although they may fare worse than the home parent. It is possible that, with fur-

ther rounds of recombination, later generation hybrids would suffer much greater loss of fitness from the disruption of closely linked interacting genes. In *C. fasciculata*, fitness is reduced between the F2 and F3 generation hybrids. This indicates an empirical need to determine

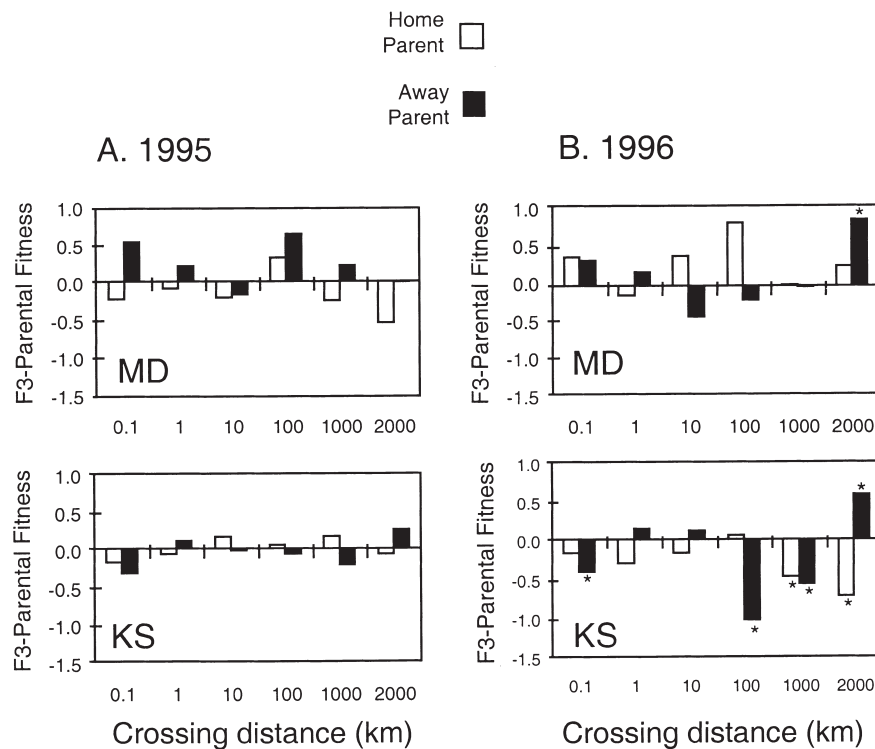


Figure 2. Fitness of the F3 hybrids between populations of *Chamaecrista fasciculata* 0.1–2000 km apart relative to each of the parental populations (MD, Maryland; KS, Kansas) contributing to the cross. See Fig. 1 for details.

the fitness consequences of even later-generation hybrids.

The variable expression of heterosis and hybrid breakdown across years and sites that we have documented may reflect several different phenomena acting simultaneously. Dilution of genes associated with local adaptation may vary because the selective advantage of having a full complement of home-site genes also varies. In addition, variation in environmental conditions may be associated with different selection intensities acting upon deleterious mutations (e.g., Dudash 1990; Pray et al. 1994) and coadapted gene complexes (reviewed by Lynch & Walsh 1997). Strong environmental effects imply that we can expect greenhouse or laboratory studies of heterosis and hybrid breakdown to demonstrate different magnitudes of effect compared with field studies. In general, field-based studies of inbreeding depression depict a greater negative consequence of inbreeding (reviewed by Lynch & Walsh 1997). There are few studies of environmental influences on the expression of hybrid breakdown, but the few that have been conducted suggest that as the environment deteriorates the amount of hybrid breakdown expressed increases (reviewed by Lynch & Walsh 1997). Spatiotemporal variation in the amount of heterosis and hybrid breakdown expressed suggests that there may be great uncertainty in the long-term consequences of genetic manipulation of any particular population.

There is always temptation to generalize results, but here the consequences might have enormous influence on policy. Thus it seems especially prudent to place limits on the interpretation of our findings. Our study focused on an annual organism that possesses a limited seed-bank and colonizes disturbed habitat. Thus, it is likely that population turnover is rapid. Different results may have been observed with a perennial organism with lower population extinction rates, because selective pressures might result in more consistent adaptive divergence (e.g., Waser & Price 1985). In this case, mixing gene pools might have much greater deleterious consequences because of the effect of diluting genes associated with local adaptation. Furthermore, hybrid breakdown due to the disruption of coadapted genes is more likely to occur following interpopulation crosses of organisms that have restricted recombination (Fenster & Dudash 1994; Dudash & Fenster 2000).

It has also been suggested that gene interaction and hence the evolution of coadaptation of genes will be more important in polyploid organisms (Breese & Mather 1960; Honne 1982; Dudash & Fenster 2000) because there are more genes and hence a greater opportunity for their interaction to contribute to genetic variation. Thus, the degree of hybrid breakdown may vary tremendously among organisms, being strongest in organisms that are highly selfing, that have facultatively asexual mating systems, and/or that are polyploid.

We also have little understanding of how quickly a hybrid population might recover fitness. There are two reasons for this: (1) there may be a greater loss of fitness in later-generation hybrids because recombination continues to disrupt coadapted complexes, and (2) we have almost no knowledge of how rapidly selectively advantageous recombinants are generated. In other words, chance combinations of genes from different populations may confer high fitness (Rieseberg et al. 1995; Rieseberg 1997). What is clear, however, is that more studies like ours are needed, in which the long-term consequences of mixing gene pools of organisms with a range of life-history traits, mating systems, and ploidy levels are examined under field conditions. But determining whether outbreeding depression poses a risk is an enormous undertaking that only emphasizes the importance of preventing populations from declining to the point where such drastic measures are necessary.

Acknowledgments

M. Dudash, K. Holsinger, M. Johnston, and an anonymous reviewer made helpful comments on a previous version of the manuscript. The help of staff at U.S. Department of Agriculture Beltsville, Gooselake Prairie (Illinois), The Illinois Department of Natural Resources, Konza Prairie (Kansas), and the University of Maryland greenhouse, along with many undergraduate and graduate students, is greatly appreciated. In particular, we thank B. Glass, L. Hellman, A. Kuhl, M. Sanchez, J. Nyhoff, and T. Van Slyke for their efforts. This work was funded by National Science Foundation grants DEB-9312067 and DEB-9815780 to C. Fenster.

Literature Cited

- Barrett, S. C. H., and J. R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pages 3–30 in D. A. Falk and K. E. Holsinger, editors. *Genetics and conservation of rare plants*. Oxford University Press, Oxford, United Kingdom.
- Bowles, M. L., and C. J. Whelan, editors. 1994. *Restoration of endangered species. Conceptual issues, planning and implementation*. Cambridge University Press, Cambridge, United Kingdom.
- Breese, E. L., and K. Mather. 1960. The organisation of polygenic activity within a chromosome in *Drosophila*. II. Viability. *Heredity* 14: 375–400.
- 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.
- Dudash, M. R., and C. B. Fenster. 2000. Inbreeding and outbreeding depression in fragmented populations. Pages 35–53 in A. Young and G. Clarke, editors. *Genetics, demography and viability of fragmented populations*. Cambridge University Press, Cambridge, United Kingdom.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*. Longman, London.
- Fenster, C. B. 1988. Gene flow and population differentiation in *Cbamaecrista fasciculata* (Leguminosae). Ph.D. thesis. The University of Chicago, Chicago.

- Fenster, C. B. 1991a. Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution* **45**:398-409.
- Fenster, C. B. 1991b. Gene flow in *Chamaecrista fasciculata* (Leguminosae). II. Gene establishment. *Evolution* **45**:410-422.
- Fenster, C. B. 1995. Mirror image flowers and mating system in *Chamaecrista fasciculata* (Leguminosae). *American Journal of Botany* **82**:46-50.
- Fenster, C. B., and M. R. Dudash. 1994. Genetic considerations for plant population restoration and conservation. Pages 34-62 in M. L. Bowles and C. J. Whelan, editors. *Restoration of endangered species*. Cambridge University Press, Cambridge, United Kingdom.
- Fenster, C. B., and L. F. Galloway. 2000a. The contribution of epistasis to the evolution of natural populations: a case study of an annual plant. Pages 232-244 in J. B. Wolf, E. D. Brodie III, and M. J. Wade, editors. *Epistasis and the evolutionary process*. Oxford University Press, Oxford, United Kingdom.
- Fenster, C. B., and L. F. Galloway. 2000b. Population differentiation in an annual legume: genetic architecture. *Evolution* **54**:1157-1172.
- Fenster, C. B., L. F. Galloway, and L. Chao. 1997. Epistasis and its consequences for the evolution of natural populations. *Trends in Ecology and Evolution* **12**:282-286.
- Frankham, R. 1995. Conservation genetics. *Annual Review of Genetics* **29**:305-327.
- Galloway, L. F., and C. B. Fenster. 1999. The role of nuclear and cytoplasmic factors in the adaptive evolution of populations of *Chamaecrista fasciculata* (Fabaceae). *Evolution* **53**:1734-1743.
- Galloway, L. F., and C. B. Fenster. 2000. Population differentiation in an annual legume: local adaptation. *Evolution* **54**:1173-1181.
- Honne, B. I. 1982. On components of the genotypic variance in autotetraploid populations. Pages 217-226 in A. Gallais Inra, editor. *Proceedings of the fourth meeting of the section biometrics in plant breeding*. European Association for Research on Plant Breeding, Rheims, France.
- Lynch, M. 1991. The interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**:622-629.
- Lynch, M., and B. Walsh. 1997. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, Massachusetts.
- Pray, L. A., J. M. Schwartz, C. J. Goodnight, and L. Stevens. 1994. Environmental dependency of inbreeding depression: implications for conservation biology. *Conservation Biology* **8**:562-568.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**:223-225.
- Rieseberg, L. H. 1997. Hybrid origins of plant species. *Annual Review of Ecology and Systematics* **28**:359-389.
- Rieseberg, L. H., B. Sinervo, C. R. Linder, M. C. Ungerer, and D. M. Arias. 1995. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. *Science* **272**:741-745.
- Waser, N. M., and M. V. Price. 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae): evidence for local adaptation. *American Journal of Botany* **72**:1726-1732.
- Whitlock, M. C., P. C. Phillips, F. G.-G. Moore, and S. J. Tonsor. 1995. Multiple fitness peaks and epistasis. *Annual Review of Ecology and Systematics* **26**:601-629.

