Genetics, Demography and Viability of Fragmented Populations

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Inbreeding and outbreeding depression in fragmented populations

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ABSTRACT

The goal of this chapter is to review inbreeding and outbreeding depression in the context of habitat fragmentation and to show how smaller, fewer populations of any organism separated by distance may exasperate the effects of these two genetic phenomena. We review the genetic basis of each, provide examples, and discuss specific empirical issues that need to be addressed in future research. We conclude with an illustrative case study of how both genetic phenomena can act simultaneously in a single species.

INTRODUCTION

Most rare and endangered species exist as small, isolated populations (Holsinger & Gottlieb, 1989). Unfortunately this seems to be the fate of even common species as natural populations are becoming increasingly fragmented. Fragmentation reduces the number of breeding individuals within a population while reducing gene flow between populations. Consequently, mating between individuals in fragmented populations is more likely to represent selfing (if genetically feasible) and/or biparental inbreeding (matings between related individuals) resulting in inbred offspring. The deleterious consequences of inbreeding are manifold. Inbred progeny may suffer from inbreeding depression, i.e. a decline in fitness, where the relative performance of the resulting inbred progeny is lower compared to progeny produced from matings between unrelated individuals within a population (Falconer & Mackay, 1996). Continued inbreeding associated with small populations also results in the loss of within-population genetic diversity (e.g. Schoen & Brown, 1991). Genetic diversity may influence the colonising ability and persistence of a population (Barrett & Kohn, 1991; Lande, 1994). Decreased genetic diversity may also be associated with increased susceptibility to pathogens and pests (Frankham, 1995*b*). Further-

more, as deleterious mutations are introduced to populations at a relatively high rate (Lynch, 1988) their accumulation and fixation are much more likely in small populations (Lande, 1994; Lynch *et al.*, 1995a). In sum, fragmented 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 pre-fragmentation population structure.

A positive relationship between population size and genetic diversity is often observed (e.g. van Treuren *et al.*, 1991; Leberg, 1993; Sun, 1996). Isolated populations frequently possess limited neutral molecular variation relative to less isolated populations (Brussard, 1984; Bayer, 1991; Holderegger & Schneller, 1994; Siikamaki & Lammi, 1998), providing empirical circumstantial evidence that isolation can also lead to the loss of genetic diversity and perhaps increase the expression of inbreeding depression. Manipulation of population size demonstrates that the fitness of a population will decrease following a bottleneck (Polans & Allard, 1989; Newman & Pilson, 1997). In a few cases, a direct relationship between reduced local population levels of heterozygosity (implying inbreeding) and the probability of local population extinction has been noted in natural populations of Granville fritillary butterfly (Saccheri *et al.*, 1998) and the greater prairie chicken (Bouzat *et al.*, 1998a).

Ecological considerations resulting from fragmentation may include difficulty in obtaining mates (i.e. Allee effect: Groom, 1998) and a lack of genetically compatible genotypes. For example, DeMauro's (1993) study of the self-incompatible lakeside daisy (*Hymenoxys acaulis* var. *glabra*) in Illinois, USA, demonstrates the potential role of chance/genetic drift on the population genetics of an endangered organism. The self-incompatibility system played a key role in obtaining successful seed production once the last native population had become so small that natural recovery was impossible. DeMauro found that only a handful of individuals remained and all possessed the same self-incompatibility type. Hand cross-pollinations between plants from Ohio and Illinois successfully produced viable progeny that were initially maintained in glasshouses. Following seedling establishment they were transplanted into protected nature preserves to re-establish this species in Illinois.

Clearly, if endangered populations are small and inbred then consideration has to be given to manipulation of the remaining populations to counteract the erosion of their fitness. Two methods to restore a population's vigour whose decline is due to inbreeding are (1) purge the popula-

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tion of its mutational load and (2) seed populations with progeny from interpopulation crosses (e.g. Fenster & Dudash, 1994). The success of both methods depends on the genetic basis of variation within and among populations. Purging a population of its genetic load by selecting among high performance (most fit) progeny following intense inbreeding will only be successful if inbreeding depression is due to the expression of recessive or partly recessive deleterious alleles expressed in the homozygous state (Fenster & Dudash, 1994). However, because purging requires close inbreeding, it is likely that weakly deleterious alleles will be fixed during the purging process owing to an increase in the role of drift relative to selection, leading to an overall reduction of population vigour (Hedrick, 1994; Lynch *et al.*, 1995*a*). An alternative method to restore heterosis is to make interpopulation crosses. However, this raises the issue of outbreeding depression, or the loss of vigour that may result from crossing individuals from different/and or distant populations (e.g. Fenster & Dudash, 1994; Frankham, 1995*b*).

The goal of this chapter is to review inbreeding and outbreeding depression, and their potential role in population restoration within fragmented landscapes. We will briefly review what theory and empirical data suggest at this time, and propose future directions for research and management strategies. The empirical emphasis will be on flowering plants; however, we will make comparisons wherever possible with animal systems.

INBREEDING DEPRESSION

Inbreeding depression is defined as the reduction in the mean phenotype of a population associated with increasing homozygosity which results from matings between relatives, i.e. biparental inbreeding and selfing (mating occurs within an individual) (Falconer & Mackay, 1996). Traits known to exhibit inbreeding depression include such components of fitness as seed production of the parent, germination, juvenile survival and growth/reproduction of the offspring (e.g. Charlesworth & Charlesworth, 1987; Husband & Schemske, 1996), pollen and ovule production (e.g. Dudash *et al.*, 1997), plant physiological traits (Norman *et al.*, 1995), sperm production (e.g. O'Brien *et al.*, 1987), egg-hatching rates (e.g. Westemeier *et al.*, 1998) and long-term survival (e.g. Jiménez *et al.*, 1994). Individual fitness is ultimately measured by both the quantity and quality of progeny produced by an individual that contributes to the next generation (e.g. Dudash, 1990). Comparisons of progeny performance utilising a multiplicative fit-

ness function, incorporating all measured components of fitness into a single value for each cross type, indicate that the magnitude of inbreeding depression experienced in populations is often great (e.g. Sakai *et al.*, 1989; Dudash, 1990; Fenster, 1991b; Carr & Dudash, 1996). However, the environment in which one chooses to examine inbreeding depression can influence the magnitude of detection in both plants (e.g. Schemske, 1983; Dudash, 1990) and animals (e.g. Miller, 1994; Pray *et al.*, 1994) and needs to be considered when assessing the state of any population. Thus concerns raised about the consequences of inbreeding in fragmented populations should be addressed in the appropriate environment, i.e. nature, if maintenance of natural populations is of primary importance.

Darwin (1876) documented inbreeding depression in both cultivated and native plant species. His results have been confirmed across the range of diversity of the plant kingdom including cultivated plants such as maize (e.g. Hallauer & Miranda, 1985), naturally occurring annual plants *Gilia achilleifolia* (Schoen, 1983), *Chamaecrista fasciculata* (Fenster, 1991b) and *Impatiens capensis* (McCall *et al.*, 1994), hermaphroditic obligate biennials *Sabatia angularis* (Dudash, 1990) and *Hydrophyllum appendiculatum* (Wolfe, 1993), gynodioecious shrubs *Hebe subalpina* (Delph & Lloyd, 1996) and *Schiedea* spp. (Sakai *et al.*, 1989, 1997; Culley *et al.*, 1999), herbaceous perennials *Costus* (Schemske, 1983) and *Lobelia* spp. (Johnston, 1992), ferns (e.g. Kirkpatrick *et al.*, 1990; Soltis & Soltis, 1990, 1992), gymnosperms (e.g. Bush *et al.*, 1987; Williams & Savolainen, 1996; Sorensen, 1999) and flowering trees (e.g. Eldridge & Griffin, 1983; Brown *et al.*, 1985). Investigations of the impact of inbreeding depression in natural animal populations include studies of prairie dogs (Hoogland, 1992), mice (Jiménez *et al.*, 1994) and song sparrows (Keller, 1998), as well as captive populations of various animals (e.g. Ralls & Ballou, 1983; Lacy, 1993a; Lacy *et al.*, 1993). Given its widespread occurrence it is likely that we need to factor inbreeding depression into the management of natural populations.

Plants especially display a range of breeding systems, from selfing to complete outcrossing. We would like to know whether our concerns about inbreeding depression should be applied equally to all taxa. Selfing is the most extreme form of inbreeding since each generation of selfing results in a 50% increase in homozygosity of the progeny or in other words a 50% decrease in heterozygosity each generation (Falconer, 1981). Biparental inbreeding refers to matings between two related individuals. In this situation, increase in homozygosity levels will be slower than from selfing, thus decreasing the immediate potential expression of inbreeding depression. Although the rate of increase in homozygosity levels differs among the

Table 3.1. Expected homozygosity levels for different modes of inbreeding

Generation	Mode of inbreeding		
	Half-sib	Full-sib	Selfing
1	0.125	0.250	0.500
2	0.219	0.375	0.750
3	0.305	0.500	0.875
4	0.381	0.594	0.938
5	0.448	0.672	0.969
10	0.692	0.886	0.992

various mating strategies, ultimately all familial lines could become homozygous to the same degree over time regardless of the mating system (Table 3.1). How rapidly one can generate inbred lines influences the balance between the role of selection in removing deleterious alleles and the random fixation of deleterious alleles. If inbreeding is intense, then the role of drift is predicted to be more important than if inbreeding is weakly enforced across many generations (Lynch, 1988; Ehiobu et al., 1989).

With no gene flow between fragmented populations individuals will eventually become inbred within a population. In animals, biparental inbreeding is the most common form of inbreeding since dioecy is the norm (separate male and female individuals within a population). Some plants also exhibit dioecy where related individuals are mated via a pollen vector (biotic or abiotic) owing to proximity between two individuals. In some hermaphroditic plant species there exist mechanisms that prevent inbreeding which include self-incompatibility systems, and temporal and physical separation of male and female function within a flower (dichogamy and herkogamy, respectively) as well as monoecy where there are separate male and female flowers on the same individual (all above reviewed in Briggs & Walters, 1997). Selfing contributes substantially to mating patterns in plants (Schemske & Lande, 1985) and can occur both within a single hermaphroditic flower or between two flowers on the same individual, i.e. geitonogamy. Some animals also exhibit selfing including freshwater snails *Lymnaea peregra* (Jarne & Delay, 1990) and *Physa heterostropha* (Wethington & Dillon, 1997). Jarne & Charlesworth (1993) recently reviewed the presence of selfing and its potential evolutionary path in both hermaphroditic plants and animals. As we shall see below, the interaction between inbreeding depression and mating system largely depends on the genetic basis of inbreeding depression.

There are two genetic mechanisms thought to be responsible for inbreeding depression; however, they are not mutually exclusive, confound-

ing empirical elucidation of their genetic basis (Charlesworth & Charlesworth, 1987). The first is dominance, in which loss of fitness is due to increased expression of recessive or partially recessive deleterious alleles as homozygosity accumulates (Wright, 1977; Falconer, 1981; also known as partial dominance in Charlesworth & Charlesworth, 1987). The second is overdominance or heterozygote advantage. Under this mechanism, heterozygosity is advantageous *per se*, and inbreeding depression results from a breakdown of this advantage as heterozygosity declines (Wright, 1977; Lande & Schemske, 1985). The genetic basis of inbreeding depression will directly affect the ability to purge the genetic load of a population. Utilising the simplest scenario, the dominance hypothesis of inbreeding depression predicts that the amount of inbreeding depression *decreases* with increasing self-fertilisation in the presence of selection. This occurs because inbreeding increases both homozygosity and the efficiency of selection in removing deleterious recessive alleles from the population. A population's genetic load is expected to be more difficult to purge if overdominance is responsible for the observed inbreeding depression (Lande & Schemske, 1985; Charlesworth *et al.*, 1990), since the most-fit heterozygous genotypes continue to re-generate the less-fit homozygous genotypes. The overdominance hypothesis predicts that the amount of inbreeding depression *increases* with increasing self-fertilisation unless selection on viability against homozygotes is asymmetrical (Charlesworth & Charlesworth, 1987, 1990; Ziehe & Roberds, 1989). Other factors, however, such as epistasis (e.g. Crow & Kimura, 1970; Bulmer, 1985; Lynch, 1991), linkage (or pseudo-overdominance: e.g. Comstock & Robinson, 1952; Wright, 1977 and references therein), selection and drift may all influence the subsequent expression of inbreeding depression and consequently affect the ability of a population to purge its genetic load as well.

Understanding the genetic basis of inbreeding depression is important in predicting the success of a purging programme. The genetic basis of inbreeding depression has historically been examined primarily in crop plants. Evidence exists for dominance-based inbreeding depression in alfalfa (El-Nahrway & Bingham, 1989) and maize (Moll *et al.*, 1965; Hallauer & Miranda, 1985). Overdominance-based inbreeding depression has been suggested from data on orchard grass (Aprion & Zohary, 1961), cherry (Williams & Brown, 1956), barley (Gustafson, 1950) and maize (Hallauer & Miranda, 1985). The relative importance of dominance-vs. overdominance-based inbreeding depression in natural populations is largely unknown. Studies of allozyme variation in pitch pine suggested overdominance as the mechanism to explain fitness differentials between self and outcross

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progeny (Bush et al., 1987). However, a growing number of other studies points to dominance-based inbreeding depression. Indirect evidence of dominance-based inbreeding depression has been found in studies of *Eichhornia paniculata* (Barrett & Charlesworth, 1991). Furthermore, approximate levels of dominance in two largely self-fertilising *Amsinkia* species provide further evidence for the role of deleterious recessive alleles in natural populations (Johnston & Schoen, 1995). Finally, a quantitative genetic study of two *Mimulus* species with contrasting breeding systems primarily supports dominance-based inbreeding depression as well (Dudash & Carr, 1998).

Can genetic load be purged? In other words, can the genetic load responsible for inbreeding depression be purged following either a natural population bottleneck (i.e. dramatic reduction in population size owing to declining habitat and/or fragmentation) or through a controlled inbreeding programme? Examination of population bottlenecks in nature has provided controversial support for reduction of genetic load in Speke's gazelle (Templeton & Read, 1984; but see Lacy, 1997) and the European bison (Simberloff, 1988; Lacy et al., 1993; Ballou, 1995).

To conduct a purging experiment one must simultaneously inbreed to increase levels of homozygosity while selecting for high-performance genotypes across generations. A purging experiment assumes that the genetic mechanism responsible for the reduced fitness is deleterious recessive alleles which in a homozygous state can be eliminated, which has been largely demonstrated (see above). Additionally, epistasis can either enhance ('reinforcing') or inhibit ('diminishing') inbreeding depression as a function of increasing inbreeding coefficients (F) (Wright, 1977) and is documented by the non-linear decline in fitness because the expression of partially deleterious mutations is not independent (Crow & Kimura, 1970). Ideally one would want to compare the performance of elite inbred lines purged of their genetic load with the performance of outcrossed progeny in a natural environment.

Numerous empirical investigations of natural plant populations demonstrate significant variation among maternal lines in expression of inbreeding depression (e.g. Sakai et al., 1989, 1997; Dudash, 1990; Norman et al., 1995; Carr & Dudash, 1997; Dudash et al., 1997; Mutikainen & Delph, 1998; Culley et al., 1999). This information suggests that purging by selecting among maternal lines can be accomplished. However, we are unaware of any ideal purging studies. We can gain some insight from a number of serial inbreeding depression studies that have tried to assess the likelihood of purging in their study systems. At the population level Barrett

& Charlesworth (1991) and McCall *et al.* (1994) suggest that purging has occurred in populations of *Eichhornia paniculata* and *Impatiens capensis*, respectively. For example, individuals from an outcrossing, tristylous population in Brazil of *E. paniculata* were self-pollinated for five generations and then matings were performed between the inbred lines. Barrett & Charlesworth (1991) suggest that purging has occurred owing to an increase in flower production following intercrossing the inbred lines when compared to flower production following random mating (prior to any artificial inbreeding) in the parental population. In contrast, population-level purging was not indicated in other serial inbreeding and outcrossing studies of *Mimulus guttatus* (Carr & Dudash, 1997; Dudash *et al.*, 1997) or *Tribolium* (Pray & Goodnight, 1995). However, in the studies of *M. guttatus* and *Tribolium* cited above purging in some maternal families was demonstrated because self progeny were produced that outperformed the outcrossed progeny from the same female. Other maternal lines exhibited a consistent performance advantage of outcrossed progeny compared to self progeny, while still others revealed no pattern in performance, illustrating the importance of drift, i.e. the random fixation of a trait. Furthermore, a laboratory serial inbreeding depression study (without selection) of three subspecies of *Peromyscus* suggested purging of the genetic load, no purging and an increase in the genetic load for the three different subspecies examined (Lacy & Ballou, 1998). Additionally, Ballou (1997) found little evidence of purging after examining pedigrees of 25 captive populations of mammals. These investigations provide further support for the need to conduct true purging studies as described above.

In an attempt to generate inbred lines and enhance purging of a population's genetic load numerous family lines may be lost (e.g. McCall *et al.*, 1994; Dudash *et al.*, 1997). Losses can range from 30% to 80% of the original families and this is an important issue when one is dealing with a threatened or endangered organism where every individual is valuable. Nonetheless, loss of lines in nature is quite common through death and unequal reproduction among individuals from mammals and birds (e.g. Gompper *et al.*, 1997) as well as plants (e.g. Dudash, 1990, 1991; Dudash & Fenster, 1997), which suggests that purging may not decrease overall population vigour to a great extent. However, the loss of maternal lines increases the likelihood of drift leading to the fixation of slightly deleterious alleles as well as an overall general loss of genetic diversity.

Inbreeding depression can be reduced as the mating system of a population evolves toward selfing (Lande & Schemske, 1985), supporting the efficacy of purging. Thus, if selfing species exhibit reduced inbreeding de-

pression compared to a related outcrossing species this suggests an increased efficiency through inbreeding to reduce the genetic load. Theoretical expectations in favour of dominance/partial dominance predict that inbreeding depression should decrease with increased inbreeding as deleterious recessive alleles are expressed and purged via natural selection (e.g. Charlesworth & Charlesworth, 1987). A review of 54 species of vascular plants by Husband & Schemske (1996) demonstrated a significant reduction in inbreeding depression in selfing species compared to outcrossing species. Their review also revealed that outcrossing species exhibited greater inbreeding depression in early life-history stages while selfing species tended to exhibit greater inbreeding depression in later life-history stages. This trend suggests that inbreeding depression expressed during the early life-history stages is caused by mutations of major effect while inbreeding depression expressed in later life-history traits is caused by mutations of small effect. In addition they found a general lack of correlation in the expression of inbreeding depression between traits thought to be associated with an individual's fitness throughout the life history. This final point also suggests that numerous genes are responsible for inbreeding depression throughout the life history. One study examined critical reproductive traits (pollen and ovule production), and demonstrated that natural selection had been effective in removing the genetic load associated with these fitness traits in the selfing *Mimulus micranthus* when compared to its mixed-mating congener species, *M. guttatus* (Carr & Dudash, 1996). However, given that some traits still exhibit inbreeding depression in selfing species also indicates that selection may be unable to purge the complete genetic load because (1) overdominance may be acting at some loci, (2) mutations introducing new deleterious alleles are fixed by drift and (3) the load of some traits may be polygenic, i.e. composed of alleles at many loci of slight deleterious effect making purging difficult. Additionally, research on *Drosophila* indicates that about one-half of inbreeding depression is due to lethals and the other half of inbreeding depression is due to detrimentals of smaller effect (Simmons & Crow, 1977).

The ability to purge genetic load may also be dependent on ploidy levels (e.g. Ronfort, 1999). This is an important issue in plants since upwards of 50% of all plant species are polyploids (Lewis, 1979; Grant, 1981; Masterson, 1994). Polyploidy is relatively rare in animals, being confined to self-compatible hermaphrodites (e.g. flatworms, earthworms, and freshwater snails) and parthenogens that are capable of producing offspring without fertilisation, (e.g. some species of shrimp, goldfish and salamanders) (Lewis, 1979). Polyploidy, i.e. having more than two sets of homologous

chromosomes, can occur from independent doubling (autopolyploidy) or through matings between two individuals of different species that subsequently results in a doubling of genetic material (allopolyploidy). Allopolyploidy, the predominant mode of polyploidy, results in fixed heterozygosity which in part may explain the success of allopolyploids compared to their diploid ancestors. Fixed heterozygosity implies that allopolyploids will be less affected by inbreeding depression than their diploid ancestors. However, this would seem to be dependent on the age of the polyploid and the rate of accumulation of mutations. Whether allopolyploids actually express less inbreeding depression after long-term inbreeding than their diploid ancestors is an important empirical question. The case is certainly far more complex for autopolyploids which are now recognised to be much more evolutionarily common (Soltis & Soltis, 1989). The assumption of partial dominance/dominance predicts a reduction (or slowing) in the expression of inbreeding depression since homozygosity increases following selfing by only 17%–20% in an autopolyploid compared to 50% in a diploid (e.g. Haldane, 1930; Mayo, 1987). Thus purging may be slower in autopolyploid species compared to diploids. However, this is conditional on the degree to which deleterious mutations are recessive and how closely linked they are to the centromere, and is also related to the interactions among the mutations in the various heterozygous states (fitness of $Aaaa$ vs. $AAaa$ vs. $AAAa$, where a is the deleterious recessive allele) and homozygous ($aaaa$) states (Ronfort, 1999). Overdominance-based inbreeding depression predicts an increase in inbreeding depression in polyploids compared to diploids, owing to a decrease in potential higher-order heterotic allelic interactions following selfing (Bever & Felber, 1992). Empirical data on natural populations of flowering plants are inconclusive. Overdominance-based inbreeding depression is supported by increased expression of inbreeding depression in polyploid populations compared to diploid populations of *Amsinckia* (Johnston & Schoen, 1996). However, dominance-based inbreeding depression is supported by the observation of decreased inbreeding depression in polyploid populations compared to diploid populations of *Epilobium angustifolium* (Husband & Schemske, 1997). Even if we assume that dominance-based inbreeding depression is the basis for inbreeding depression we still have no empirical understanding of the fitness of the various heterozygous classes relative to one another. Thus we can make little prediction as to the success of purging in polyploids at this point. Clearly we need comparative studies that assess the ability to purge the genetic load of related diploid and polyploid congeners.

An alternative approach to purging is to conduct crosses among popula-

tions to combat inbreeding due to fragmentation. However, this latter approach raises the issue of outbreeding depression, which is discussed below.

OUTBREEDING DEPRESSION

Following the definition for inbreeding depression it seems reasonable to define outbreeding depression as the phenomenon where outcrossed offspring have lower relative performance or fitness than the parents (Lynch & Walsh, 1998). The term has been used to reflect the loss of fitness following crosses among individuals within a single population (Parker, 1991), between individuals in nearby and distant populations (Fenster, 1991b; Waser, 1993) and the product of interspecific hybridisation (Orr, 1995). Recall that fitness is a relative measure of performance of genotypes (Crow & Kimura, 1970). In most studies (reviewed in Waser, 1993), the term outbreeding depression is used slightly differently than as defined above with the difference in usage focusing on the word 'relative'. Thus in many studies, outbreeding depression has also been used to describe loss of fitness relative to some optimum crossing distance, which itself may reflect a cross not normally observed in nature, e.g. an interpopulation or higher cross. There is no inherent reason to restrict usage of the term outbreeding depression but none the less it is important to be clear on what is actually being described when measuring outbreeding depression. Using the definition of Lynch & Walsh (1998) above seems most useful for conservation purposes where interest focuses on whether to keep the genotypes of parental populations intact or to introduce hybrids in order to combat the cumulative effects of inbreeding depression (see discussion above).

There are further problems with the Lynch & Walsh (1998) definition of outbreeding depression because outbreeding depression may reflect complex interactions among ecological, evolutionary and genetic processes and the nature of these interactions may change with the scale of cross between the parents. The inherent confusion arising from using the term outbreeding depression is perhaps more clearly illustrated by a more detailed description of how outbreeding depression arises. Firstly, dilution of genes associated with local adaptation may lead to loss of fitness of the hybrid offspring. For example, if each parental population represents an ecotype where a certain number of loci confer local adaptation then where these loci are fixed for alternative alleles between populations, hybrids will have on average only half the genes of either parent. Consequently, hybrids may be less fit than either parent in either parental environment. Secondly, hybrid-

isation may also result in the disruption of coadapted gene complexes (Fenster & Dudash, 1994). Genetic coadaptation reflects epistatic gene action, i.e. the interaction among loci that enhance 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.

The problems associated with using the term outbreeding depression arise when we realise that both hybrid vigour and hybrid breakdown can be simultaneously expressed in hybrid populations, due to the simultaneous masking of recessive deleterious alleles, dilution of genes that confer local adaptation and disruption of coadapted gene interactions (Lynch, 1991). Furthermore, the interaction among these genetic effects is likely to change depending on which particular hybrid generation one is comparing with the parents. Thus we expect the F_1 generation to express the most heterosis, because all individuals are heterozygous at the maximum number of loci. With continued random mating heterozygosity is reduced by one-half (as a consequence of Mendelian $1:2:1$ segregation); thus the expression of heterosis in F_2 and later segregating generations is expected to be one-half that of the F_1. Compounding the loss of heterozygosity in these later segregating hybrid generations is the continued action of recombination which will result in gene combinations among the populations at smaller and smaller intervals of the chromosome. If coadapted gene complexes reflect linked genes, as predicted by theory (reviewed in Fenster *et al.*, 1997), then the loss of fitness due to the disruption of coadapted gene complexes is expected to be greater with each passing generation. Since populations exist on an ecological time-scale and gene flow is often restricted, populations may be inherently limited in their response to selection pressures because what represents an adaptive genotype in any given population may not reflect the testing of all combinations of genes found in a species. Thus by chance we might expect that a small proportion of the gene combinations that arise via interpopulation crosses may actually confer higher fitness than either parent. This latter scenario has been invoked in several recently described cases of recombinational speciation among a number of plant taxa (e.g. Rieseberg, 1997). The spread of these successful hybrid combinations will then depend on how often these chance positive associations arise and the selective advantage they confer (Kruuk *et al.*, 1999).

The environmental context may also increase the complexity of the interpretation of what is and is not outbreeding depression. For example, is it

outbreeding depression if the hybrid performs poorly relative to the home parent, but better than the transplanted parent? The answer depends on the interest of the investigator. For a conservation biologist or land manager interested in maintaining the highest-performing population, lower hybrid performance relative to any one parent would constitute outbreeding depression. Thus the obvious strategy would be to keep population genotypes intact and not allow hybridisation. To summarise, when measuring outbreeding depression, we must be cognisant of the particular hybrid generation we are measuring relative to the parents and in what environment we are making the comparisons.

Mating system and ploidy levels should theoretically have a large influence on the expression of outbreeding depression. As the degree of sexuality decreases either through increasing asexuality or selfing, it is more and more likely that adaptation to very local environmental conditions will occur (e.g. Antonovics & Bradshaw, 1970; Endler, 1977; Schmitt & Gamble, 1990). Thus, gene dilution effects are likely to be greater in more highly selfing organisms following artificial production of hybrids. Furthermore, as discussed above, there is expected to be decreased expression of heterosis because of purging of the genetic load, and the loss of fitness through the disruption of coadapted gene complexes is likely to be greater. Any mating system which effectively reduces recombination will also in turn promote the evolution of coadapted gene complexes (Fenster *et al.*, 1997), thus crosses among populations with reduced tendencies of sex and recombination will be more likely to increase the chances of disrupting coadapted gene complexes. The evolution of gene combinations may also be more important in polyploid organisms (Breese & Mather, 1960; Honne, 1982), because there are more genes and hence a greater opportunity for their interaction to contribute to genetic variation. Thus crosses among polyploid populations may also suffer the increased chance of disrupting coadapted gene complexes. We also expect an optimal outcrossing distance where the maximum amount of heterosis might be conferred on the progeny while minimising dilution of gene effects and disruption of coadapted gene complexes.

For comprehensive reviews on outbreeding depression see Waser (1993), Fenster & Dudash (1994), Frankham (1995*b*), Whitlock *et al.* (1995) and Fenster *et al.* (1997). The loss of fitness of hybrid generations relative to their parents first received prominence with the experiments of the early *Drosophila* geneticists who demonstrated the role of coadaptation in population divergence (Wallace, 1953; Brnic, 1954; Wallace & Vetukhiv, 1955;

Anderson, 1968) (but see McFarquhar & Robertson, 1963). Recent studies using marker-assisted techniques have demonstrated the contribution of coadaptation among genes to the adaptation of experimental populations of cultivated barley (Clegg et al., 1978), to reproductive isolation among sibling species of Drosophila (Palapoli & Wu, 1994), to introgressive hybridisation in Helianthus (Rieseberg et al., 1995), to genetic differentiation among cultivars for traits correlated to yield (Doebley et al., 1995; Lark et al., 1995; Li et al., 1997) and to differences among mouse strains for body weight (Routman & Cheverud, 1997). Furthermore, the role of coadaptation in adaptive evolution has been investigated by quantifying epistasis in laboratory environments which simulate natural conditions and testing the contribution of epistasis to the divergence of adaptive characters. Examples include the evolution of alcohol tolerance in laboratory populations of Drosophila melanogaster (Cavner & Clegg, 1981), population differentiation of photoperiod requirements for diapause in the pitcher-plant mosquito (Hard et al., 1992, 1993; Armbruster et al., 1997), osmoregulation of the tidepool copepod Tigriopus californicus (Burton, 1987, 1990), gill-raker length differences between sympatric species of stickleback (Hatfield, 1997) and others (reviewed in Whitlock et al., 1995; Fenster et al., 1997).

Outbreeding depression can also occur at a very local scale. In some studies loss of fitness occurs in crosses among asexual or inbred lines within the same population (Templeton et al., 1976; Parker, 1991; Deng & Lynch, 1996) and in others (Burton, 1987, 1990) disruption of coadapted gene complexes occurs among populations as close as 10 km apart. In several other studies (notably Price & Waser, 1979; Waser & Price, 1989, 1994) outbreeding depression in the F_1 of crosses among plants only tens of metres apart have been quantified, but in this case breakdown may reflect a loss of fitness relative to other outbred progeny and not necessarily to the original parental population. The breakdown of presumably adaptive characters in the progeny of interpopulation crosses also provides examples of outbreeding depression. For example, between-population crosses result in larger retina size in the blind cave fish (Wilkens 1971), breakdown of pesticide resistance in houseflies (King, 1955) and the recovery of the wild-type breeding system in Eichhornia paniculata (Fenster & Barrett, 1994).

While an impressive number of studies indicates the presence of outbreeding depression as defined in a number of different ways, we still need studies that address the following issues. Can heterosis offset hybrid breakdown? At what spatial scale can crosses be conducted where the beneficial consequences of heterosis are stronger than hybrid breakdown? And what are the relative magnitudes of heterosis and hybrid breakdown when com-

pared to the performance of the original parental populations? These remain important unanswered questions. Thus, 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).

CASE STUDY: SIMULTANEOUS INVESTIGATION OF INBREEDING AND OUTBREEDING DEPRESSION IN *CHAMAECRISTA FASCICULATA*

A long-term project by Fenster and colleagues investigated the questions posed above by examining genetic structure and the role of inbreeding and outbreeding depression in population differentiation of the native North American, highly outcrossing legume, *Chamaecrista fasciculata*. The species is discretely distributed across eastern North America so it represents a model system to examine the consequences of fragmentation in terms of inbreeding and the consequences of crossing among populations to combat inbreeding effects. To maintain brevity we refer the reader to other publications where a detailed description of methods and results and much deeper discussion is presented (Fenster, 1991*a*, *b*, *c*; Fenster & Galloway, in press *a*, *b*, *c*; Galloway & Fenster, 1999, 2000).

Chamaecrista fasciculata's breeding unit (i.e. deme or neighbourhood) is small (radius <5 m) and corresponds to a density of approximately 100 individuals (Fenster, 1991*a*, *b*). Proximity-dependent inbreeding depression (Fenster, 1991*b*), suggests that most mating events within the neighbourhood are between close relatives. Heterosis in the progeny increased rapidly with interparent distance and then plateaued (by 10–40 m), with little increase of fitness following crosses between parents in different neighbourhoods.

To further evaluate how far crosses can be conducted and still obtain heterosis in the offspring, populations were crossed over six spatial distances, ranging from 100 m to 2000 km. For each population pair, three generations of hybrids (F_1, F_2, F_3) were created and within population crosses were used to produce parental seed under greenhouse conditions. The design was replicated across three regions of the United States (Maryland, Illinois and Kansas). Field-study plots were established at each location in each region and hybrid and parental seed for all crosses were planted in them for two years. Each generation and crossing distance included at least 360 planted seeds each year for a total of at least 8000 seeds per site per year. Fitness was estimated as the number of fruit produced per seed planted.

Because of limited genetic effects expressed in Illinois we present the results from only the Maryland and Kansas sites. Progeny from crosses between populations from 100 m to 2000 km apart express F_1 heterosis. Often the F_1 outperformed both parents, even for longest-distance crosses (Fig. 3.1). Since the expression of heterosis in the progeny depends on genetic differentiation between the parents (Falconer, 1981), these results further demonstrate population differentiation in *C. fasciculata*. The spatial pattern of genetic variation, determined by an extensive isozyme survey (Fenster, 1988; Fenster & Dudash, 1994: Fig. 2.3; L. F. Galloway & C. B. Fenster, unpublished data), support this conclusion.

The F_2 generation overall performed intermediately between the parents and F_1, conforming to a model of heterosis but no coadaptation (data not shown). However, the F_3 suffered a drop-off in fitness that reflects both the loss of heterozygosity as well as the disruption of coadapted gene complexes (Fig. 3.2). The F_3 performance was still often equal to those of the parents, suggesting that heterosis outweighs the loss of coadaptation except for the longest-distance crosses. However, at the longer distances, greater than or equal to 1000 km, interpopulation crosses often resulted in F_3 performance less than that of either parent, and indicated hybrid breakdown and true outbreeding depression.

For *C. fasciculata*, it appears that crossing populations originating from distances of up to intermediate length (100 km) has a short-term beneficial effect (owing to proximity-based inbreeding depression) on progeny performance through the F_1, and that longer-term effects are not necessarily disruptive of fitness, at least relative to parental performance for crosses less than or equal to 100 km. Because negative epistasis is mostly observed in the F_3 and less consistently in the F_2 generation, an additional round of recombination appears to be necessary to disrupt putatively linked epistatically interacting genes. These results conform to theoretical predictions that the evolution of interacting gene systems is facilitated where recombination is limited either through restricted gene flow or linkage as discussed above. The results suggest that deleterious alleles are likely to become fixed in populations through drift and that crossing populations results in the recovery of heterosis. The lack of an expected scale effect here may simply indicate that populations of *C. fasciculata* become so differentiated from one another so quickly with distance that we cannot detect increased heterosis with increased crossing distance. It is still unclear what the ultimate performance of the hybrids will be since further recombination may lead to even greater disruption of fitness, while chance might also bring together favourable gene combinations.
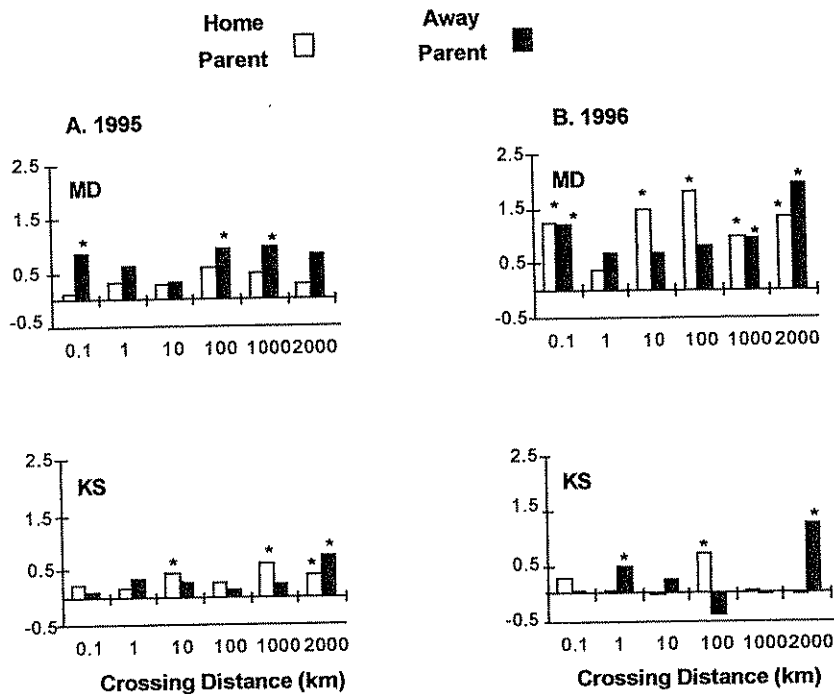
Home Parent ☐   Away Parent ■

A. 1995

B. 1996

Fig. 3.1. Fitness of the F_1 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) in the years 1995 and 1996. Analysis of covariance was used to evaluate the performance of the F_1 relative to the parents (details in Fenster & Galloway, in press, *a*, *b*). We quantified the performance of F_1 hybrids by constructing a priori contrasts of the F_1 with the parents contributing to the cross. The contrasts took the following form: F_1 fitness – Home Parent fitness; F_1 fitness – Away Parent fitness and were conducted for each crossing distance for each site and year. Square-root transformed means adjusted for initial seed size are presented. Comparisons that differ at $P < 0.05$ following sequential Bonferroni correction are indicated by *. Open bar: F_1 fitness – Home Parent fitness; dark bar: F_1 fitness – Away Parent fitness. If bar < 0 (horizontal line), then performance of $F_1 <$ parent. If bar > 0, then performance of $F_1 >$ parent.

RECOMMENDATIONS

In order to determine the consequences of fragmentation on population viability, researchers planning investigations of inbreeding depression need to quantify its effects at as many life-history stages as feasible at both the population and family level, and in the field if possible. Knowledge of
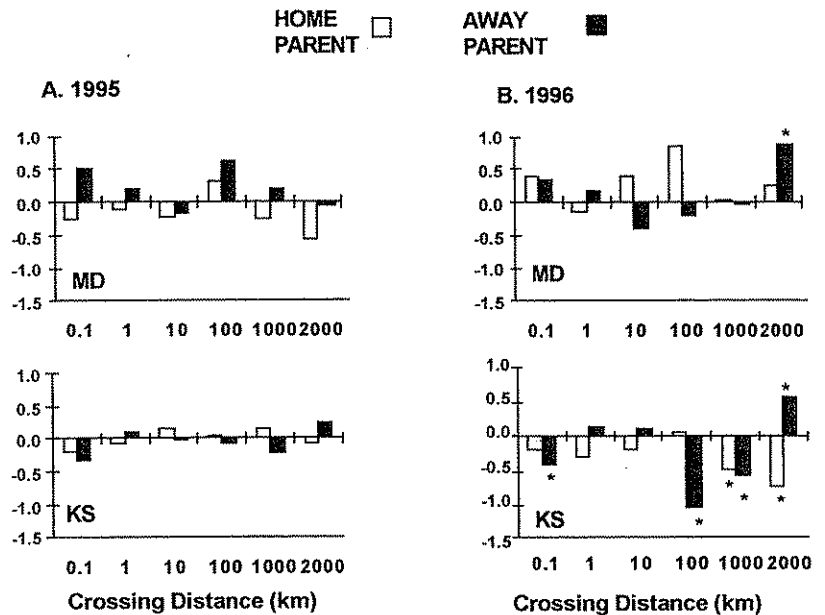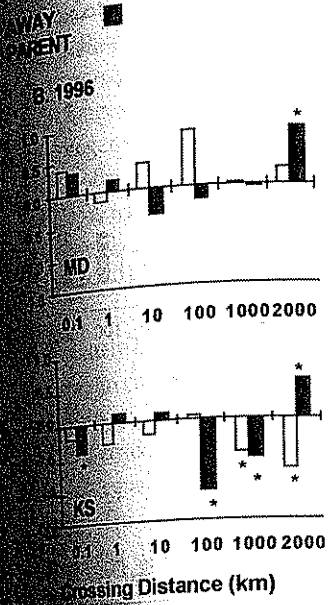
HOME PARENT □   AWAY PARENT ■

A. 1995

B. 1996

Fig. 3.2. Fitness of the F₃ hybrids between populations of *Chamaecrista fasciculata* 0.1–2000 km apart relative to each of the parental populations contributing to the cross. Open bar: F₃ fitness − Home Parent fitness; dark bar: F₃ fitness − Away Parent fitness. If bar < o (horizontal line), then performance of F₃ < parent. If bar > o, then performance of F₃ > parent. See Fig. 3.1 for details.

the mating system, basic reproductive biology and the genetics underlying the expression of inbreeding depression is also desirable. The purging of genetic load through enforced inbreeding and selection of superior genotypes may be successful in some maternal lines or populations but the random fixation of traits whether favourable or not and the loss of maternal lines in the inbreeding process should be weighed carefully in light of any potential benefits. Ideal purging studies are needed to gain insights into artificial breeding programmes and their likelihood of success. Given the lack of detail that we now have on the genetic basis of inbreeding depression, it seems prudent to encourage further research on these questions using model organisms.

Whether one can successfully combine distant gene pools to produce viable persistent populations is still in great need of further empirical work. We would like to see investigations that quantify outbreeding depression in natural study systems which vary in mating system, ploidy level and degree of fragmentation, etc. If possible both inbreeding and outbreeding depress-

ion should be jointly investigated to understand how a fragmented landscape may magnify their effects and influence long-term persistence of the organism or population in question.

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