INVITED REVIEW

Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management

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Abstract

As populations become increasingly fragmented, managers are often faced with the dilemma that intentional hybridization might save a population from inbreeding depression but it might also induce outbreeding depression. While empirical evidence for inbreeding depression is vastly greater than that for outbreeding depression, the available data suggest that risks of outbreeding, particularly in the second generation, are on par with the risks of inbreeding. Predicting the relative risks in any particular situation is complicated by variation among taxa, characters being measured, level of divergence between hybridizing populations, mating history, environmental conditions and the potential for inbreeding and outbreeding effects to be occurring simultaneously. Further work on consequences of interpopulation hybridization is sorely needed with particular emphasis on the taxonomic scope, the duration of fitness problems and the joint effects of inbreeding and outbreeding. Meanwhile, managers can minimize the risks of both inbreeding and outbreeding by using intentional hybridization only for populations clearly suffering from inbreeding depression, maximizing the genetic and adaptive similarity between populations, and testing the effects of hybridization for at least two generations whenever possible.

Keywords: fitness, hybridization, inbreeding depression, outbreeding depression

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Introduction

Anthropogenic assaults on the environment are causing both wild and captive populations to become increasingly fragmented and vulnerable to inbreeding depression and loss of evolutionary potential. A possible solution to this problem is to infuse the ailing population with individuals from a genetically and demographically healthy population. Several recent studies have shown that inbred populations can be 'rescued' by the introduction of surprisingly small numbers of migrants (reviewed in Tallmon *et al.* 2005). For example, experimental introduction of immigrants into a metapopulation of the water flea *Daphnia magna* resulted in lineages that were over 35 times more fit than the resident lineages (Ebert *et al.* 2002). Natural populations have reportedly experienced similarly impressive rescues. For example, the introduction of a single immigrant is credited with reviving the growth of a dwindling population of the Scandinavian wolf *Canis lupus* (Vila *et al.* 2002; Ingvarsson 2003). Similarly, the introduction of migrants into a remnant population of greater prairie chickens (*Tympanuchus cupido*) is reported to have restored fertility, hatching rates and genetic diversity (Westemeier *et al.* 1998). The burgeoning hybrid zone literature also demonstrates that both intraspecific and interspecific hybrids can have fitness exceeding their parents in certain environments (e.g. Burke & Arnold 2001; Campbell 2004).

Enthusiasm for the benefits of intentional hybridization is tempered by the possibility that population mixing may induce outbreeding depression. That is, interpopulation hybrids could suffer a loss in fitness due either to the

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disruption of intrinsic interactions between genes, or disruption of extrinsic interactions between genes and environment. The costs and benefits of intraspecific hybridization is therefore a major concern for captive breeding programmes, artificial stocking programmes and translocation/reintroduction programmes. Managers are faced with very difficult decisions: promote hybridization to save populations from inbreeding depression, a phenomenon for which there is nearly universal evidence; or avoid hybridization for fear of inducing outbreeding depression, a phenomenon for which there is very scarce evidence.

Here I review available information on the relative risks of inbreeding and outbreeding, discuss reasons why the risks may be difficult to predict, and assess particular areas where more information is needed. The focus is on the fitness effects associated with genetic changes caused by inbreeding with close relatives and outbreeding between populations. While interspecific hybridization is also a significant conservation issue (e.g. Allendorf *et al.* 2001), it is rarely carried out intentionally. I also do not cover the many nongenetic risks of population mixing (introduction of parasites or disease, disruption of social interactions and so on), or the complex issue of preserving historical integrity. Instead, I focus specifically on the relative dangers of inbreeding depression and outbreeding depression.

Genetic basis of inbreeding and outbreeding effects

Inbreeding effects

Inbreeding depression, the reduction of fitness caused by mating between relatives, has been recognized at least since Darwin (1876). While inbreeding does not alter allele frequencies, it does redistribute genotype frequencies leading to an increase in homozygosity. The two hypotheses for the genetic basis of inbreeding depression both focus on this increase in homozygosity (Lynch & Walsh 1998). According to the overdominance hypothesis, inbreeding depression is attributed to the superiority of heterozygotes over both homozygotes. Alternatively, the dominance hypothesis posits that inbreeding depression is caused by the expression of deleterious recessives in the homozygote state. Cleanly distinguishing between these two hypotheses turns out to be extraordinarily difficult because linked sets of recessive alleles in repulsion mimic overdominance (i.e. 'associative overdominance', Keller & Waller 2002).

These two competing hypotheses for the genetic basis of inbreeding depression have very different implications for conservation and management (Lynch & Walsh 1998). Under the overdominance hypothesis, all inbred lines will eventually be inferior to the randomly mating base population due to the loss of heterozygotes. Under the dominance hypothesis, it should be possible to produce a pure inbred line equivalent to the most fit member of the base population, at least until the inbred line accrues deleterious mutations.

While the debate over the two hypotheses continues, current thought tends to favour the dominance hypothesis (e.g. Keller & Waller 2002). However, evidence suggests that overdominance may still be important under certain conditions (Charlesworth & Charlesworth 1999; Li *et al.* 2001). Even if overdominance is rare it may make a considerable contribution to inbreeding depression because the fitness effects of only a few overdominant loci can exceed the effects of a much larger number of partially dominant loci (Lynch & Walsh 1998). In addition to overdominance and dominance, inbreeding depression may be influenced by epistasis, as evidenced by nonlinear relationships between phenotype and inbreeding coefficients (Lynch & Walsh 1998).

Outbreeding effects

Like the detrimental effects of inbreeding, the beneficial effects of crossbreeding have also been known for centuries (e.g. Darwin 1876). Interpopulation hybridization sometimes results in an increase in fitness termed heterosis or hybrid vigour. This is the inverse of inbreeding depression and is generally attributed to overdominance or the masking of deleterious recessives, although epistasis can also be involved (Lynch 1991).

However in some cases, interpopulation crosses cause reduced fitness in first generation (F_1) hybrids that possess a complete haploid set of each parental genome. F_1 fitness reductions can be attributed to the disruption of local adaptation (i.e. 'extrinsic isolation'), underdominance, or epistatic interactions (heterozygote–heterozygote interactions or interactions involving sex chromosomes). Often, fitness reductions are delayed until the F_2 or later generations when deleterious interactions between homozygous loci become exposed. It has become widely accepted that the evolution of epistatic incompatibility is explained by the model proposed by Bateson (1909), Dobzhansky (1937) and Muller (1940). The so-called Dobzhansky–Muller model explains the observation that isolated populations gradually accumulate neutral or advantageous mutations over time. Furthermore, selection for positive epistasis may result in the development of unique co-adapted gene complexes within each isolated population (Whitlock *et al.* 1995; Fenster *et al.* 1997). When mating occurs between populations, segregation and recombination can break-up these co-adapted gene complexes and bring together mutations that have not been 'tested' together and potentially have harmful effects (Orr 1996; Turelli *et al.* 2001).

Box 1 Measuring the cost of inbreeding

Controlled crosses

The cost of inbreeding is typically measured by comparing the mean phenotype of progeny from outbred or random mating ($X_{CONTROL}$) to those from a specific class of inbred mating (X_{INBRED}). This is typically expressed as the coefficient of inbreeding depression δ (Lande & Schemske 1985), where $δ = 1 − (X_{INBRED}/X_{CONTROL})$. Inbreeding effects in Table 2 are instead calculated as $(X_{INBRED}/X_{CONTROL}) − 1$, so that deleterious effects of inbreeding will be negative. Similarly, traits such as mortality, which are inversely related to fitness, were changed to survivorship so depressed fitness yields a negative value.

The magnitude of inbreeding effects will of course depend on the inbreeding coefficient, *F*. If mutations at different loci have independent effects, the logarithm of overall fitness is expected to decline linearly with *F* (Keller & Waller 2002). The slope of this line (−*B*) serves as an estimate of inbreeding load, typically measured in terms of the number of lethal equivalents (a group of alleles which would be lethal if made fully homozygous). Inbreeding effects are related to the inbreeding coefficient by the equation: $δ = 1 − e^{BF}$. A standardized cost of inbreeding could thus theoretically be obtained by adjusting for a given level of inbreeding (e.g. Crnokrak & Roff 1999). However, this relies on a linear relationship between inbreeding costs and inbreeding coefficient, an assumption that is sometimes violated (Lynch & Walsh 1998; Wang *et al*. 2002).

Genetic estimates from natural populations

Levels of inbreeding and inbreeding depression can also be estimated using molecular markers (reviewed in Keller & Waller 2002). While this is generally not as accurate as measuring fitness in controlled crosses, it has the distinct advantage of being applicable to natural populations. For example, inbreeding depression can be estimated from multilocus heterozygosity in those organisms (particularly bivalves) where heterozygosity correlates with individual fitness (Mitton 1997). A related method specifically for microsatellite data relies on mean d^2, a measure of the genetic distance between parental gametes. Mean d^2 assumes a stepwise mutation model and is calculated as the squared difference in microsatellite repeat units between alleles averaged across all loci. However, studies suggest fitness is better correlated with microsatellite heterozygosity than with mean d^2 in most situations (Coltman & Slate 2003). A third method uses shifts in the inbreeding coefficient *F*, to infer selection against inbred individuals (Ritland 1990). Similarly, pairwise estimates of relatedness (through bandsharing for example) can be used to estimate inbreeding, and the correlation between fitness and relatedness can then be used to infer inbreeding depression (e.g. Amos *et al*. 2001). Large sample sizes and marker numbers (ideally codominant markers) are critical for all of these methods for estimating inbreeding and inbreeding effects (e.g. Balloux *et al*. 2004). With the current profusion and high throughput scoring of such markers this is likely to become less of a limitation than it has been in the past.

Empirical evidence

Inbreeding depression (Box 1)

The evidence for inbreeding depression is overwhelming and has been reviewed extensively (e.g. Ralls *et al*. 1988; Crnokrak & Roff 1999; Keller & Waller 2002). While there is considerable variation in its expression among taxa and traits, very few organisms are spared from its effects. In an early compilation of inbreeding effects, Ralls *et al*. (1988) assessed 40 captive mammal populations and found that full sibling or parent–offspring mating (*F* = 0.25) reduced juvenile survivorship by an average of 33%. For plants, extreme inbreeding in the form of self-fertilization (*F* = 0.50) caused mean cumulative fitness to decline by 23% in predominantly selfing species and 53% in predominantly outcrossing species (Husband & Schemske 1996).

Despite the undeniable evidence for inbreeding depression in captive or domestic populations, there has been

considerable debate over its importance in wild populations (reviewed in Frankham 1995; Pusey & Wolf 1996). This skepticism stems from three main arguments. First, both animals and plants are known to have a variety of mechanisms for avoiding inbreeding. Second, even if inbreeding does occur, wild species are able to limit the phenotypic effects through behavioural mechanisms while captive species are not free to exercise the same strategies. Third, populations that have experienced bottlenecks or have a history of inbreeding should purge themselves of some portion of their load of deleterious recessive alleles. Recent reviews of the effects of inbreeding in wild populations (Crnokrak & Roff 1999; Keller & Waller 2002), suggest that the skepticism is unfounded and that inbreeding depression in the wild may be at least as great as in captivity. This suggests that many populations are not able to avoid inbreeding or limit its phenotypic effects, and that purging may be inefficient (discussed below).

Box 2 Measuring the cost of outbreeding

Controlled crosses

Most studies of outbreeding effects depend on controlled crosses, most often conducted in the laboratory or greenhouse. Studies are too few to have converged on a standard definition of outbreeding depression. Some define outbreeding depression as a significant decline in hybrid fitness relative to *either* parent (Sagvik et al. 2005). This can result from purely additive gene action and is a relatively minor problem from a conservation perspective as long as robust populations are used for supplementation. In cases where disruption of local adaptation is tested *in situ*, it may be most appropriate to compare hybrid fitness to that of the home parent. Alternatively, second generation hybrid breakdown due specifically to deleterious epistasis can be defined as a decline in fitness below [(midparent + F_1)/2] (Fenster & Galloway 2000a; Marr et al. 2002; Erickson & Fenster 2006). Most often, outbreeding depression is defined as a reduction in fitness below the midparent. This is the metric used in Table 2 whenever data for both parents are available. As with inbreeding,

effects of outbreeding in Table 2 are calculated as $(X_{OUTBRED}/X_{CONTROL}) - 1$, so that deleterious effects of outbreeding will be negative. It is important that there be temporal controls. That is, F_2 fitness should be compared to parental fitness in the second generation. Or with plants, seeds from multiple generations can be grown simultaneously.

Genetic estimates from natural populations

Levels of outbreeding and outbreeding depression in natural populations can be estimated using some of the same molecular methods used for inbreeding (Box 1). While multilocus heterozygosity may be a more sensitive measure of recent inbreeding (Coltman & Slate 2003), mean d^2 may be a more appropriate measure of outbreeding (Neff 2004b). Using these two metrics, Marshall & Spalton (2000) inferred that simultaneous inbreeding and outbreeding depression have approximately equivalent effects on juvenile survival in Arabian oryx (*Oryx leucoryx*). LeBas (2002) used the same two metrics to conclude that survivorship in ornate dragon lizards (*Ctenophorus ornatus*) was impacted by outbreeding depression but not by inbreeding depression.

Outbreeding depression (Box 2)

Outbreeding depression has received comparatively little attention. An ISI Web of Science literature search on the term 'inbreeding depression' received 2142 hits, while a search on 'outbreeding depression' received only 174 hits. The paucity of reports on outbreeding depression may be due to our limited knowledge of the appropriate spatial scale, our failure to measure fitness over the whole life cycle, and especially to the rarity of hybridization studies that extend beyond the first generation.

Despite the relative scarcity of studies specifically focusing on outbreeding depression, the phenomenon has been recognized in a diversity of plants, invertebrates and vertebrates. Table 1 gives a series of examples, including many that were first summarized by Endler (1977). Some of these cases are extreme. Crossing over a distance of only 30 m in the perennial plant *Delphinium nelsonii* is reported to have caused a 48% reduction in F_1 body size (Waser & Price 1994). Crossing over a genetic distance of only G_{ST} = 0.05 in largemouth bass (*Micropterus salmoides*) caused F_2 viral resistance to drop by 58% (Goldberg et al. 2005). Some of the known cases of outbreeding depression appear driven by disrupted interactions between genes and environment (e.g. Greig 1979; Waser & Price 1994), others are largely attributable to disrupted epistatic interactions

(e.g. Edmands 1999; Gharrett et al. 1999; Edmands & Deimler 2004; Galloway & Etterson 2005), and for many other cases the underlying mechanism is unknown. While the pattern of fitness problems being delayed until the second generation has been known and understood since at least the 1950s, studies of the F_2 and later generations have remained rare. In at least one known case, outbreeding depression was delayed until the F_3 where it was attributed to the additional generation of recombination (Fenster & Galloway 2000a, b).

Relative risks of intentional hybridization

While inbreeding depression is often thought to be more severe than outbreeding depression (e.g. Lacy et al. 1993; Sheffer et al. 1999) the data on relative effects are few. Table 2 includes examples of species where both inbreeding and outbreeding effects have been quantified and are accessible in the literature. Importantly, inbreeding and outbreeding effects were frequently assayed in different studies and under different conditions. Inbreeding effects listed are for mating between close relatives, with a mean inbreeding coefficient (*F*) of 0.311 (SE 0.028). For these examples the mean cost of outbreeding in the first generation (−0.090, SE 0.047) is somewhat lower than the mean cost for the second generation (−0.125, SE 0.045),

Table 1 Intraspecific crosses resulting in outbreeding depression (OBD)

Species	Character(s)	1st hybrid generation showing OBD	Reference(s)
Plants			
Anchusa crispa	fecundity	1	Quilichini *et al*. (2001)
Calylophus serrulatus	body size, fecundity	1	Heiser & Shaw (2006)
Campanula americana	development time, fecundity, fertility, body size, viability	1	Galloway & Etterson (2005)
Chamaecrista fasciculata	cumulative fitness	3	Fenster & Galloway (2000a, b)
Delphinium nelsonii	body size, fertility, lifespan, population growth, viability	1	Waser & Price (1991); Waser & Price (1994)
Gentianella germanica	germination rate, survival	1	Fischer & Matthies (1997)
Ipomopsis aggregata	fertility	1	Waser & Price (1989)
Lotus scoparius	cumulative fitness	1	Montalvo & Ellstrand (2001)
Mimulus guttatus	fertility	1	Lindsay & Vickery (1967)
Steptanthus glandulosus	fertility	1	Kruckeberg (1957)
Invertebrates			
Boloria toddi	fertility	2	Oliver (1972)
	viability	1	Oliver (1972)
Botryllus schlosseri	fertility, viability	1	Grosberg (1987)
Cisseps fulvicollis	fertility, viability	1	Oliver (1972)
Drosophila melanogaster	viability	2	Wallace (1955)
Drosophila paulistorum	fecundity, viability	2	Vetukhiv (1954); Wallace & Vetukhiv (1955)
Drosophila pavani	viability	2	Brncic (1961)
Drosophila persimilis	viability	2	Spiess (1959)
Drosophila prosaltans	viability	2	Dobzhansky *et al*. (1959)
Drosophila pseudoobscura	longevity, fecundity, viability	2	Vetukhiv (1954), (1956), (1957); Wallace & Vetukhiv (1955); Vetukhiv & Beardmore (1959)
Drosophila willistoni	fecundity, viability	2	Vetukhiv (1954); Wallace & Vetukhiv (1955)
Echinostoma caproni	fecundity	2	Trouvé *et al*. (1998)
Eurytemora affinis	development time, viability	1	Lee (2000)
Hyperia postica	fertility, viability	1	Blickenstaff (1965)
	fecundity	2	Blickenstaff (1965)
Nuculaspis californica	viability	1	Alstad & Edmunds (1983)
Phyciodes tharos	fertility, viability	1	Oliver (1972)
Scottolana canadensis	population growth, fertility	1	Lonsdale *et al*. (1988)
Tigriopus californicus	fecundity, fertility	1	Brown (1991); Ganz & Burton (1995)
	development time, viability	2	Burton (1990); Edmands (1999)
Vertebrates			
Callimico goeldii	viability	2	Lacy *et al*. (1993)
Capra ibex	viability	2	Turcek (1951); Greig (1979)
Oncorhynchus gorbuscha	return rate	1	Gharrett *et al*. (1999); Gilk *et al*. (2004)
Lepomis macrochirus	reproductive success	1	Neff (2004a)
Melospiza melodia	lifetime reproductive success, viability	2	Marr *et al*. (2002)
Micropterus salmoides	viral resistance	2	Goldberg *et al*. (2005)
Rana pipiens	development time, fertility, viability	1	Moore (1946); Ruibal (1955); Sasa *et al*. (1998)
Triturus cristatus	fertility	1	Callan & Spurway (1951); Spurway (1953)
	viability	2	Callan & Spurway (1951); Spurway (1953)

Table 2 Effects of inbreeding between relatives vs. outbreeding between populations. Effects calculated as [(inbred or outbred character value/control character value)-1]. See Boxes 1 and 2 for methods for measuring breeding effects. —, same as above

Species	Character	F	Inbreeding effect	Outbreeding effect in F_1	Outbreeding effect in F_2 or backcross	Distance between populations	Inbreeding references	Outbreeding references
Plants								
Arnica montana	seed production	0.50	− 0.597	0.493			Luijten *et al.* (2002)	Luijten *et al.* (2002)
—	seed set	—	− 0.625	0.136			—	—
Delphinium nelsonii	population growth rate	0.06	− 0.286	− 0.810		30 m	Waser & Price (1994)	Waser & Price (1994)
—	lifespan	—	− 0.117	− 0.175		—	—	—
—	body size	—	− 0.365	− 0.476		—	—	—
Gentianella germanica	germination	0.50	− 0.314	− 0.366		25 km	Fischer & Matthies (1997)	Fischer & Matthies (1997)
—	early growth	—	− 0.306	− 0.123		—	—	—
—	survival	—	− 0.538	− 0.579		—	—	—
Ipomopsis aggregata	seed set	—	− 0.941	− 0.110		100 m	Waser & Price (1989)	Waser & Price (1989)
—	population growth rate	—	− 0.530	− 0.324		—	—	—
Invertebrates								
Boloria selene	egg fertility	0.25	− 0.115	− 0.085	− 0.330	2735 km	Oliver (1972)	Oliver (1972)
—	embryo viability	—	− 0.058	− 0.058	− 0.249	2735 km	—	—
Boloria toddi	egg fertility	—	− 0.082	0.009	− 0.170	~800 km	—	—
—	embryo viability	—	− 0.138	− 0.025	− 0.058	~800 km	—	—
Cisseps fulvicollis	egg fertility	—	− 0.020	− 0.096	0.081	1450 km	—	—
—	embryo viability	—	− 0.349	− 0.096	0.153	1450 km	—	—
Phyciodes tharos	egg fertility	—	− 0.262	− 0.022	− 0.152	724 km	—	—
—	embryo viability	—	− 0.166	− 0.012	− 0.467	724 km	—	—
Tigriopus californicus	hatching	—	− 0.026	0.038	− 0.224	$G_{ST} = 0.754$	Palmer & Edmands (2000)	Edmands *et al.* (2005), Edmands & Harrison 2003, unpub. data
—	survivorship	—	− 0.261	0.005	− 0.304	—	—	—
—	metamor-phosis	—	− 0.252	0.006	− 0.228	—	—	—
Xylosandrus germanus	hatching	0.50	0.124	− 0.001			Peer & Taborsky (2005)	Peer & Taborsky (2005)
—	larval survival	—	− 0.167	− 0.125			—	—
—	pupation rate	—	− 0.488	0.312			—	—
Vertebrates								
Callimico goeldii	viability	0.25	− 0.795		− 0.216		Lacy *et al.* (1993)	Lacy *et al.* (1993)
Oncorhynchus kisutch	weight	0.265	− 0.250	− 0.033	0.135	$G_{ST} = 0.043$	Myers *et al.* 2001	McClelland *et al.* (2005); Teel *et al.* (2003)
Poeciliopsis occidentalis	survival	0.125 to 0.25	0.000	− 0.042	− 0.022	$G_{ST} = 0.223$ to 0.712	Sheffer *et al.* (1999), Parker *et al.* (1999)	Sheffer *et al.* (1999), Parker *et al.* (1999)
—	female body size	—	0.029	0.018	0.054	—	—	—
—	male body size	—	− 0.030	0.034	− 0.006	—	—	—

which is in turn approximately half the cost of inbreeding (-0.273; SE 0.047). Using paired comparisons, first-generation hybridization effects are significantly less severe than both second-generation hybridization effects (paired t value = 1.841, one-tailed P = 0.043) and inbreeding effects (paired t value = 2.583, two-tailed P = 0.016). However, second generation outbreeding effects are not distinguishable from inbreeding effects (paired t value = 0.766, two-tailed P = 0.455). Thus, this small data set shows a tendency for inbreeding risks to exceed outbreeding risks, yet the difference is not significant for later-generation hybrids. Given that these data are for extreme levels of inbreeding beyond that typically expected in natural populations, this at the very least argues that managers should give serious consideration to the potential risks of outbreeding.

Factors affecting relative risks

Relative risks of inbreeding and outbreeding will of course be situation-specific. Effects of population mixing are likely to vary widely depending on taxa, characters being measured, level of divergence between hybridizing populations, mating history, environmental conditions and the potential for inbreeding and outbreeding effects to be occurring simultaneously.

Taxa

There are a few known taxonomic differences in the severity of inbreeding depression. In plants, highly self-fertilizing species are expected to have reduced inbreeding costs due to selection against deleterious recessive alleles brought together in homozygotes. This pattern has been confirmed by comparative studies (Husband & Schemske 1996) as well as experimental studies (Crnokrak & Barrett 2002). Species experiencing frequent bottlenecks may also reduce segregational load as alleles go to fixation. Evidence for this is equivocal. Repeated founder events may explain the lack of detectable inbreeding depression in cases such as the endangered Gila topminnow, *Poeciliopsis occidentalis occidentalis* (Sheffer *et al*. 1999). However, endangered mammal taxa presumably impacted by bottlenecks showed levels of inbreeding depression similar to more abundant species (Ralls *et al*. 1988; Lacy *et al*. 1996). In addition to variation among species, genetic load can vary substantially among populations. Lacy *et al*. (1996) found substantial variation in sensitivity to inbreeding among populations of the beach mouse *Peromyscus polionotus*, a pattern they attributed to the action of few genes of large effect.

There are also a number of specific genetic systems that alter the effects of inbreeding. For example, a variety of plants (Heiser & Shaw 2006) and animals (White 1973) persist as permanent translocation heterozygotes. In the case of the yellow primrose *Calylophus serrulatus* this results in significant F_1 outbreeding depression but no inbreeding depression because the species is protected from loss of heterozygosity (Heiser & Shaw 2006). Haplodiploids are also expected to be relatively immune from inbreeding depression due to rapid purging of deleterious recessives in haploid males (Werren 1993; Henter 2003; Peer & Taborsky 2005; but see Zayed & Packer 2005).

Little is known about species-specific differences in the consequences of outbreeding. Reports of first generation outbreeding depression appear to be more common in plants than in animals (Table 1). It is not clear if this is due to ascertainment bias or if it is an authentic pattern, perhaps due to higher levels of population subdivision and habitat heterogeneity in plants.

Characters measured

The impacts of inbreeding and outbreeding also vary among characters. Life history traits in animals have been found to be over fivefold more sensitive to inbreeding than morphological traits (DeRose & Roff 1999), a pattern originally noted by Falconer (1989). Explanations for this pattern involve the contributions of dominance and directional dominance. That is, while mutations affecting fitness-related traits are typically deleterious and recessive resulting in directional dominance, this may not be true of mutations affecting morphology. Within life history traits, inbreeding had a more detrimental effect on survival than on adult body size (DeRose & Roff 1999).

For both inbreeding and outbreeding it is important to study the entire life cycle. Self-fertilizing species tend to exhibit a large fraction of their inbreeding depression late in the life cycle (Husband & Schemske 1996). One explanation for this pattern is that early-acting inbreeding depression is due to lethal recessives that get purged by selfing, while late-acting fitness problems are driven by more weakly deleterious mutations. Waser & Price (1994) point out that outbreeding depression may also be expressed late in the life cycle, as their study of the perennial plant *Delphinium nelsonii* shows differences in size and survival that were not apparent until 5 years after seed planting. Detrimental effects of hybridization early in the life cycle may be particularly prone to masking by maternal effects. This is especially true for F_2 effects when F_1 dams are heterotic (e.g. Tave *et al*. 1990). Conversely, some studies show stronger outbreeding depression early in the life cycle. In the herbaceous plant Campanula americana F_1 fitness problems were almost entirely restricted to juvenile traits (Galloway & Etterson 2005). A similar pattern in haplodiploid ambrosia (*Xylosandrus germanus*) beetles was attributed to disruption of maternal–offspring co-

adaptation (Peer & Taborsky 2005). Regardless of the ontogenetic timing of inbreeding and outbreeding effects, it is important to caution against reliance on a single fitness component. Leberg (1993) makes this point based on his study of mosquitofish (*Gambusia holbrooki*) where substantial heterotic effects in brood size did not translate into detectable differences in population size or growth rate.

Population divergence

There is little doubt that increased divergence between populations tends to reduce hybrid fitness. This has been shown for both intraspecific and interspecific comparisons within a wide range of taxa (reviewed in Edmands 2002; Coyne & Orr 2004; Mendelson *et al*. 2004). The trouble is that this 'incompatibility clock' ticks at wildly different rates in different groups. For example, pink salmon populations separated by only $G_{ST} = 0.02$ (Beacham *et al*. 1988) exhibit substantial F_2 fitness problems (Gharrett *et al*. 1999) while Gila topminnow populations that are 10 times more divergent ($G_{ST} = 0.223$ Parker *et al*. 1999) exhibit no significant problems in the F_2 (Sheffer *et al*. 1999). This variation is typical and makes it impossible to define a level of divergence that is 'safe' for interpopulation hybridization.

Part of the discrepancy is due to the fact that molecules evolve at very different rates in different taxa. A given level of sequence divergence, even for the same gene, does not imply the same level of evolutionary divergence in frogs and birds, for example. This problem might be reduced by converting molecular metrics into time estimates. However, even with these corrections we are left with some glaring incongruities. For example, broad surveys reveal that birds typically lose reproductive compatibility after only 8 million years (Myr) of divergence, while amphibians maintain the capacity for hybridization for 55–60 Myr (Prager & Wilson 1975; Zeh & Zeh 2000). Considerable variation can be found even within closely related taxa. For example, some pairs of *Drosophila* species suffer F_2 fitness reductions after ~0.35 Myr of divergence (Coyne & Orr 1989), while other species pairs that have been isolated for approximately 10 times as long (3–4.3 Myr; Kelemen & Moritz 1999) exhibit F_2 heterosis (Hercus & Hoffmann 1999). Certainly these time estimates could be refined through better molecular metrics (e.g. Archibald *et al*. 2005) and more nuanced corrections for rate heterogeneity (e.g. Bolnick & Near 2005) but this is unlikely to eliminate the huge variation in the rate at which hybrid incompatibilities evolve.

So why is the incompatibility clock so inconstant? Part of the variation comes from differential vulnerability to hybridization among broad taxonomic groups. Hybrid incompatibility is generally found to evolve quickly in mammals and frogs, more slowly in birds, and perhaps even more slowly in plants (Lijtmaer *et al*. 2003; Coyne & Orr 2004). The rapid accumulation of incompatibility in mammals may be related to more stringent regulatory controls (Prager & Wilson 1975) or, alternatively, to accelerated evolution of mother–offspring conflicts in viviparous organisms relative to egg-laying organisms (Zeh & Zeh 2000). Another factor causing variation in the accumulation of hybrid incompatibility may be sex chromosome differentiation. According to Haldane's Rule, sterility and inviability tend to evolve first in the heterogametic sex. While there is much debate about the exact mechanisms driving this pattern, most explanations involve incompatibilities between autosomal genes and recessive alleles exposed on the hemizygous sex chromosome. It follows that genic incompatibilities should evolve most rapidly in species with a large X chromosome and a highly degenerate Y chromosome (Rieseberg 2001; Coyne & Orr 2004). This may explain why species like *Tigriopus californicus*, which lacks degenerate sex chromosomes, may show only moderate levels of outbreeding depression at divergence levels vastly exceeding those ordinarily found between conspecific populations (Edmands 1999).

Mating history

A prior history of inbreeding is expected to reduce the risk of inbreeding depression by exposing deleterious recessives to selection. As discussed above, this has been established for self-fertilizing species (Husband & Schemske 1996; Crnokrak & Barrett 2002) and to a lesser extent for small and bottlenecked populations (reviewed in Keller & Waller 2002). The efficiency of purging is likely to depend on a number of genetic and demographic factors (Keller & Waller 2002). First, deleterious mutational effects must be high relative to effective population size (s must be greater than $1/2N_e$). Second, selective interference among loci must be minimal so that strong selection at one locus does not disrupt selection against more mildly deleterious alleles at linked loci. Third, inbreeding must occur gradually over successive generations so that the strength of selection is not debilitated by reduced effective population size. And finally, effective dispersal must be sufficiently low that immigrants do not re-introduce deleterious alleles to locally purged populations. Empirical evidence for purging is equivocal. A study of 25 captive mammalian populations (Ballou 1987) showed that a prior history of inbreeding had a slight effect on neonatal survival but concluded that effects were not sufficiently strong to provide a practical strategy for eliminating inbreeding depression. Similarly, a meta-analysis of results from 45 studies of plant populations showed no overall evidence of purging, prompting the conclusion that deliberate attempts to purge genetic load may be misguided (Byers & Waller 1999).

Mating history is also a significant issue for expected fitness consequences of outbreeding. It is well known that fitness effects can change between generations, with problems often being delayed until the second generation (see Table 2). For conservation purposes we really need to know how hybridization affects subsequent generations and yet very little is known about what happens beyond the F_2 or first backcross generations. Fitness could continue to decline as successive bouts of recombination further disrupt beneficial epistatic interactions. This is the proposed explanation for hybrid breakdown being delayed until the F_3 in *Chamaecrista fasciculata* (Fenster & Galloway 2000a, b), although it is possible that fitness problems began in the F_2 but were obscured by maternal heterosis in the F_1. Alternatively, fitness could increase in later generation hybrids as selection promotes the rare beneficial gene combinations created by recombination. Indeed, there are several reports of rapid recovery from severe fitness problems in hybrid sunflowers (Heiser 1947; Rieseberg *et al.* 1996; Carney *et al.* 2000). Similarly, crosses between highly divergent copepod populations show evidence of recovery from outbreeding depression within a maximum of 15 generations (Edmands *et al.* 2005). A third example comes from a study of hybridization between locally adapted legume populations in which fitness appeared to have recovered within six generations (Erickson & Fenster 2006). Studies such as these raise the hope that outbreeding depression might be cured in some situations if hybridizing populations can survive the initial phase of fitness problems.

Environmental conditions

It is long been assumed that deleterious effects of inbreeding are aggravated by stressful conditions. A review of 34 studies of a range of taxa by Armbruster & Reed (2005) confirms this belief, showing an approximately 69% increase in inbreeding depression in stressful vs. benign environments. While the precise environmental triggers are unknown, more stressful conditions are the likely mechanism behind the observed pattern of higher inbreeding depression in wild populations than captive populations (Crnokrak & Roff 1999).

Like inbreeding depression, outbreeding depression also appears to be environmentally dependent. While studies are few, there is some evidence that detrimental hybridization effects are reduced under stress. Several studies show that stress enhances heterosis (Pederson 1968; Barlow 1981; Armbruster *et al.* 1997). This pattern might be explained either by the masking of inbreeding depression exacerbated by stress (Hoffman & Parsons 1991) or by the higher buffering capacity of heterozygotes (e.g. 'developmental homeostasis', Lerner 1954). In one study the reduction of outbreeding depression under stress was due not to an increase in beneficial dominance effects but instead to a reduction in detrimental epistatic effects (Edmands & Deimler 2004).

Joint effects of inbreeding and outbreeding

A final difficulty in evaluating the relative risks of inbreeding and outbreeding is that the effects cannot always be distinguished. There are many practical situations in which inbreeding and outbreeding occur simultaneously (Templeton & Read 1984). Individuals can be inbred within loci while being crossbred between loci. That is, a later-generation hybrid could be homozygous for alleles identical by descent from one population at one locus, and homozygous for alleles from a second population at another locus. This is particularly likely in managed populations (zoo, aquaculture, agriculture) where highly, inbred populations are often supplemented by individuals from other inbred populations. Fitness problems attributed to inbreeding depression may therefore actually result from outbreeding depression (Lynch 1991). Conversely, apparent outbreeding depression can be influenced by inbreeding depression, particularly if a single F_1 population is intercrossed to produce the F_2 (e.g. Burton 1990).

Attempts have been made to estimate simultaneous effects of inbreeding and outbreeding in wild populations using molecular measures of hybridity (Boxes 1 and 2). However, these methods tend to require extraordinarily large number of molecular markers for accurate estimates (e.g. Balloux *et al.* 2004). For individuals of known pedigree Templeton & Read (1984) developed a model to partition effects of inbreeding and crossbreeding in which the fitness of an individual is determined by its inbreeding coefficient and the hybridity of its parents. According to Lynch (1991) this model does not consider the effect of epistasis on inbreeding depression and also suffers from a faulty treatment of cross-population epistasis. Perhaps the most explicit method for partitioning the joint effects of inbreeding and outbreeding is by line-cross analysis (Lynch 1991). However, a full partitioning of effects requires measurements of at least 16 different cohorts and this has yet to be carried out for even a single species.

Recommendations

While the data on outbreeding depression are dwarfed by those on inbreeding depression, the few studies that exist suggest that concerns over outbreeding should be taken seriously, as the effects can in some cases be as damaging as severe inbreeding. The list of areas needing further study is long. We certainly need studies of outbreeding in a broader range of taxa, with fitness measured over multiple generations, ideally under natural conditions. And we particularly need careful studies of the simultaneous

effects of inbreeding and outbreeding. Of course, major conservation decisions concerning the relative dangers of inbreeding and outbreeding will need to be made long before these data are in. What do we do in the meantime?

As a start, managers should strive to do no harm. That is, we should intentionally hybridize populations only when there is hard evidence that a population is suffering from inbreeding depression. We should not adopt the practice of promoting 'one migrant per generation' as a general rule (Mills & Allendorf 1996), without regard to natural levels of gene flow or the potential genetic repercussions of artificial mixing. Just as very small levels of gene flow can have remarkably beneficial effects on populations vulnerable to inbreeding, low levels of gene flow are predicted to have disastrous effects on populations vulnerable to outbreeding (Edmands & Timmerman 2003).

In cases where population mixing is critically needed to restore genetic and demographic health we should choose source populations that are as genetically and adaptively similar as possible. In situations where disruption of intrinsic co-adaptation is a concern, it may be particularly important to focus on genetic differences between populations. While the uneven accumulation of hybrid incompatibilities among taxa makes it impossible to define a 'safe' genetic distance, there is little doubt that lower divergence is typically better. For situations where disruption of extrinsic co-adaptation is a concern, the adaptive distance between populations may be more important than genetic distance (e.g. Gravuer *et al.* 2005). The new emphasis on adaptive variation (e.g. Delaney & Wayne 2005; Storz 2005) holds promise for identifying taxa where disruption of local adaptation is a concern, as well as for choosing source populations that adaptively match the population of concern.

Lastly, if at all possible, fitness consequences of interpopulation hybridization should be tested for at least two generations before inflicting this management strategy on the population of concern. We should be particularly concerned with the fitness of backcross hybrids as these are likely to be more common than F_2 hybrids in most real scenarios. Certainly, there are many conservation situations where such controlled breeding studies are not practical or even possible. However, what little we know about outbreeding depression shows that it is highly unpredictable, with fitness effects in the F_1 sometimes being entirely decoupled from effects in the F_2 or first backcross generations (e.g. Edmands 1999). It is therefore entirely possible that intentional mixing will cure inbreeding depression in the first generation, only to induce comparable levels of outbreeding depression in the second generation.

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