Predicting the Probability of Outbreeding Depression

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Abstract: *Fragmentation of animal and plant populations typically leads to genetic erosion and increased probability of extirpation. Although these effects can usually be reversed by re-establishing gene flow between population fragments, managers sometimes fail to do so due to fears of outbreeding depression (OD). Rapid development of OD is due primarily to adaptive differentiation from selection or fixation of chromosomal variants. Fixed chromosomal variants can be detected empirically. We used an extended form of the breeders' equation to predict the probability of OD due to adaptive differentiation between recently isolated population fragments as a function of intensity of selection, genetic diversity, effective population sizes, and generations of isolation. Empirical data indicated that populations in similar environments had not developed OD even after thousands of generations of isolation. To predict the probability of OD, we developed a decision tree that was based on the four variables from the breeders' equation, taxonomic status, and gene flow within the last 500 years. The predicted probability of OD in crosses between two populations is elevated when the populations have at least one of the following characteristics: are distinct species, have fixed chromosomal differences, exchanged no genes in the last 500 years, or inhabit different environments. Conversely, the predicted probability of OD in crosses between two populations of the same species is low for populations with the same karyotype, isolated for <500 years, and that occupy similar environments. In the former case, we recommend crossing be avoided or tried on a limited, experimental basis. In the latter case, crossing can be carried out with low probability of OD. We used crosses with known results to test the decision tree and found that it correctly identified cases where OD occurred. Current concerns about OD in recently fragmented populations are almost certainly excessive.*

Keywords: adaptive differentiation, chromosomes, effective population size, genetic diversity, genetic rescue, habitat fragmentation, outbreeding depression, polyploid

Predicción de la Depresión por Exogámica

Resumen: *La fragmentación de poblaciones animales y vegetales típicamente lleva a la erosión genética y al incremento de la probabilidad de extirpación. Aunque estos efectos generalmente se pueden revertir mediante el restablecimiento del flujo genético entre los fragmentos de poblaciones, los manejadores a veces fallan debido al temor a la depresión exogámica (DEX). El rápido desarrollo de la DEX se debe principalmente a la diferenciación adaptativa de la selección o fijación de variantes cromosómicas. Las variantes cromosómicas fijadas pueden ser detectadas empíricamente. Utilizamos una forma extendida de la ecuación de criadores para predecir la probabilidad de DEX debido a la diferenciación adaptativa entre fragmentos de poblaciones aisladas recientemente como una función de la intensidad de selección, la diversidad genética, el tamaño poblacional efectivo y las generaciones en aislamiento. Los datos empíricos indicaron que poblaciones en*

ambientes similares no habían desarrollado DEX aun después de mil generaciones en aislamiento. Para predecir la probabilidad de DEX, desarrollamos un árbol dedecisiones basado en las 4 variables de la ecuación de criadores, el estatus taxonómico y el flujo génico durante los últimos 500 años. La probabilidad predicha de DEX es alta en cruzas entre dos poblaciones cuando las poblaciones tienen por lo menos una de las siguientes características: son especies diferentes, tienen diferencias en cromosomas fijados, no intercambiaron genes durante los últimos 500 años o habitan en ambientes diferentes. Por el contrario, la probabilidad predicha de DEX es baja en cruzas entre dos poblacionesde la misma especie cuando las poblaciones tienen el mismo cariotipo, han estado aisladas por <500 años y ocupan ambientes similares. En el primer caso, recomendamos evitar la cruza o probarla en un nivel limitado, experimental. En el segundo caso, la cruza puede llevarse a cabo con baja probabilidad de DEX. Utilizamos cruzas con resultados conocidos para probar el árbol de decisiones y encontramos que este identifico casos correctamente cuando ocurrió DEX. Las preocupaciones actuales sobre DEX en poblaciones fragmentadas recientemente con toda seguridad son excesivas.

Palabras Clave: cromosomas, depresión exogámica, diferenciación adaptativa, diversidad genética, fragmentación de hábitat, poliploide, rescate genético, tamaño poblacional efectivo

Introduction

Fragmented populations of many species have reduced genetic diversity relative to historic levels (Tallmon et al. 2004; Aguilar et al. 2008). Inbreeding depression and loss of genetic diversity will ultimately contribute to the extirpation of many small populations (Fenster & Dudash 1994; Moritz 1999; Frankham et al. 2010). The adverse effects of genetic isolation can often be reversed by re-establishing gene flow between populations (Tallmon et al. 2004; Frankham et al. 2010). However, we are aware of only 19 cases in which augmentation of gene flow between isolated populations may have been implemented for conservation purposes in threatened and near threatened (IUCN 2010) populations throughout the world (see Supporting Information). These represent a small fraction of the small, fragmented populations that might benefit from management of gene flow (Supporting Information). By contrast, diverged lineages have in many cases merged naturally following environmental fluctuations, such as glacial cycles (Supporting Information).

Use of management actions to accomplish genetic rescue (gene flow between populations that reverses inbreeding, recovers genetic diversity, and improves reproductive fitness) is sometimes limited due to concerns about outbreeding depression (OD) (Templeton 1986; Thornhill 1993; Edmands 2007). Outbreeding depression is a reduction in reproductive fitness (reduced ability to mate [pollinate], fertilize, produce offspring, survive, or reproduce) in the first or later generations following attempted crossing of populations. Thus, it is critical to develop means to predict the probability of OD in crosses between fragmented populations of a species that previously had a continuous distribution (Frankham 2010).

The decision to augment gene flow in fragmented populations should be based on a cost-benefit analysis in which the currency is reproductive fitness of a population. The costs are inbreeding depression and loss of genetic diversity when gene flow is inhibited or poten-

tially OD when gene flow is augmented. The benefits are genetic rescue when gene flow is augmented or avoidance of OD when gene flow is prevented. Inbreeding depression has been observed in essentially all well-studied outbreeding species for which it has been investigated, and the occurrence and level of depression are relatively predictable (Keller & Waller 2002; Frankham et al. 2010). Many habitually inbreeding species also exhibit inbreeding depression, although to a lesser degree than in outbreeding species (Byers & Waller 1999).

By contrast, OD occurs in only some crosses (Supporting Information) and its occurrence to date has been poorly predicted (Edmands 2002; McClelland & Naish 2007). Edmands (2007) listed 35 species, including plants, invertebrates, and vertebrates, in which OD occurred but did not list species in which OD did not occur. Some cases of OD involve crosses between different species or populations exhibiting fixed chromosomal differences (indicating that the crossed populations are of different species) where OD is not unexpected (Ryder et al. 1989; Osborne et al. 1996).

We reviewed evidence concerning proposed mechanisms that generate OD and developed theory for estimating the probability of rapidly evolving OD. Using empirical data, we estimated the number of generations required to develop OD. To aid in practical implementation of our findings, we developed a decision tree to predict the probability of OD if two recently isolated populations are crossed. We evaluated the predictive ability of our decision tree against known cases exhibiting or not exhibiting OD. We concentrated on the probability of rapidly developing OD in allopatry by focusing on populations fragmented and potentially isolated genetically within the last 500 years. This conservative and pragmatic time frame encompasses the increased fragmentation associated with a 13-fold increase in the world's human population (U.S. Census Bureau 2010).

We did not address the consequences of supportive breeding (release of individuals from captive populations

to augment wild populations) or genetic swamping due to hybridization between species (Frankham et al. 2010).

Mechanisms Generating Outbreeding Depression

We use the terms OD and reproductive isolation interchangeably to encompass reductions in reproductive fitness of crossed populations due to any combination of prezygotic and postzygotic isolation. Three main mechanisms for generating OD that have been proposed are chromosomal differences resulting in partial or complete sterility of F_1 hybrids, adaptive differentiation among populations, and population bottlenecks and genetic drift.

Chromosomal Differences

The effects of fixed chromosomal differences on reproductive isolation are well documented (Table 1) (White 1978; Rieseberg 2001). Populations with a fixed chromosomal difference have elevated probabilities of OD when crossed. The effects on fitness are greatest for polyploids, intermediate for translocations, and modest for centric fusions and inversions. Adverse effects of inversions increase as the size of the inversion increases (Supporting Information). Duplications and deletions of heterochromatin are unlikely to have adverse effects on fitness in crosses. Shared chromosomal rearrangement polymorphisms, are unlikely to cause OD. Adverse effects typically increase as the number of fixed differences increases (White 1973). Fixation of chromosomal rearrangements results from either genetic drift overwhelming heterozygote disadvantage (Lande 1979; Coyne 1984) or from natural selection (White 1973; Rieseberg 2001).

Adaptive Differentiation in Allopatry

Darwin (1859) concluded that natural selection causes reproductive isolation as a secondary consequence of genetic adaptation to different environments (ecological speciation). There is now compelling theoretical and empirical evidence from many plants and animals that rapid development of reproductive isolation (not associated with chromosomal aberrations) develops primarily by this mechanism (Coyne & Orr 2004; Rieseberg & Willis 2007; Nosil et al. 2009; plus 11 references in Supporting Information). For example, Funk et al. (2006) report positive associations between ecological divergence and reproductive isolation for over 500 species. In three isolated lakes in British Columbia, stickleback fishes (*Gasterosteus* spp.) independently evolved benthic and lymnetic forms. Crosses between benthic and lymnetic forms within and between lakes have low spawning rates, whereas spawning rates in crosses between the same forms between lakes are normal (Rundle et al. 2000). In *Drosophila* partial reproductive isolation has evolved between isolated populations that have adapted to differ-

ent captive environments, but not between populations maintained in the same laboratory environment (Kilias et al. 1980; Dodd 1989). Most investigated speciation genes involved in prezygotic isolation have molecular signals of positive selection (McCartney & Lessios 2004; Orr et al. 2007). Theory indicates that reproductive isolation is more likely to arise and occurs more rapidly with selection than genetic drift (Gavrilets 2004). It is unclear whether adaptive differentiation is associated with all cases of reproductive isolation (Coyne & Orr 2004; Templeton 2008; Presgraves 2010), but adaptation is generally involved in cases where reproductive isolation has rapidly evolved (excluding chromosomal differences).

Prezygotic reproductive isolation arises from either pleiotropic effects on reproductive traits of alleles involved in adaptive differentiation or from linkage disequilibrium between them and alleles affecting reproductive isolation (Rice & Hostert 1993). Adaptive changes in timing or location of reproduction in both plants and animals can result in prezygotic reproductive isolation (Hall & Willis 2006; Savolainen et al. 2006; Nosil 2007).
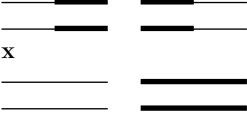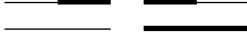
Some postzygotic reproductive isolation (lower F1, F2, and backcross fitness) may also arise directly from adaptive differentiation (Rundle & Whitlock 2001; Hereford 2009). Outbreeding depression in which the F1 and later generations are approximately intermediate in fitness between the two parents in parental environments and lower than the home parent has been found in *Drosophila*, stickleback fishes, and leaf beetles (*Neochlamisus bebbianae*) (de Oliveira & Cordeiro 1980; Rundle 2002; Egan & Funk 2009).

Genetic Drift and Population Bottlenecks

Population bottlenecks were previously proposed as a major force in the development of reproductive isolation (Mayr 1963; Templeton 2008). However, both theory and empirical studies favor selection over genetic drift as the dominant mechanism producing reproductive isolation, apart from the role of drift in chromosomal differentiation (Coyne & Orr 2004; Gavrilets 2004; Templeton 2008 and references in "Adaptive Differentiation in Allopatry"). For example, empirical data on poison-dart frogs (*Dendrobates pumilio*), anole lizards (*Anolis roquet*), and a cichlid fish (*Pseudocrenilabrus philander*) indicate that divergence due to natural selection better explains levels of reproductive isolation among populations than divergence caused by drift (Stelkens & Seehausen 2009; Thorpe et al. 2010; Wang & Summers 2010).

Drift plus natural selection may lead to the evolution of different coadapted gene complexes in completely isolated populations within the same environment (Whitlock et al. 1995; Supporting Information). However, inbreeding will increase in populations subject to bottlenecks, and when the populations are crossed they are likely to show genetic rescue effects that tend to mask

Table 1. Associations of fixed chromosomal differences with outbreeding depression when populations are crossed.

Chromosomal variant	Examples of population cross[a]	Genetic effect[a]	Fitness effect
Polyploid	$4n$ (tetraploid) $\times 2n$ (diploid)	$3n$ (triploid)	high level of F1 sterility
Translocation			~ 0.5 reduction in gamete (plants) or zygote (animals) viability
	x	F1 normal, but meiosis with unbalanced chromosomal constitution	
Centric fusion (chromosome formed by fusion at the centromere of two previously acrocentric chromosomes)	$(2n - 2) \times 2n$ V^b V $\times$ I^c I I I	$2n - 1$ V I I F1 normal, but meiosis results in some unbalanced gametes	modest reduction in gamete (plants) or zygote (animals) viability
Inversion	A*DCB*E $\times$ ABCDE A*DCB*E ABCDE	A*DCB*E ABCDE F1 normal, but meioses with crossovers in the inverted region yield $\sim$ 0.5 gametes with unbalanced chromosomal constitution (a minority of meioses)	modest reduction in gamete (plants) or zygote (animals) viability
Duplication (or deletion) of heterochromatin		F1 and F2 have normal complement of functional loci	little or no change in reproductive fitness of F1 or F2 generation

[a]*The* n *in this column is number of haploid sets of chromosomes. The lines are representations of chromosomes.*
[b]*A metacentric chromosome (formed by centric fusion).*
[c]*An acrocentric chromosome.*
[d]*Letters represent loci and sequence represents gene order within a chromosome.*

OD due to coadaptive differences (Fenster & Galloway 2000a). Similarly, when small populations are isolated in the same environment, drift plus sexual selection can lead to reproductive isolation and speciation, but again genetic rescue effects tend to mask adverse effects if the populations are crossed (Coyne & Orr 2004; Sobel et al. 2010).

Other cases of postzygotic reproductive isolation beyond those discussed above are believed to develop, often slowly (Supporting Information), mainly through the interactions of two or more loci (Dobzhansky–Muller incompatibilities) (Coyne & Orr 2004).

Predicting the Probability of Outbreeding Depression

Prior predictions of OD (Emlen 1991; Edmands & Timmerman 2003; Edmands 2007) have not been sufficiently accurate to be useful in conservation. Fixed chromosomal differences between populations are a risk factor for OD that can be assessed by karyotyping, so new theory is not required for them.

Focusing on adaptive differentiation, we applied quantitative genetic theory to predict the extent of OD for crosses between diploid populations with the same karyotypes. Quantitative genetic models have been used extensively to investigate conditions for speciation (Gavrilets 2004). Because reproductive fitness is a quantitative character, the cumulative genetic adaptation (ΣAD_t) over t generations in a population due to pre-existing quantitative genetic variation can be predicted by using an extension of the breeders' equation (Frankham 2008; Supporting Information):

$$\sum_{i=1,}^{t} AD_i \sim h^2 \sum_{i=1,}^{t} S_i (1 - 1/[2N_e])^{t-1}, \qquad (1)$$

where h^2 is heritability of reproductive fitness, S_i is the selection differential in the ith generation, and N_e is effective population size. Cumulative adaptation is $h^2 S_1$ in the first generation and $h^2 S_1 + h^2 S_2 (1 - [1/2N_e])$ in the second. Although loss of genetic diversity for fitness characters can be slower initially than predicted by Eq. 1 due to nonadditive genetic variation in well-adapted populations (Van Buskirk & Willi 2006), this is unlikely in the circumstances we considered because most genetic variation is additive when populations move to new environments or when environmental conditions change in a given location (Supporting Information).

Table 2. Equations to predict adaptive differentiation under a range of scenarios.

Scenario	Prediction equation[*]
1. Two completely isolated populations from the same source, one in the original environment, and the other adapting to a new environment	$\Sigma AD_t \sim GD + M$
2. Populations from same source move into two new and different environments (a, b) and undergo adaptive divergence (source now extinct)	$\Sigma AD_t \sim E\,(GD_a + M_a + GD_b + M_b)$
3. Partly diverged populations from once connected range with gene flow (cline), isolated in different environments and locally adapting	$\Sigma AD_t \sim AD_0 + E\,(GD_a + M_a + GD_b + M_b)$

[*]*Variables: ΣAD_t is the cumulative adaptive genetic divergence between the populations over t generations, GD is adaptation from selection on initial genetic diversity (given by Eq. 1 in the text), M is adaptation from selection on new mutations (given by Eq. 2), and E is the proportion of the adaptations of the two new populations that is to different new features in their environments.*

New mutations also contribute to genetic variation and adaptation (Hill 1982):

$$\sum_{i=1,}^{t} AD_i \sim \sum_{i=1,}^{t} 2N_e S_i \sigma_m^2 / \sigma_P^2, \qquad (2)$$

where σ_m^2 is the increase in additive genetic variation due to mutation in each generation and σ_P^2 is phenotypic variation. Summations are again over t generations. Adaptation increases as values of the same variables as in Eq. 1 increase, except that quantitative genetic variation is due to new mutations in Eq. 2, rather than pre-existing (standing) variation in Eq. 1. The quantity σ_m^2 / σ_P^2 is approximately 10^{-4} in natural environments (Rutter et al. 2010).

The total adaptive genetic change is the sum of contributions from preexisting genetic diversity (GD) and new mutations (M):

$$\sum_{i=1,}^{t} AD_i \sim h^2 \sum_{i=1,}^{t} S_i (1 - 1/[2N_e])^{t-1}$$
$$+ \sum_{i=1,}^{t} 2N_e S_i \sigma_m^2 / \sigma_P^2 = GD + M. \qquad (3)$$

We considered several adaptive scenarios, all of which predicted that similar variables would affect differential adaptation and the development of OD (Table 2). In scenario 1, a population splits, one part remains in the original environment to which it is adapted (i.e., it shows no further directional evolution in fitness), and the other moves to a new environment. Adaptive differentiation for this scenario is given by Eq. 3. In scenario 2, two populations originate from one adapted source population and move to different, new environments, and the source population is extirpated. Genetic differentiation between them is the sum of adaptation in the two environments multiplied by E, the proportion of the adaptations of the new populations that is to different new features in their environments ($E = 0$ when the two environments are the same and 1.0 when they are entirely different) (Table 2). If both populations move to new but identical environments, their adaptive differentiation from the source population will be similar (given similar N_e and no severe population-size bottlenecks) (as given by Eq. 3), but small relative to each other ($E \sim 0$) (Rundle et al. 2000).

In scenario 3, where populations from a cline (or with some level of initial adaptive divergence) become isolated in different environments, the total genetic differentiation between any two populations is as in scenario 2 plus the initial adaptive difference (AD_0). If two populations split and are totally isolated, but experience sexual selection in similar environments, response to sexual selection is given by the equation in scenario 2, except that S now refers to antagonistic selection between females and males and E now measures the difference in sexual selection between the populations. Gene flow from the source population into an adapting population in any generation dilutes cumulative adaptation by the proportion of migrant alleles (Frankham & Loebel 1992).

These scenarios show that the degree of adaptive differentiation and the probability of OD is an increasing function of four factors: selection differential (which increases as a function of the difference between the environment to which the population was previously adapted and the current environment), heritability (a function of genetic diversity for reproductive fitness), effective population size, and number of generations since the populations became isolated. Empirical evidence confirms the roles of these variables in determining genetic adaptation (Frankham 2008; Leimu & Fischer 2008). Supporting Information contains evaluations of further predictions and alterations to our predictions required for species with other breeding systems.

Generations to Develop Outbreeding Depression

To apply our theory, we required empirical estimates of the number of generations necessary for initial evolution of OD under allopatry. In similar environments, a minimum of thousands of generations of evolution in isolated populations is required to initiate OD in the absence of fixed chromosomal differences (Supporting Information). For example, stickleback fish populations isolated in similar niches in different lakes for more than 6000 generations are not reproductively isolated (Rundle et al. 2000), despite having sufficient genetic diversity and adequate population sizes to evolve benthic and lymnetic forms in each lake.
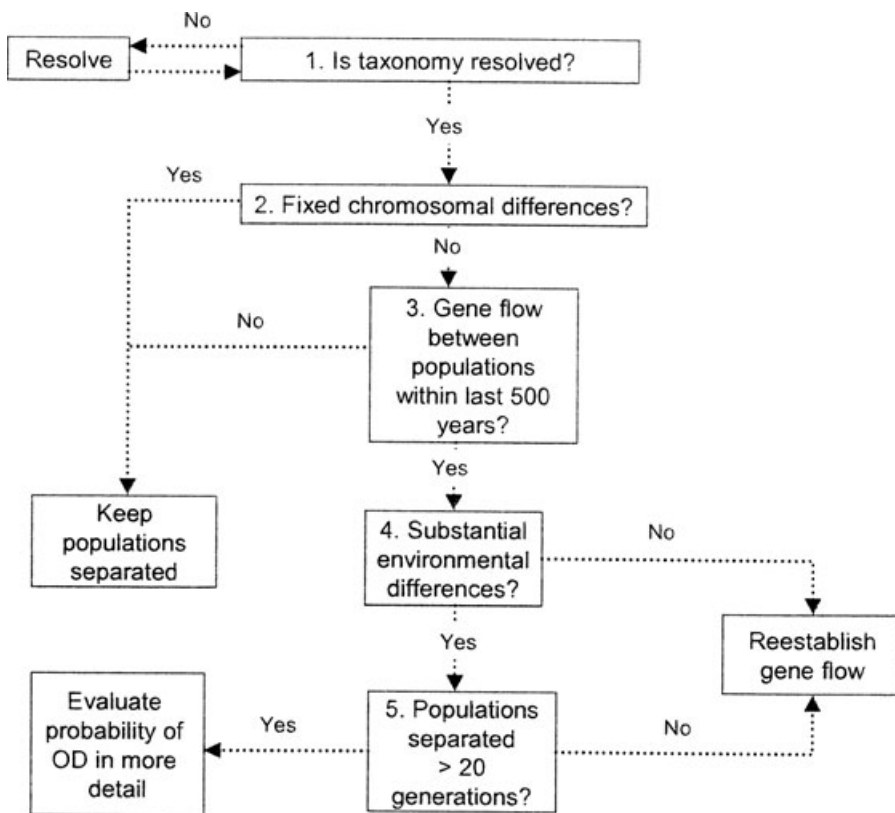
Figure 1. Decision tree for determining the probability of outbreeding depression (OD) between two populations.

In different natural environments, signs of reproductive isolation between populations of plants, invertebrates, and vertebrates can commence within dozens of generations (Hendry et al. 2007).

Decision Tree

We developed a decision tree to facilitate the application of our prediction of OD to management of fragmented populations (Fig. 1). Three of the five questions in the tree (2, 4, and 5) were based on our considerations above. If the taxonomic status of the two populations is unclear (question 1), the taxonomy should be resolved before OD is predicted. We do not recommend augmentation of gene flow between species defined according to the biological species concept (Mayr 1963) because crosses between different species are expected to exhibit OD.

The question about gene flow between the populations within the last 500 years has two purposes. First, long-isolated populations may represent unrecognized species (e.g., Brown Kiwis [*Apteryx australis*]; Burbidge et al. 2003). Second, populations may have in the past adaptively differentiated while they were allopatric (Churikov & Gharrett 2002). If populations have been isolated for 500 years or more, we recommend managing them as separate populations.

If gene flow between populations within 500 years is unknown, it can be inferred from either past geograph-

ical distribution (as indicated by the collection localities recorded for specimens in museums or herbaria) or genetic markers (Wright 1969; Crooks & Sanjayan 2006; Hey 2006). Population genetic theory and empirical evidence (Wright 1969; Wang 2004) indicate that very low levels of gene flow (>1 effective "migrants" per generation) suffice to prevent two populations from developing meaningful genetic differentiation in the absence of selection.

Small populations that have low probabilities of OD have clearly resolved taxonomies, no fixed chromosomal differences, gene flow between the populations within the last 500 years, and inhabit similar environments or have been in different environments for <20 generations (Hendry et al. 2007). Conversely, populations that have modest to high probabilities of OD have one or more of the following characteristics: fixed chromosomal differences, isolated for ≥500 years, or inhabit substantially different environments and have been present in them for >20 generations. We recommend that any augmentation of gene flow between such populations be conducted on an experimental basis. For populations with >20 generations of adaptation to different environments, we recommend a detailed assessment of the probability of OD on the basis of the variables in Eq. 3.

Additional considerations are required for species that previously exhibited clines across environmental gradients, but now exist in isolated fragments. For example, the Red-cockaded Woodpecker (*Picoides borealis*)

shows isolation by distance in molecular markers (indicating previous gene flow), but gene flow is now absent between many of the existing populations (Haig & Avise 1996). This species occurs from North Carolina to Florida and Texas (U.S.A.) and shows clines in wing and tail lengths along gradients of air temperature (Mengel & Jackson 1977). Because there may be adaptive differences among distant populations but not nearby populations, our recommendation is to re-establish gene flow, but only between near-neighbor populations. This has been recommended by others and implemented (U.S. Fish and Wildlife Service 2003). Similar recommendations apply in other related clinical situations in which taxonomy is clear and there are no fixed chromosomal differences between populations.

Beyond the question of taxonomy, if information is missing, we recommend progressing through the remaining questions in the decision tree and completing a preliminary assessment. If the assessment yields a low qualitative probability of OD, it is advisable to obtain the missing information or to proceed to augment gene flow only on an experimental basis.

Guidance on what constitutes a meaningful difference in environmental conditions for a species can be obtained from literature on planning translocations and reintroductions, where such assessments are routinely made and are generally not controversial (Fiedler & Laven 1996; Bremner-Harrison & Cypher 2007). Consideration should be given to the range of variation of key features of the two environments, such as differences in environmental features to which a species is either adapted or sensitive and whether the species is a narrow specialist (e.g., butterfly species restricted to a single host plant species) or a generalist species (cosmopolitan in its distribution; e.g., large carnivores).

Evaluation of Predictions

The status of cases with known OD was correctly predicted on the basis of information on one or more questions in our decision tree (Table 3). For example, we placed corroboree frogs (*Pseudophryne corroboree* and *P. pengilleyi*), ibex (*Capra ibex*), and dik-diks (*Madoqua kirki*) in the high probability of OD category because the crossed populations belong to separate species. We placed owl monkeys, dik-diks, and button wrinkleworts (*Rutidosis leptorhynchoides*) into the high probability category because they have fixed chromosomal differences.

We placed the *Tigriopus californicus* copepods studied by Edmands (2007) in the high-risk category on the basis of answers to several questions. Edmands & Harrison (2003) describe these copepod populations as exhibiting "extreme molecular subdivision," which indicates that they belong to several species (Supporting Information). The mtDNA divergence of these populations indicate that they have been isolated for between 100,000 and 9.2 million years (Edmands 2002), well beyond our 500-year criterion. There is little OD between the copepod populations with <5% sequence divergence in mtDNA, which corresponds to approximately 2 million years of isolation (Edmands 1999). Because the crossed copepod populations range from Alaska to Baja California, there are also substantial differences in environments between many of the source locations (indicating a high probability of OD), and F2 OD is related to geographic distance between populations (Edmands 1999).

Ibex and crosses between distant populations of partridge peas (*Chamaecrista fasciculata*) fell into the high probability of OD category because the crossed populations inhabited different environments (Table 3). Lacy (1998, unpublished data) found large genetic rescue effects and small OD effects in crosses between three well-differentiated subspecies of oldfield mouse (*Peromyscus polionotus*). Outbreeding depression was found when subspecies from different environments were crossed, but not when subspecies from similar environments were crossed, which is consistent with our probability categorization.

Outbreeding depression has been reported in F2 crosses between sympatric even- and odd-year populations of pink salmon (*Oncorhynchus gorbuscha*) coexisting in the same environment (Gharrett et al. 1999). However, there may not be OD for total fitness in the F2 generation of pink salmon because differences in survival and body size (a correlate of fecundity in fishes) deviate from controls (even-year × even-year or odd-year × odd-year crosses) in opposite directions (Supporting Information). Furthermore, there is substantial reproductive isolation between the even- and odd-year forms due to strict 2-year life cycles, including differentiation in karyotypes, allozymes, and mtDNA. Churikov & Gharrett (2002) used mtDNA analysis to estimate that the two forms have been isolated for at least 23,600 years. Additionally, the two forms may have diverged in slightly different environments (Supporting Information). We predicted a high probability of OD in crosses between the even-year and odd-year forms because there has been no gene flow between them for over 500 years.

The golden lion tamarin (*Leontopithecus rosalia*) does not exhibit OD (Ballou 1995), and we categorized it as having a low probability of OD. It has a well-established taxonomy, no reported fixed chromosomal difference, previously had gene flow across its range in Brazil, and the remaining populations exist in similar environments.

Use of our tree indicated a high probability of OD in a number of known cases that did not exhibit OD, but all involved uncertainties in the data (Supporting Information).

Table 3. Retrospective evaluation of probability of outbreeding depression in crosses between populations exhibiting or not exhibiting outbreeding depression.

Taxa	Taxonomic status of populations	Fixed chromosome differences	Gene flow in last 500 years	Similarity of environments	Predicted probability of outbreeding depression	Observed outbreeding depressions	Ref[a]
Corroboree frog (*Pseudophryne corroboree* and *P. pengilleyi*)	two species		no	similar	high	yes	1
Ibex (*Capra ibex ibex*)	three species[b]	unlikely	no	different	high	yes	2
Owl monkey (*Aotus trivirgatus*)	some consider them two species	yes	unlikely		high	yes	3
Dik-dik (*Madoqua kirki*)	>1 species	yes	unlikely	similar	high	yes	4
Button wrinklewort (*Rutidosis leptorrhynchoides*)	tetraploid and diploid	yes			high	yes	5
Peromyscus (*P. polionotus leucocephalus* × *P. p. subgriseus* or *P. p. rhoadsi*)	beach mouse subspecies × oldfield mouse subspecies	no	no	different	high	yes, modest	6
Peromyscus (*P. p. subgriseus* × *P. p. rhoadsi*)	subspecies of oldfield mouse	no	uncertain	similar	low?[c]	no	6
Partridge pea (*Chamaecrista fasciculata*)	crosses between distant populations	no	unlikely	different	high	yes	7
Copepod (*Tigriopus californicus*)	one species or species complex	no	no	some different, some similar	high	yes	8
Golden lion tamarin (*Leontopithecus rosalia*)	one species	no	yes	similar	low	no	9
Florida panther (*Puma concolor coryi*)	one species[d]	no	yes	moderately different	low or modest	no	10
Pink salmon (*Oncorhynchus gorbuscha*)	one species (odd and even years)	no (52 vs. 52, 53, and 54 chromosomes)	no	similar[e]	high	unclear[f]	11

[a]*References: 1, Osborne et al. 1996; 2, Turcek 1951; Templeton 1986; Wilson & Reeder 2005; 3, De Boer 1982; 4, Ryder et al. 1989; 5, Young & Murray 2000; 6, Lacy 1998; 7, Fenster & Galloway 2000; 8, Edmands 1999, Edmands 2002, Lee 2000; 9, Ballou 1995; 10, Culver et al. 2000, Hedrick & Frederickson 2010; 11, Phillips & Kapuscinski 1988, Allendorf & Waples 1996, Gharrett et al. 1999, Churikov & Gharrett 2002.*
[b]*Populations used are uncertain, but probable candidates that were crossed are now classified as separate species.*
[c]*Preliminary assessment conducted with incomplete information (gene flow uncertain).*
[d]*Previously classified as two subspecies, but subsequently revised on the basis of molecular studies.*
[e]*Distributions partially different so may be adapted to partially different environments.*
[f]*See the text for details.*

Discussion

We concluded that the probability of OD in crosses between populations with the same karyotype separated in the last 500 years and located in similar environments is small. These probabilities are much less than the probability of population extirpation due to inbreeding depression and loss of genetic diversity in separate, small, isolated populations. Thus gene flow can be augmented between many fragmented populations, potentially reducing population extirpation rates for many species. Details of levels and frequencies of augmented gene flow are considered briefly in Tallmon et al. (2004) and Crooks & Sanjayan (2006).

Consequences of the occasional incorrect assessment of the probability of OD would often be temporary, especially in large populations. Natural selection acts on the enhanced genetic diversity in partially depressed pooled populations to eliminate OD (Edmands et al. 2005; Erickson & Fenster 2006). In some cases, crossed populations evolved fitness greater than that of either of their parent populations.

Our investigation of the probability of OD is related partially to defining evolutionarily significant units (ESUs) and related concepts within species, but our work and work with ESUs have different objectives (Supporting Information).

Edmands (2007:463) recommended an extremely conservative approach to augmentation of gene flow among fragmented populations: [use] "intentional hybridization only for populations clearly suffering inbreeding depression, maximizing the genetic and adaptive similarity between populations, and testing the effects of hybridization for at least two generations." We endorse Edmands' recommendation to maximize similarity between crossed populations, but consider the other recommendations inappropriate because they will result in unnecessarily high extirpation rates for two reasons. First, many small populations are likely to be extirpated while awaiting collection of data on the effects of inbreeding. Inbred populations may have undocumented inbreeding depression, decreasing abundance, and a high probability of extirpation (Fenster & Dudash 1994). Second, testing effects of hybridization requires resources generally not available in low-income countries or for noncharismatic species in high-income countries, and we do not recommend that such testing be required in cases with low probability of OD. To act promptly on the best current information (Soulé 1985), we recommend a more active approach to augmenting gene flow to minimize population extirpations, as do Moritz (1999) and Hedrick & Frederickson (2010).

Our decision tree may be overly cautious in identifying crosses with a high probability of OD. Where the need for genetic rescue is pressing (e.g., Florida panther [*Puma concolor coryi*]; Hedrick & Frederickson 2010),

we would not wish to preclude augmentation of gene flow if use of the decision tree were to suggest the possibility of OD. In such cases, we recommend gene flow be augmented wherever possible on an experimental basis so that its effects can be monitored thoroughly. We urge publication of results independent of outcome, and archiving of data in a central database, to facilitate future refinement of predictions. We advocate an increased emphasis on re-establishing gene flow between fragmented populations when there is low probability of OD. Overall, current concerns about OD are almost certainly excessive.

Supporting Information

The following is available online: a table of species and populations that have had gene flow augmented and text sections titled Species That Would Potentially Benefit from Augmentation of Gene Flow; Merging of Previously Long-Isolated Populations in the Wild; Occurrence of Inbreeding and Outbreeding Depression; Impacts on Fitness in Crosses of Fixed Chromosomal Differences; Additional References on the Role of Differential Adaptation in the Evolution of Reproductive Isolation; Coadapted Gene Complexes and Genetic Drift; Postzygotic Reproductive Isolation; Prediction Equations; Additivity in New Environments; Evaluating Other Predictions; Probabilities of Inbreeding and Outbreeding Depression in Species with Different Breeding Systems; Generations to Develop Outbreeding Depression in Similar Environments; Evaluation of Predictions; and Relationship to Predictions of Evolutionarily Significant Units. The authors are solely responsible for the content and functionality of these materials. Queries (other than the absence of the material) should be directed to the corresponding author.

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