Gene Flow and Genetic Restoration: The Florida Panther as a Case Study

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Abstract: *Populations of some endangered species have become so small that they have lost genetic variation and appear to have become fixed for deleterious genetic variants. To avoid extinction from this genetic deterioration individuals from related subspecies or populations may have to be introduced for genetic restoration i.e., elimination of deleterious variants and recovery to a normal level of genetic variation. I construct a general population genetics framework from which to evaluate the potential for genetic restoration, and I discuss its specific application to the Florida panther. The translocation of Texas cougars into the free-ranging Florida panther population has been recommended to genetically restore the Florida panther, a subspecies of* Felis concolor *that appears to have both a low level of genetic variation and low fitness. Specific recommendations recently given by a scientific panel are to introduce enough animals so that there is approximately 20% gene flow in the first generation of translocation and approximately 2–4% in the generations thereafter. I evaluated these recommendations in a theoretical population genetics framework and found that they should result in the removal of most detrimental genetic variation and an increase in the standing genetic variation without a high probability of loss of any adaptive Florida panther alleles. Unless the population of the free-ranging Florida panthers is very small, the planned translocation should result in genetic restoration of the Florida panther.*

Flujo de genes y restauración genética: La pantera de la Florida como un estudio de caso

Resumen: *Las poblaciones de algunas especies en peligro se han hecho tan pequeñas que han perdido la variabilidad genética y parecen haberse transformado en monomórficas para ciertas variantes genéticas dáninas. A los efectos de evitar la extinción debida a este deterioro genético, podría ser necesario introducir individuos de subespecies o poblaciones relacionadas para restaurar la integridad genética, es decir eliminar las variantes dáninas y recuperar los niveles normales de variabilidad genética. El presente trabajo provee un marco de acción general basado en la genética de poblaciones con el cual evaluar el potencial para la restauración genética y discute un ejemplo usando la pantera de la Florida. A los efectos de restaurar genéticamente a la pantera de la Florida, una subespecie de* Felis concolor *que parece tener bajos niveles de variabilidad genética y pobre condición, se ha recomendado la translocación de pumas de Texas a las poblaciones libres de las panteras de la Florida. Las recomendaciones específicas recientemente dadas por un panel científico son introducir un número suficiente de animales de tal forma que exista un 20% de flujo genético en la primera generación de traslocación y aproximadamente 2-4% en las generaciones subsecuentes. He evaluado estas recomendaciones dentro del marco de la teoría de genética de poblaciones y encontré que estas recomendaciones resultarían en la remoción de la mayor parte de la variación genética perjudicial y en un incremento de la variación genética sin que exista una alta probabilidad de la pérdida de alelos adaptativos de la pantera de la Florida. A menos que el tamaño poblacional efectivo en las panteras libres de la Florida sea muy pequeño, la translocación planeada debera resultar en la restauración genética de la pantera de la Florida.*

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Introduction

A number of endangered species exist only as a small natural population that may be suffering from genetic deterioration either from the loss of genetic variation due to genetic drift or to lowered fitness resulting from chance fixation of detrimental alleles or inbreeding depression (Lande & Barrowclough 1987; Hedrick & Miller 1992). In such situations the main strategies have been to try to prevent further genetic deterioration in the natural population and/or to establish a captive population in which the individuals can be more intensely managed genetically. In adopting these strategies it has been implicitly assumed there was no source of individuals that could be used to help such a population overcome these genetic problems, and it has been thought to be just a matter of time until the population declined further and went extinct (Gilpin & Soule 1986). However, because of the arguments of O'Brien and Mayr (1991), Dowling et al. (1992), Avise (1994), and others that different subspecies or populations of a species naturally exchange genes, there now is the opportunity (because of changes in federal and state policies) to introduce genetic variation from other subspecies or populations to help alleviate the problems of genetic drift and inbreeding depression in a particular population and thereby result in the genetic restoration of the population.

The Florida panther (*Felis concolor coryi*), a subspecies of the widespread mountain lion (also known as the cougar or puma), was given legal protection by the Florida Game and Fresh Water Fish Commission in 1958 and was listed as endangered by the U.S. Fish and Wildlife Service in 1967. Protected from hunting for nearly 30 years, the Florida panther now appears to be suffering from low fitness, mainly in male fitness components such as sperm viability and male sterility, but there also has been an increase in heart defects and also appears to display less genetic variation than other mountain lion subspecies. A series of genetics workshops (Seal 1991, 1992, 1994) have recommended that Texas cougars (*Felis concolor stanleyana*), another subspecies of the mountain lion, be translocated into the Florida panther population to alleviate the problems associated with low fitness and low genetic variation. Specific recommendations were made in the most recent workshop (Seal 1994) to ensure that the detrimental alleles associated with low fitness would be eliminated, the level of genetic variation be restored, and any adaptive alleles present in the Florida panther population be retained.

When there is no selection on a locus and the population has a simple structure, gene flow allows straightforward predictions about the change in the allele frequency and the maintenance of genetic variation (Slatkin 1985; Lacy 1987). But the combination of selection and gene flow can result in a variety of potential outcomes, depending primarily upon the type and level of selection that is occurring on the alleles of interest. After reviewing the situation of gene flow and no selection in a population with simple structure, I consider two different types of selection: detrimental alleles that may have reached a high frequency in the endangered population and are causing low fitness, and adaptive alleles that have a high frequency in the endangered population because of their selective advantage in that environment or population. Generally, it is thought that molecular variants have small selective differences and that their frequencies are indicative of nonselective factors such as genetic drift and gene flow and may therefore be used to monitor the actual level of gene flow. Other variation, such as morphological differences, may be much harder to categorize as to its selective effects. For example, several unusual traits used to distinguish Florida panthers from other *F. concolor* subspecies, such as a kinked tail and a cowlick in the middle of the back, may be in high frequency because of genetic drift and could be unrelated to fitness or could possibly result in some reduction in fitness.

Even with a given type of selection and amount of gene flow, in an endangered species there may be only a probability that a certain outcome will occur because of its small population size. That is, the influence of genetic drift must be considered along with the type and extent of gene flow and selection. Such chance effects are important to consider over different genes, because alleles at different genes may have different dynamics, and over different populations (if there are several populations) for alleles at the same gene. For example, an adaptive allele may be lost by chance in one finite population but not in another.

I introduce the context of general population genetics in which to evaluate the impact of gene flow into a population for neutral, detrimental, or adaptive alleles. I then examine recommendations for the genetic restoration of the Florida panther, specifically to determine what the potential effect of gene flow is expected to be and how long it should take for genetic restoration to occur.

Model and Methods

No Selection and No Genetic Drift

There are a number of different models of gene flow that have been used to understand the impact of gene flow on the genetic variation in a population (Hedrick 1985; Hartl & Clark 1989). Because we are concerned here with the influence of gene flow from a large outside source (another subspecies) into a single, small population, we can use the simplest model of gene flow, called the continent-island model, in which there is gene flow from a source population (referred to as the conti-

nent) into the population of interest (the island). In the following discussion, the Florida panther is considered the island population and the Texas cougar the continental population. We examine the change for a locus with two alleles, A_1 and A_2, which have frequencies of p and q, respectively, in the island population. (Here I generally assume that A_2 is the allele of high frequency in the Florida panthers and A_1 is the allele of high frequency in the Texas cougars.) Assume, for the moment, that there are no selective differences between the genotypes at the locus and that there is one-way gene flow from the continental population to the island populations at a rate (proportion) m per generation, so that the change in the allele frequency per generation on the island is

$$\Delta q = -m(q - q_m), \quad (1a)$$

where q is the frequency of A_2 in the island population before gene flow and q_m is the frequency of A_2 in the migrants (and the source population). If we assume that allele A_2 is absent in the source population, then $q_m = 0$ and expression 1a becomes

$$\Delta q = -mq. \quad (1b)$$

In other words, the reduction of the frequency of the allele in the population is a function of the rate of gene flow and the frequency of the allele in the population.

We can give an expression for the change of allele frequency over time in this case as

$$q_t = (1 - m)^t q_0 + [1 - (1 - m)^t] q_m, \quad (2a)$$

where q_0 is the initial allele frequency and q_t is the allele frequency after t generations. As t becomes large, q_t approaches q_m and the alleles in the population are replaced by alleles from the source population. Again, if we assume that the frequency of A_2 is zero in the source population, then this expression becomes

$$q_t = (1 - m)^t q_0 \quad (2b)$$

For example, after 10 generations in which the rate of gene flow is 0.1, $q_t = 0.349q_0$. Therefore, the island has only 34.9% of its ancestry from the island population and 65.1% of its ancestry from the continental population.

To determine the amount of genetic variation in the island population (I assume three alleles here), let us assume that the heterozygosity in generation $t + 1$ is defined as

$$H_{t+1} = 1 - p_{t+1}^2 - q_{t+1}^2 - r_{t+1}^2,$$

where the frequencies of alleles A_1, A_2, and A_3 in generation $t + 1$ are p_{t+1}, q_{t+1}, and r_{t+1}, respectively, which are calculated as

$$p_{t+1} = (1 - m) p_t + mp_m$$
$$q_{t+1} = (1 - m) q_t + mq_m,$$
$$r_{t+1} = (1 - m) r_t + mr_m.$$

The frequency of the three alleles, A_1, A_2, and A_3, among the migrants are p_m, q_m, and r_m, respectively.

Selection with No Genetic Drift

If selection occurs along with gene flow, then different outcomes may result depending upon the type and extent of gene flow and selection. For example, Li (1976) (see also Hedrick 1985) has shown for two alleles that if the level of selection is high compared to the rate of gene flow, the equilibrium allele frequency will be determined primarily by the type of selection in the island population. For example, when there is strong selection against an allele it will be nearly eliminated except for introduction from gene flow. If an allele is selectively advantageous, it will nearly go to fixation except for the introduction of the other allele by gene flow. On the other hand, if the rate of gene flow is much greater than the level of selection, then the island will eventually come to resemble the source population in allele frequency. When the level of gene flow and selection are similar, then the rate of change and equilibrium are influenced by both forces.

Let us consider this general case for two alleles with the assumption that the allele frequency in migrants is zero, $q_m = 0$. First, let us assume that there is selection against A_2 where the fitnesses of the three genotypes, A_1A_1, A_1A_2, and A_2A_2, are 1, $1 - hs$, and $1 - s$, respectively, where h indicates the level of dominance. In this situation, the change in allele frequency is

$$\Delta q = -mq - \frac{sq(1 - q)[h - (2h - 1)q]}{1 - 2q(1 - q)hs + q^2 s} \quad (3)$$

Because both gene flow (first term on right side of the equation) and selection (second term) are acting to decrease the frequency of A_2, the rate of elimination of A_2 will be faster than with no selection, and the eventual frequency of A_2 will be zero. The denominator of the right-hand term is the mean fitness in both expressions 3 and 4.

Second, let us consider the situation in which selection and gene flow are acting in opposite directions, where gene flow is causing the reduction of A_2 and selection is causing an increase in A_2. In this case, let us assume the fitnesses of the three genotypes, A_1A_1, A_1A_2, and A_2A_2, are 1, $1 + hs$, and $1 + s$, respectively, where A_2A_2 now has the highest fitness, so that the change in allele frequency is

$$\Delta q = -mq + \frac{sq(1 - q)[q + h(1 - 2q)]}{1 + 2q(1 - q)hs + q^2 s} \quad (4)$$

Depending upon the level of selection and gene flow, there may be a stable equilibrium as the result of the bal-

ance between the effects of gene flow and selection. If we set $\Delta q = 0$, then the equilibrium allele frequency, when there is an equilibrium, is

$$q_e = \{ -s(2hm - 1 + 3h) \pm \{ [s(2hm - 1 + 3h)]^2 - 4s(1 - 2h)(m + 1)(m - sh) \}^{1/2} \} / 2s(1 - 2h)(m + 1) \quad (5a)$$

In general, there is only one feasible solution to this equation (an allelic frequency between zero and one). When $h = 0$ (or is close to zero), however, the expression has two feasible solutions; the lower value is an unstable equilibrium and the one with the higher value is a stable equilibrium (for a discussion, see Hedrick 1985). For example, if $h = 0$, $s = 0.2$, and $m = 0.04$, there is an unstable equilibrium at 0.28 and a stable one at 0.68. When the heterozygote is exactly intermediate between the two homozygotes in fitness (additive gene action), $h = 0.5$, then the equilibrium allele frequency is given by

$$q_e = \frac{s - 2m}{s(1 + 2m)} \quad (5b)$$

No Selection or Selection with Genetic Drift

The predictions based on the above deterministic equations, assuming no genetic drift, may be different than what may happen in a small population. We can determine the expected effect of genetic drift in specific generations by using the probability matrix approach (Hedrick 1985). The elements in the matrix are the probability of i A_2 alleles in generation $t + 1$, given that j A_2 alleles in generation t are the binomial probabilities

$$x_{ij} = \frac{(2N)!}{(2N - 1)!i!}(1 - q)^{2N - i}(q)^i, \quad (6)$$

where $q = j/(2N)$ and the population size is N (in this case, $N = N_e$, the effective population size). This matrix can then be multiplied by the vector of possible states of the population to give the expected distribution of states in the next generation. This process can be continued for any number of generations to determine, for example, the expected distribution of alleles in any generation or the expected proportion of genes that are fixed for a given allele at a particular time. When selection and gene flow are present, then the allele frequency after selection and gene flow (q') can be substituted for q as $q' = q + \Delta q$, where Δq is given by one of the expressions above. Also, different levels of gene flow can be allowed by changing Δq to reflect various levels of gene flow in different generations. An analytical approach can be used to calculate the eventual probability of fixation of an allele, given constant selection values (Kimura & Ohta 1971), but the transition matrix approach gives more flexibility in determining the short-term effects of selection and gene flow on the allele frequency distribution.

Florida Panther

Background

The Florida panther now exists only in a small population in southern Florida in and near the Big Cypress Swamp (Belden 1986). It is generally recognized to be different morphologically from other subspecies because of a flatter skull, darker color, and longer legs (Belden 1986). In addition, a whorl of hair or cowlick in the middle of the back was found in 83% of 35 Florida panthers and only 4.8% in other subspecies (Wilkins 1995), and a distinctive kink near the end of the tail (the third from the last vertebra is angled at 90° from the next vertebra) is found in nearly every Florida panther but is unusual in other mountain lions (Belden 1986).

The Florida panther was formerly widespread throughout Florida and the southeastern United States and was contiguous in distribution to other cougar subspecies. By the mid to late part of the nineteenth century, the Florida panther had been extirpated from most of its range and could be found only in a few remote areas. It has been suggested that the Florida panther is adapted to the hot, damp, thickly vegetated South (Fergus 1991), although it also has been noted that panthers prefer higher, drier ground within the swamps (Radetsky 1992). In other words, it is not really known whether the swamps of southern Florida are the only remote place where panthers were able to avoid hunters or an environment to which they are specifically adapted. The present natural population is extremely isolated and has no possibility of natural gene exchange with the closest U.S. subspecies, which occurs nearly 2000 kilometers away by land in west Texas.

There is substantial evidence that the Florida panther has reduced fitness, probably resulting from random fixation of detrimental alleles that can occur in a small, finite population. Because the Florida panther population has been small for a number of generations, say 15 to 25 given the time of isolation discussed above and a generation length of six years, there probably has been some inbreeding, which occurs by chance in any population that has been small for some time. In fact, there are several known instances of matings between close relatives (Roelke et al. 1993), but it is not known if the extent of inbreeding is greater than that expected by chance in a population of the size and structure of the Florida panthers.

The reduction in fitness appears to be mainly in the male fitness components from both cryptorchidism, in

which one or both testicles are not descended, and poor sperm quality. Before 1975, the incidence of cryptorchidism was reported to be 0% (although this was based on only two individuals) but has increased to 80% in the males born after 1989 (Roelke et al. 1993). In the latest survey 65% of the free-ranging male panthers are cryptorchid, nearly all of them unilaterally cryptorchid (Dunbar 1994). In 1992, however, one of four newly captured males was bilaterally cryptorchid, and another one had a single, significantly smaller, descended testicle (Roelke & Glass 1992). Although the mode of inheritance of cryptorchidism in cats is not known, cryptorchidism is known to be genetic in other species, varying from apparent single-gene determination to polygenic control (McPhee & Buckley 1934; Claxton & Yeates 1972; Rothchild et al. 1988; Romagnou 1991), and inbreeding appears to result in an increase in the incidence of cryptorchidism (Cox et al. 1978). It is not clear, however, how such a detrimental trait could increase so much in frequency over just a few generations unless the effective population size were extremely small.

The quality of semen in the Florida panthers is the worst ever recorded in any felid, with both very low total motile sperm per ejaculate and a very high frequency of malformed spermatozoa, both in (unilateral) cryptorchids and normal males (Barone et al. 1994; bilateral cryptorchids are thought to be sterile). There is also an indication that juvenile survival may be low, with 23 pregnancies resulting in only 10 offspring surviving beyond six months (Roelke 1990), although it is not clear what proportion of this mortality is genetic. In addition, there is some suggestion of an elevated rate of a cardiac defect known as atrial septal defect and an elevated number of infectious disease agents in Florida panthers (Roelke et al. 1993). All these effects may be due to chance fixation of detrimental alleles in a small population, but they have been referred to as the result of inbreeding depression (O'Brien et al. 1990; Roelke et al. 1993). Calling these fitness problems inbreeding depression is not really correct, so I refer to them as lowered fitness.

In addition, there is evidence that the Florida panther has a lower rate of genetic variation than western cougar populations (O'Brien et al. 1990; Roelke et al. 1993). For example, the portion of the Florida panther population that has not had any introductions from outside has a lower level of mitochondrial DNA variation (only one haplotype), a lower level of allozyme heterozygosity (0.018), and a higher level of band sharing for DNA fingerprints than other cougar populations. Significantly for the translocation effort, these molecular studies appear to show fairly low levels of divergence among subspecies of *Felis concolor*, suggesting substantial gene flow among them (O'Brien et al. 1990; Roelke et al. 1993).

I use two general estimates of population parameters, effective population size and generation length. The present population of Florida panthers is estimated to be approximately 30 to 50 breeding individuals (Hines et al. 1987), and these numbers were used by Seal (1994) to indicate the effective population size (as one estimate, I use an effective population size of 40). Because the effective population size is often even less than the number of breeding adults (Mace & Lande 1991), I also use a second estimate of the effective population size of 20. An estimate of the generation length of the Florida panther is six years (Seal 1994), so examining the impact of the translocation program over 10 generations means over approximately 60 years. Because of the concern about the short-term consequences of the translocations, I will concentrate on the early generations of gene flow.

Recommendation of Florida Panther Workshop

Because of the high rate of cryptorchidism, the poor quality of sperm, and other possible negative fitness consequences, it is apparent that some action is necessary to overcome these detrimental characteristics. In addition, the low level of genetic variation may limit the potential for future adaptation. Hence, the most recent workshop on the Florida panther recommended that eight young, nonpregnant, female Texas cougars be translocated as soon as possible into the Florida panther population (Seal 1994). The effect of this initial effort would be to cause approximately 20% of the gene pool of the Florida panthers to descend from Texas cougars. Further translocation was recommended if some of the initial females did not contribute to the gene pool, in an effort to make the level of gene flow from this first introduction close to 0.2. In addition, one new breeder per generation would be added after the initial generation, producing a per-generation level of gene flow of approximately 2–4% (I use a level of gene flow of 1 out of 40 individuals = 0.025). The plan suggested that the ongoing gene flow would lead to the replacement of locally adapted traits "only if the selective advantage of the locally adapted trait over the non-local variant of the gene was less than the rate of immigration (2–4%)," a point I discuss below.

Results and Predictions

No Genetic Drift

To examine the consequences of Seal's recommendations (1994), I first calculated the expected change of allelic frequency over 10 generations for a number of different relative fitness arrays. In the first generation, I assumed a 0.2 gene-flow rate into the population; there-

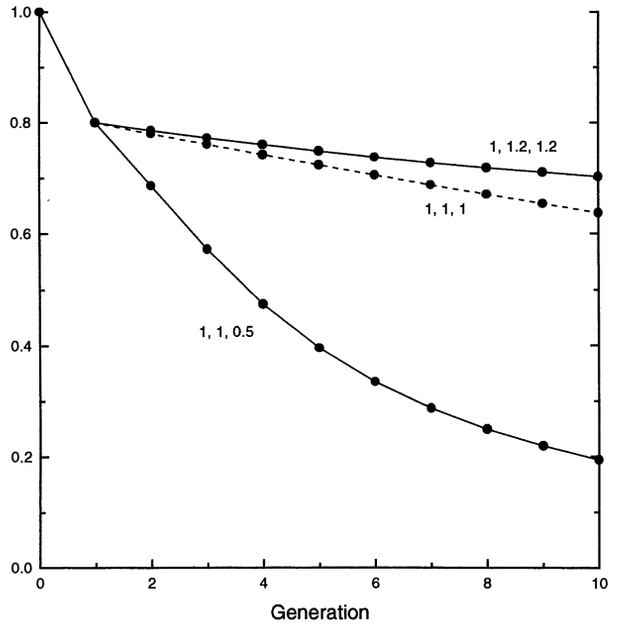

Figure 1. The change in frequency of allele A_2 ("Florida panther allele") over 10 generations with gene flow of 0.2 in the first generation and 0.025 thereafter, and selection either causing lowered fitness (1, 1, 0.5), an adaptive advantage (1, 1.2, 1.2), or no selection (1, 1, 1).

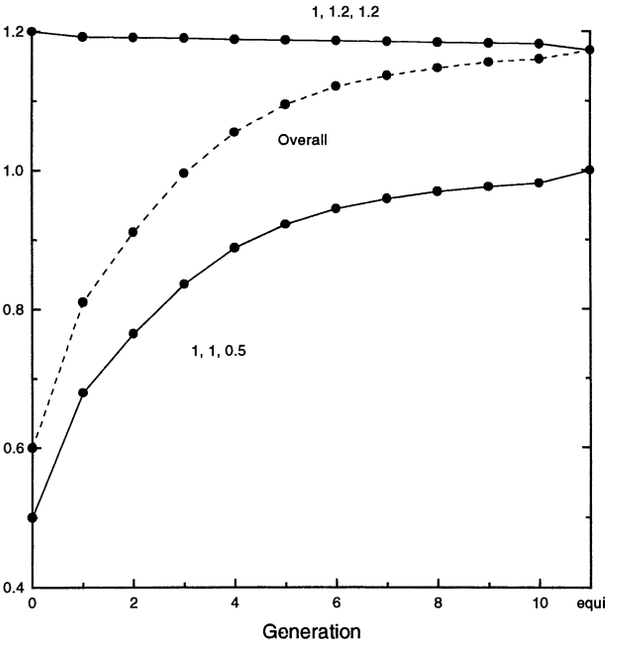

Figure 2. The change in relative fitness over 10 generations with gene flow of 0.2 in the first generation and 0.025 thereafter, and selection either causing lowered fitness (1, 1, 0.5) or an adaptive advantage (1, 1.2, 1.2) and the overall effect of these two loci.

after, gene flow was 0.025 each generation so as to closely approximate the intended rate of gene flow.

As an example, Fig. 1 gives the frequency of allele A_2 (the "Florida panther allele") for a neutral locus (no selection), a locus at which there is lower fitness because it was fixed for a detrimental allele (lowered fitness), and a locus that was fixed for an allele with an adaptive advantage. For the locus showing lowered fitness, I have assumed that it is fixed for an allele that has only half the fitness of the other genotypes. This is intended to generally simulate the situation that may be present in Florida panthers in which males appear to have a very low fitness for a locus while the females are unaffected by this locus. For the locus that has an adaptive advantage, I have assumed for illustration a selective advantage of 0.2, a fairly large effect.

First, for the neutral locus, the allele frequency was reduced to 0.8 in the first generation and declined in a nearly linear fashion to 0.637 in generation 10. The change in frequency for the adaptive locus is actually very similar to that for the neutral locus, even though it has a 20% selective advantage and reached an allele frequency of 0.704 in generation 10. This small difference occurs because at these high allele frequencies, selection is not very effective in countering the influence of gene flow in reducing the allele frequency. On the other hand, the frequency for the locus showing lowered fitness declines rapidly to only 0.193 after 10 generations.

For the two different selective regimes in Fig. 1, Fig. 2 gives the expected change in fitness for a locus showing lowered fitness and for a locus showing a selective advantage. For the locus showing lowered fitness, the fitness increases quickly from an initial value of 0.5 and by generation 10 reaches 97.6% of the maximum possible. On the other hand, the adaptive locus is only gradually and slightly lowered in fitness and at generation 10 is 98.6% of the maximum possible. This occurs because even though the allele frequency declines to 0.704, there are almost no homozygotes with the lower fitness at this frequency, and dominance masks the effect in heterozygotes. Figure 2 also gives the overall effect of these two loci, assuming multiplicative gene action over loci. By generation 5 the fitness value is quite high; by generation 10 the overall fitness is close to the maximum possible even though it began at a low value. In other words, if there were two loci as given here, one responsible for lowered fitness and one for adaptation, then the fitness would be restored in just a few generations.

Figure 3 illustrates the effects of adaptive loci with different parameters by showing the change in allele frequency when there are two extremes of dominance at the adaptive locus, $h = 0$ or 1. First, if the level of dominance is the lowest possible, $h = 0$, then selection has a greater effect relative to gene flow, both in keeping the adaptive allele frequency high and resulting in a higher

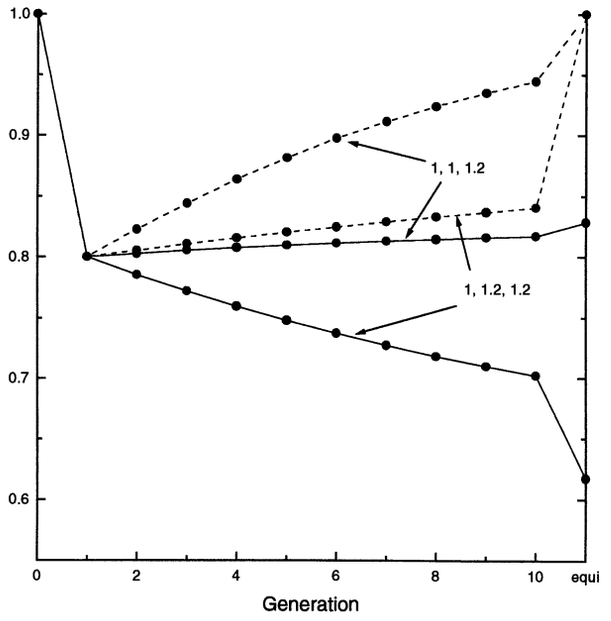

Figure 3. The change in frequency of A_2 over 10 generations with either gene flow of 0.2 in the first generation and 0.025 thereafter (solid lines) or no gene flow after the first generation (broken lines) and selection for an adaptive advantage with h = 1 (1, 1.2, 1.2) or h = 0 (1, 1, 1.2).

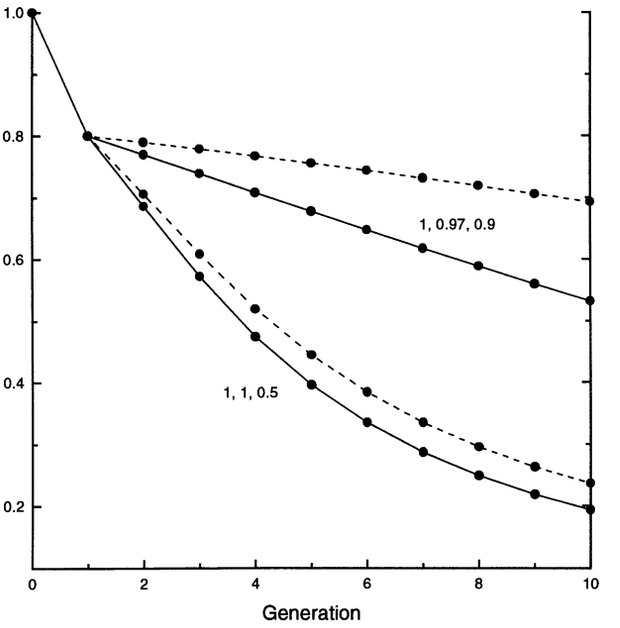

Figure 4. The change in frequency of A_2 over 10 generations with either gene flow of 0.2 in the first generation and 0.025 thereafter (solid lines) or no gene flow after the first generation (broken lines), and selection causing lowered fitness with a large effect (1, 1, 0.5) or a smaller effect (1, 0.97, 0.9).

equilibrium frequency—given at the right of Fig. 3. (If h = 0.5, then the results are almost exactly intermediate to these two levels of dominance.)

If the effect of the locus is lower, say 0.05, then there is no equilibrium for h = 0.5 or h = 0, and the equilibrium for h = 1 is 0.283 (from equation 5a). For all levels of dominance when s = 0.05, the change in allelic frequency over the first 10 generations is similar and is governed by gene flow. There is a difference in this long-term prediction and the prediction of Seal (1994): here if the adaptive value s is bigger than the level of gene flow m, the allele may be lost from the population. Seal (1994) stated that the allele would be retained if s is bigger than m.

Let us assume that there is only 0.2 gene flow in the first generation and that there is no further gene flow in the rest of the generations. Under this scenario—no gene flow after the first generation—Fig. 3 gives the expected change in allele frequency for the adaptive allele. For h = 0 the allele frequency rebounds to 0.945 by generation 10, and for h = 1 it returns to 0.840. As expected, the discontinuance of gene flow after the first generation allows a faster return to a high frequency for these adaptive alleles.

There is some information about the effects and dominance of loci that cause lowered fitness (Simmons & Crow 1977; Charlesworth & Charlesworth 1987). In general, loci of large detrimental effect, such as lethals, have very low dominance, near h = 0, such as given in Fig. 1. If the selective effect of the locus is lower, then the level of dominance is higher, averaging approximately 0.3. To illustrate the pattern of change when s is smaller and h is 0.3, the upper solid line in Fig. 4 gives the expected allele frequency change for s = 0.1. (If s is smaller than this, the change is very close to that for the neutral locus because the selective effect is less than the rate of gene flow.) As expected, the change reducing the allele frequency in this case is close to but slightly faster than the neutral locus.

Again, let us examine the effect of gene flow only in the first generation. In this case, the change in frequency for the detrimental allele is illustrated by the broken lines (no gene flow after the first generation) and reaches 0.237 and 0.693 for the two selective regimes in generation 10. As before, the allele frequency is higher than if gene flow is continued, a negative effect in this case. The allele frequency is continuing to decline, however, and will eventually be eliminated due to the selective effect against the allele.

Now let us examine the potential influence of gene flow on the level of heterozygosity when no selection occurs on a given locus in the Florida panther. Again I will assume that the initial frequency of the A_2 allele (the "Florida panther allele") is 1, so that the initial amount of heterozygosity is 0. I will consider three different scenarios, first one in which the Texas cougars are fixed for an-

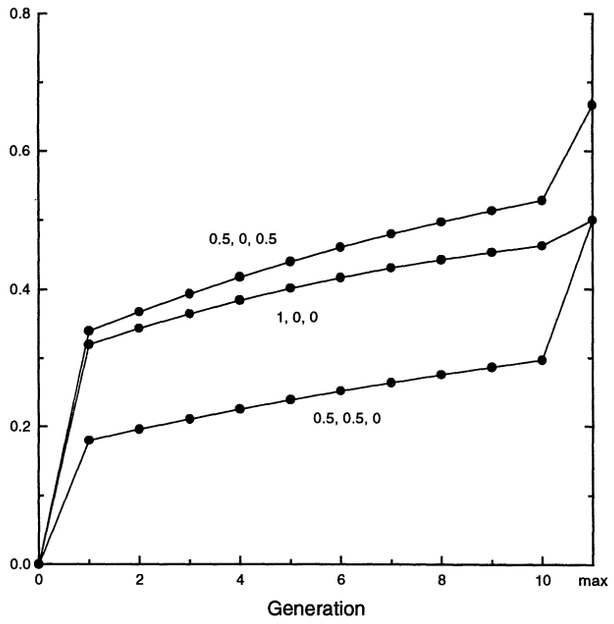

Figure 5. The change in heterozygosity over 10 generations with gene flow of 0.2 in the first generation and 0.025 thereafter for three different scenarios. The numbers indicate the frequency of the three alleles, A_1, A_2, and A_3, in the migrants (p_m, q_m, r_m).

other allele, say A_1 as above, so that $p_m = 1$ (this is referred to as 1, 0, 0 in Fig. 5 to indicate the allele frequencies of A_1, A_2, and A_3 in the migrants). In the second one, the Texas cougars are polymorphic for A_1 and A_2 (as an example, let $p_m = q_m = 0.5$, which gives the maximum heterozygosity for two alleles, referred to as 0.5, 0.5, 0 in Fig. 5). In the third example, the Texas cougars are polymorphic for two alleles not present in the Florida panther, say A_1 and A_3 (again let us assume that they are equal in frequency, so that $p_m = r_m = 0.5$, referred to as 0.5, 0, 0.5 in Figure 5).

Figure 5 gives the change in heterozygosity for these three scenarios over 10 generations and, on the right, the maximum heterozygosity obtainable. In the first case, where the Texas cougars are fixed for a different allele, there is an immediate increase in heterozygosity, with an increase to 0.462 by generation 10. In generation 19 the heterozygosity reaches a maximum of 0.5 and then eventually declines to zero as the population gets replaced with Texas cougar genes. In the second scenario in which the Texas cougars are polymorphic for the allele in the Florida panthers and another one, the heterozygosity increases to 0.297 by generation 10 and eventually reaches a maximum of 0.5, when there is complete replacement. In the third case, in which the Texas cougars are polymorphic for two other alleles, the heterozygosity again quickly increases and by generation 10 reaches 0.528. The maximum in this case is 0.667, reached in generation 36, a value higher than in either original population and possible when the three alleles reach equal frequency. Eventually the heterozygosity becomes asymptotic at 0.5. Obviously, under all these scenarios, which generally encompass all the possibilities (except when there is monomorphism in both populations for the same allele), genetic variation is quickly restored.

Genetic Drift

If there is a finite population, then the allele frequency may be greater or lower than the expected value for an infinite population. For example, Fig. 6 gives the 95% confidence limits of the allele frequency when there is no selection and the effective population size, N_e, is 20 or 40. These limits are of course larger for the smaller population size, but even after 10 generations they do not include 0 or 1. In other words, the finite population size is unlikely to result in the loss of either the introduced allele or the resident allele over 10 generations for these population sizes. For both these population sizes, the level of gene flow here is assumed to be 0.2 and 0.025 in the first and later generations, respectively, which implies that, for $N_e = 20$, migrants are half as likely to contribute to the population as for $N_e = 40$.

When selection causes lowered fitness, as in Fig. 1, the finite population size results in similar-sized confidence

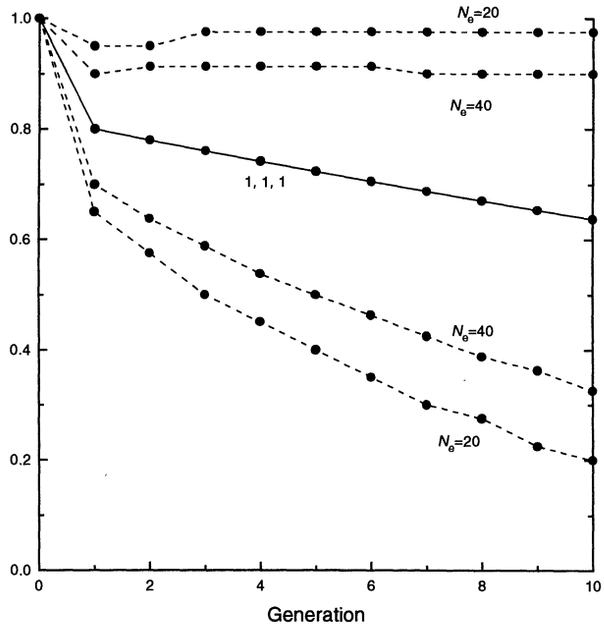

Figure 6. The change in frequency of A_2 over 10 generations with gene flow of 0.2 in the first generation and 0.025 thereafter and no selection (solid line), and the 95% confidence limits for an effective population size of 20 or 40 (broken lines).

limits. As expected, they do not include 1 (loss of the nondetrimental introduced allele) or 0 (fixation of the introduced allele) after 10 generations, even though the mean allele frequency of the detrimental Florida panther allele is much lower than for no selection.

If there is an adaptive allele and a finite population, then confidence limits are shifted upward from the neutral locus but again do not include 0 or 1 by generation 10. If we examine a greater time span for adaptive alleles, however, there is greater long-term loss of genetic variation in a finite population than predicted by the infinite population model. For example, with 1, 1.05, and 1.05, the infinite population model predicts an equilibrium at 0.283, but in a finite population the adaptive allele is eventually lost. This occurs because 0 is an absorbing boundary while 1 is not because of the contributing gene flow into the population.

Let us determine the expected outcome as far as the eventual fixation in a finite population if gene flow is halted. Table 1 gives the probability of fixation (really refixation) for the Florida panther allele (A_2) given a variety of scenarios of selection and gene flow. In the upper section of the table, for example, the probability of fixation is given for five different levels of lowered fitness with three time periods of gene flow of 0.025 after the initial gene flow of 0.2 in the first generation. Here of course we would like the probability of fixation to be low because the A_2 allele is the detrimental one we want to eliminate. Notice that for $s = 0.2$ or greater the probability of fixation is low, particularly if the population size is larger and the time that gene flow is extended is greater. For alleles of small effect, on the other hand, such as $s = 0.01$, even with the larger population size and 10 generations of gene flow, the probability of fixation is reduced to only 0.520.

Table 1. The probability of fixation of an allele (A_2) in the Florida panther population that had a frequency of 1.0 before gene flow when the allele causes lowered fitness (upper part of table) or an adaptive advantage (lower part of table) when the effective population size (N_e) is 20 or 40.

Fitness of A_1A_1, A_1A_2, A_2A_2	$N_e = 20$			$N_e = 40$		
	ϕ*	5*	10*	ϕ*	5*	10*
1.0, 0.997, 0.99	0.765	0.670	0.571	0.725	0.612	0.520
1.0, 0.985, 0.95	0.598	0.462	0.363	0.386	0.249	0.169
1.0, 0.97, 0.9	0.385	0.244	0.160	0.134	0.053	0.023
1.0, 0.94, 0.8	0.130	0.047	0.108	0.014	0.002	0
1.0, 1.0, 0.5	0.002	0	0	0	0	0
1.0, 1.0, 1.05	0.929	0.879	0.826	0.979	0.957	0.927
1.0, 1.05, 1.05	0.909	0.861	0.813	0.971	0.955	0.936
1.0, 1.0, 1.025	0.878	0.807	0.738	0.931	0.880	0.825
1.0, 1.025, 1.025	0.860	0.789	0.723	0.911	0.864	0.818

**Three regimes of gene flow after the first generation of 0.2 gene flow are given: no more generations of gene flow (0), 5 more generations of gene flow at 0.025 and then no more gene flow (5), and 10 more generations of gene flow at 0.025 and then no more gene flow (10).*

For an adaptive allele (bottom section of Table 1), we would like the probability of fixation to be high so that the adaptive allele is retained in the Florida panther. Obviously, for all the situations given here, and even when $s = 0.025$, the same level as the gene flow rate, the probability of fixation is greater than 0.8 except when there are 10 generations of gene flow and the population size is smaller. In other words, it is unlikely in these situations that an adaptive allele would be lost from the population.

Discussion

It appears that the recommendations of the workshop for genetic restoration of the Florida panther (Seal 1994) are generally robust, assuming that the estimates of the effective population size are accurate, under examination by population genetic criteria. That is, an initial gene flow of 0.2 and thereafter a gene flow of one animal per generation (gene flow of approximately 0.025) appears sufficient to eliminate lowered fitness (inbreeding depression), restore genetic variation, and retain adaptive alleles in the Florida panther population. Of course, such models and the resulting theoretical calculations as given here are intended to provide general guidelines for the genetic restoration program for the Florida panther. As recommended in Seal (1994), the success of the introduced Texas cougars and their progeny should be carefully monitored, and the restoration program should be flexible enough to incorporate changes appropriate to the observed fitness of the introduced animals and their descendants.

There are several significant caveats to this general finding. First, the initial gene flow of 0.2 appears sufficient to introduce favorable alleles at loci showing lowered fitness because selection against the detrimental alleles can then reduce them in frequency from this initial value of 0.8. In other words, continuous gene flow may not be necessary to eliminate these alleles, but it may be of some importance in increasing the rate of elimination or in eliminating other detrimental alleles as they arise in the future and increase in frequency by genetic drift.

Second, this amount of gene flow will not reduce the frequency of adaptive alleles of fairly large effect by a great amount, but alleles with a favorable effect of twice the continuous gene flow level eventually may be lost. This is particularly true for alleles with low levels of dominance and becomes more of a factor in a small, finite population such as the Florida panther. This is somewhat in contrast to the statement by Seal (1994) that alleles with an effect equal to the amount of gene flow will be retained. If gene flow is terminated after a

few generations, however, then these alleles should not be lost.

The influence of a small population size does not seem to be of great significance when the effective population size is of the order of 30 to 50 (this amount was used by Seal [1994] but actually comes from an estimate of the number of breeding adults existing in the early 1980s). Although this is still the effective population sized assumed by the agencies involved with the Florida panther, it is likely that the effective population size may be much lower than the number of breeding individuals. This occurs partly because it has been found that the basic assumptions in determining the effective population size, such as random mating and equal probability of paternity of offspring, often are not borne out in natural populations and can result in an effective population size much smaller than the number of breeding individuals (Lande & Barrowclough 1987; Mace & Lande 1991; Cabarello 1994). If a high proportion of the male Florida panthers do not reproduce successfully for genetic reasons, then there would be a skewed sex ratio among the breeding individuals that would further reduce the effective population size. It is not out of the realm of possibility that the effective population size may be as low as 10. If it is really this small, then the effect of genetic drift after gene flow, resulting in either refixation of detrimental alleles or loss of adaptive alleles, would be even greater than suggested in Table 1. In addition, if the effective population size is less than 30 to 50, then the actual rate of gene flow could be higher than 0.2 in the first generation and higher than 2–4% in succeeding generations, if eight females were successfully introduced in the first generation and one female per generation after that as recommended in Seal (1994). A higher gene-flow rate would allow detrimental alleles to be eliminated faster but would also make it more difficult to retain any adaptive alleles in the Florida panther.

It is of great urgency to estimate, as best possible, the effective size of the current population so that the impact of the translocation can be adequately predicted. Some data exist on age-dependent reproduction and survival among Florida panthers and other subspecies of *Felis concolor*. As of 15 August 1994, there were 16 free-ranging Florida panthers that were radio-tagged, and their sex ratio (seven males and nine females), general locations, and approximate ages were known (D. Jordan personal communication) (it is assumed that a number of other animals exist on private land with suitable habitat but that are unaccessible to census). Thus, it may be possible to arrive at a reasonably accurate, current estimate of effective population size.

It is not known whether the characteristic morphological traits of Florida panthers are symptoms of fixation of detrimental characteristics due to small population size, have little influence on fitness (nearly neutral), or are somehow adaptive traits in themselves. Depending upon the type of selection influencing them, the regime of gene flow proposed may eliminate them, if they have a detrimental effect, or they may be retained if they are adaptive.

Some molecular research (O'Brien et al. 1990) has indicated that the free-ranging Florida panthers are composed of two different stocks, the ones in the Big Cypress National Preserve and nearby areas that are descended from the ancestral *F. c. coryi*, and another group that resided primarily in the Everglades National Park (this group apparently has been extirpated from the Everglades and now has only four descendants in the Big Cypress Swamp). The latter group is reported to have descended from seven mountain lions that were released from the Piper stock between 1956 and 1966 (Roelke et al. 1993). From the molecular evidence, these animals appear to have had some ancestry from an animal or animals of South or Central American origin (O'Brien et al. 1990; Roelke et al. 1993). This group does not have the male reproductive problems that the Big Cypress group does and also does not generally have the cowlick or kinked tail. This earlier introduction with some Latin American ancestry appears to have resulted in a group with higher male fitness and lack of two of the distinctive Florida panther traits. What caused this group to lack characteristics, at both the molecular and the morphological levels, of the Florida panther gene pool is not clear because the exact dynamics of this introduction and its aftermath are not known. For example, were there any Florida panthers in the area of release, and if so did they interbreed with the released animals? Furthermore, the mode of the inheritance of the morphological characters—kinked tail and cowlick (and cryptorchidism)—is not known, so the predicted effect of introgression is not possible to determine for these traits. In any case, the Everglades animals do not appear to have increased in numbers and do not appear to have interbred to any great extent with the Big Cypress group.

The above analysis assumes that all the genes discussed, those that are neutral and those resulting in either lower or higher fitness, are assumed to be randomly associated, in gametic (linkage) equilibrium (Hedrick 1985). If they are not and they are tightly linked, then changes at a given locus due to selection could result in changes at another locus, a phenomenon termed genetic hitchhiking (Hedrick 1982). Genetic hitchhiking may be of significance in Florida panthers because initial disequilibrium could be generated by gene flow and because the small population size could generate further disequilibrium. Furthermore, there is a potential for strong selection to eliminate detrimental variants, which would reduce the time available for recombination to break down associations between loci. For example, it is possible that genetic hitchhiking may have been of significance in the increase of (neutral) molecular variants

among the panthers that were in the Everglades as detrimental variants were eliminated, although the details of these changes are not known.

There are two other modes of selection—heterozygote advantage and heterozygote disadvantage—that I have not considered here because they are likely of secondary consequence. For example, the majority of loci that cause lowered fitness under inbreeding appear to be loci with selection against homozygotes (and their associated heterozygotes) and not loci with a heterozygote advantage (Charlesworth & Charlesworth 1987). Further, heterozygote disadvantage, where heterozygotes have a lower fitness than either homozygote, is also unlikely of major consequence because it is assumed that Texas cougars and Florida panthers had contiguous distributions in the recent past. The progeny of Florida panthers and Texas cougars will be monitored for traits related to fitness to determine whether they display either heterotic effects or outbreeding depression. Of course, both of these phenomena may be caused by single locus selection, but they are generally thought to be the result of multilocus phenomena in which neither heterozygote advantage nor heterozygote disadvantage at individual loci is present (Hedrick 1985; Charlesworth & Charlesworth 1987).

The introduction of genetic variation from another taxa has been suggested to both allow adaption to a new environment (Lewontin & Birch 1966) and to increase fitness (Spielman & Frankham 1992). These two studies of Diptera illustrated that the infusion of genetic variation can in fact result in genetic restoration. But it would be useful to document in detail in such model systems the situations in which genetic restoration is consistent with theoretical predictions and instances in which simple theory is inadequate—and, in these cases, to determine why.

Although I have focused on the genetic restoration of the Florida panther here, the framework of theoretical population genetics can be used to evaluate genetic restoration in other species. Many populations of species that formerly had contiguous distributions with no barriers to gene flow have been fragmented by human actions, so that translocation of individuals among these fragmented groups may result in gene flow not too different than that before human impact. Possible application of the logic presented here may be appropriate in some fishes, such as the Apache trout (*Oncorhynchus apache*), sockeye salmon (*Oncorhynchus nerka*), and desert pupfish (*Cyprinodon macularius*), or some birds, such as the Dusky Seaside Sparrow (*Ammodramus henslowii houstonensis*; already extinct), Peregrine Falcon (*Falco peregrinus*), and Mexican Duck (*Anas diazi*). Good candidates would be populations for subspecies of a species that do not, because of genetic problems, appear to be viable. If the population or subspecies has been isolated for many generations, however, it may have developed significant adaptive differences, so the extent of gene flow should be carefully monitored to avoid swamping these adaptive alleles.

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