Animal Conservation. Print ISSN 1367-9430

Estimation of the bottleneck size in Florida panthers

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Keywords
computer simulation; effective population size; endangered species; microsatellite loci; mtDNA.

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Received 13 September 2007; accepted 22 October 2007

doi:10.1111/j.1469-1795.2007.00154.x

Abstract

We have estimated the extent of genetic variation in museum (1890s) and contemporary (1980s) samples of Florida panthers *Puma concolor coryi* for both nuclear loci and mtDNA. The microsatellite heterozygosity in the contemporary sample was only 0.325 that in the museum samples although our sample size and number of loci are limited. Support for this estimate is provided by a sample of 84 microsatellite loci in contemporary Florida panthers and Idaho pumas *Puma concolor hippolestes* in which the contemporary Florida panther sample had only 0.442 the heterozygosity of Idaho pumas. The estimated diversities in mtDNA in the museum and contemporary samples were 0.600 and 0.000, respectively. Using a population genetics approach, we have estimated that to reduce either the microsatellite heterozygosity or the mtDNA diversity this much (in a period of *c*. 80 years during the 20th century when the numbers were thought to be low) that a very small bottleneck size of *c*. 2 for several generations and a small effective population size in other generations is necessary. Using demographic data from Yellowstone pumas, we estimated the ratio of effective to census population size to be 0.315. Using this ratio, the census population size in the Florida panthers necessary to explain the loss of microsatellite variation was *c*. 41 for the non-bottleneck generations and 6.2 for the two bottleneck generations. These low bottleneck population sizes and the concomitant reduced effectiveness of selection are probably responsible for the high frequency of several detrimental traits in Florida panthers, namely undescended testicles and poor sperm quality. The recent intensive monitoring both before and after the introduction of Texas pumas in 1995 will make the recovery and genetic restoration of Florida panthers a classic study of an endangered species. Our estimates of the bottleneck size responsible for the loss of genetic variation in the Florida panther completes an unknown aspect of this account.

Introduction

The decline of pumas *Puma concolor* began when the Americas were settled by Europeans (pumas are also called cougars, mountain lions, and, in Florida, panthers). Pumas were aggressively hunted and bounties were offered for hides in the eastern United States. By the late 1920s, pumas were present only in central and south Florida and possibly along some river drainages in Louisiana (Young & Goldman, 1946). Numbers in Florida continued to decline after the 1920s because of continued persecution (Tinsley, 1970). In 1967, the Florida panther *Puma concolor coryi* was federally listed as endangered.

In the early 1970s, the Florida panther was believed extinct and no breeding population was known (Nowak, 1993). However, several animals were treed by dogs in 1973 and 1974 in southern Florida (Nowak, 1993) and the numbers found over the next few years were small. For the 1980s and early 1990s, the general claim was that the census population number was between 30 and 50 (Seal, 1994; Maehr *et al*., 2002). In the late 1990s, the census population number estimated by researchers was around 70 (Land & Lacy, 2000) but the present distribution of the Florida panther still constitutes $<5\%$ of the historical range (Maehr *et al*., 2002). Thus, reliable population estimates of Florida panthers are unavailable for the mid-20th century although it is assumed that Florida panthers went through an extreme bottleneck during this period.

Obviously, it is not possible to obtain direct estimates of the effective population size of the Florida panther during much of the 20th century because there are not even reliable census numbers over this period. To estimate the effective

population size or bottleneck size during this period, consistent with the historical information cited above, we here assume numbers were relatively large in the late 1800s, declined throughout much of the 1900s, reached very low numbers in the 1960s and 1970s, and have since increased. A few museum samples exist from the 1890s when the Florida panther was first described, before much of the decline in numbers. Here we compare the extent of molecular genetic variation present in these early samples to levels found in contemporary animals and estimate the size and extent of the bottleneck necessary to account for the observed decline in genetic variation between these two samples.

Molecular methods and samples

We used two different sets of samples to estimate the impact of a bottleneck on microsatellite genetic variation. First, we compared the genetic variation in museum samples of Florida panthers, mainly from the late 1800s, to contemporary samples from the 1980s. Second, we compared genetic variation for a larger set of loci in an Idaho sample that presumably did not go through a recent bottleneck, like the Florida panthers, and contemporary Florida panthers (Driscoll *et al*., 2002).

The techniques used to determine the extent of genetic variation for microsatellite loci in contemporary and museum samples of the Florida panther were reported by Culver *et al*. (2000). For the museum samples, unfortunately there was a limited amount of DNA and amplification was not successful for a number of locus–sample combinations (see discussion below).

Culver *et al*. (2000) examined three mtDNA gene segments (891 bp) in contemporary and museum samples and found that all were identical. In fact, all 186 pumas sampled north of Nicaragua, except for four from the Pacific northwest, shared the same haplotype. Therefore, here we utilize new, unpublished sequence data for a portion of the control region (Genbank accession numbers are EU258953, EU258954 and EU258955) to estimate genetic variation. Primers used to amplify and sequence this portion of the control region are: (forward) PDL3N-GACCTCAACTGT CCAAAGG and (reverse) DLUP4-CCTGAAGTAAGAA CCAGATG. Other techniques are as described for the other mtDNA gene segments in Culver *et al*. (2000).

Museum samples were extracted and the setup of PCR took place in a separate wing of the building, with separate air supply, where only human samples were handled. All equipment for processing museum samples was purchased new and only used for these types of samples. Museum samples were never handled in the same area as other felid samples. Furthermore, appropriate negative controls were included for DNA extractions and PCR amplification. No negative controls amplified, indicating no evidence of contamination. Museum samples 777–780 were from bone, while samples 785, 787 and 792 were from hide (see Culver, 1999, for museum voucher identification and other information). To determine whether any of the alleles or sequences in the museum samples was likely to be artifactual, for each round of extraction for the seven museum samples, a DNA extraction blank was included in each round of extraction, that is, a tube that underwent DNA extraction but with no sample added. These blanks were PCR amplified and they never showed any alleles or sequences. In addition, a PCR negative was included in every round of PCR amplification, and these also never showed up with alleles or sequences. As a result, we conclude that it is quite unlikely that the alleles and sequences seen in the museum samples were artifactual.

Seven museum samples provided data for either mtDNA or microsatellite loci: four from the Florida Museum (777, 778, 779 and 780) and three from the Museum of Comparative Zoology at Harvard University (785, 787 and 792) (identification numbers and geographic information as in Culver, 1999). These animals all dated from the 1890s, except one sample from 1922. Therefore, these animals should provide an estimate of genetic variation before the numbers of Florida panthers declined. Because Culver *et al*. (2000) found little differentiation over much of North America, it is reasonable to assume that the museum samples from Florida constitute a sample from the population extant in Florida at that time. The contemporary sample consisted of six individuals from the 1980s (Culver *et al*., 2000).

Models

We used computer simulation, based on the following expressions, to determine the population size or bottleneck size necessary to explain the observed difference in the genetic variation between the museum and the contemporary samples. For nuclear loci, the expected heterozygosity in generation t, H_t, due to genetic drift is a function of the initial heterozygosity, H_0, the number of generations t and the effective population size, N_e, as

$$H_t = H_0\left(1 - \frac{1}{2N_e}\right)^t \tag{1a}$$

(e.g. Hedrick, 2005). This expression can be solved for N_e as

$$N_e = \frac{0.5}{1 - e^{[\ln(H_t/H_0)]/t}} \tag{1b}$$

For mtDNA diversity, which is haploid and only transmitted from females,

$$H_t = H_0(1 - \frac{1}{N_{e.f}})^t \tag{2a}$$

where $N_{e.f}$ is the female effective population size, then

$$N_{e.f} = \frac{1}{1 - e^{[\ln(H_t/H_0)]/t}} \tag{2b}$$

We also used data from an unpublished survey of pumas from northern Yellowstone (Murphy, 1998), and the following expressions, to estimate the effective population size in a puma population. Using the estimated census number from Murphy (1998), we then estimated the ratio of the effective population size to the census population size in pumas. In the following analysis, parents and offspring were identified

using molecular genetic markers. To estimate the effective population size for females and males, demographic information on the average number ($\bar{k}$)and variance (V_k) in the number of offspring per individual and the number of individuals of each sex (N_f and N_m) is used in

$$N_{e.f} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f - 1 + V_{k.f}/\bar{k}_f} \quad (3a)$$

$$N_{e.m} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m - 1 + V_{k.m}/\bar{k}_m} \quad (3b)$$

(e.g. Lande & Barrowclough, 1987). These estimates can be combined to give an overall estimate of the effective population size as

$$N_e = \frac{4 N_{e.f} N_{e.m}}{N_{e.f} + N_{e.m}} \quad (4)$$

Results

Microsatellite loci

Museum versus contemporary Florida panther samples

The contemporary sample of six individuals was examined at 10 loci; two individuals were heterozygous for microsatellite locus 043, one individual was heterozygous for locus 249, and the rest were homozygous (data for contemporary and museum samples for the three loci that could be amplified in the museum samples are given in Table 1). Using the correction for small sample size (Nei, 1987), the expected heterozygosity in the contemporary sample over these three loci is 0.101 (or 0.047 for all 10 loci examined).

Despite multiple PCR attempts from a limited amount of DNA (total DNA yield from museum samples allowed for only ~20 PCR attempts), only three museum samples amplified for the microsatellite loci, all from the 1890s. For the microsatellites, amplification products ranged from 107 to 255 bp. Of these, one sample amplified for three loci and the other two amplified for only locus 043 (Table 1). Two of the three samples that amplified for locus 043 were heterozygous and there were five different alleles out of a possible six. Again using the correction for small sample size, the expected heterozygosity in this sample over the three loci is 0.311, more than threefold that found in the contemporary sample (0.101).

What effective population size can explain the observed loss of microsatellite variation from the museum to contemporary samples? Assuming that the time between the samples is 80 years and the generation time is 4, 5 or 6 years (this spans generation length estimates from the data given in Maehr *et al.*, 2002), the number of generations during which genetic variation can be lost is *c.* 20, 16 or 13, respectively. Using expression (1b), we calculated the effective population sizes that can account for a loss of 67.5% of the variation over this period. If the effective population size is constant over this period, that is, no lower bottleneck population size in specific generations, the necessary effective sizes are 6.0, 7.4 and 9.1 when there are 13, 16 and 20 generations, respectively (the top three lines in Table 2, see Fig. 1 for a generation length of 5 years).

On the other hand, if there is a bottleneck in recent generations, then the effective size in the other generations need not be as small. For example, using a modification of expression (1b) when there is an extreme bottleneck of two generations with an effective size of 2, then the effective size in the other generations need only be between 10.2 and 16.7 (Table 2, see Fig. 1 for a generation length of 5 years). If there were a bottleneck of size 2 for four generations, this

Table 1 The date and location of the Florida panther *Puma concolor coryi* samples and their genotypes for microsatellite loci and haplotypes for mtDNA in contemporary and museum samples

			Microsatellite locus			
Number	Date	Location	043	090	096	mtDNA
Contemporary						
14	1980s	Big Cypress Swamp	122/122	121/121	203/203	A
67	1980s	Big Cypress Swamp	122/122	121/121	203/203	A
71	1980s	Big Cypress Swamp	122/130	121/121	203/203	A
422	1980s	Big Cypress Swamp	122/122	121/121	203/203	A
426	1980s	Big Cypress Swamp	122/130	121/121	203/203	A
428	1980s	Big Cypress Swamp	122/122	121/121	203/203	A
Museum						
777	1890s	Florida	–	–	–	C
778	1890s	Florida	–	–	–	C
779	1890s	Florida	–	–	–	C
780	1890s	Immokolee	122/124	127/127	203/203	C
785	1898	Sebastian	134/134	–	–	A
787	1898	Sebastian	104/126	–	–	–
792	1922	Allen's River	–	–	–	B

Table 2 The number of generations (t) and effective population sizes (N_e) that can explain the observed difference in microsatellite variation between the museum and contemporary samples

t	N_e	Bottleneck t	Bottleneck N_e
13	6.0	–	–
16	7.4	–	–
20	9.1	–	–
11	10.2	2	2
14	13.2	2	2
18	16.7	2	2
9	7.9	4	4
12	10.4	4	4
16	13.9	4	4

The top lines assume a constant effective population size for the entire time but different numbers of generations t while the other examples assume bottlenecks of $N_e = 2$ or 4 for two or four generations, respectively.

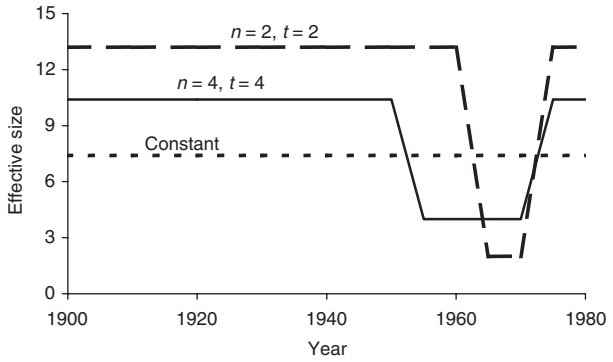

Figure 1 Three scenarios that can result in the observed loss of microsatellite variation from museum to contemporary Florida panther *Puma concolor coryi* samples when the generation length is 5 years: a bottleneck of 2 for two generations in the late 1960s with $N_e = 13.2$ in other generations (long broken line), a bottleneck of 4 for four generations in the 1950s and 1960s with $N_e = 10.4$ in other generations (solid line), and a constant effective population size of 7.4 (short broken line).

would result in a loss of 68.4% of the variation, all of the reduction in variation observed. As an intermediate possibility between lower constant population size and a bottleneck of size 2 for two generations, a bottleneck of size 4 for four generations is also given (Table 2, Fig. 1). Overall, it appears that to reduce the microsatellite variation as much as observed between the museum and contemporary samples, and consistent with the historical information, it seems likely that there were at least several generations in which the population went through an extreme bottleneck.

Contemporary Idaho versus contemporary Florida panther samples

A study by Driscoll *et al.* (2002) examined 84 microsatellite loci in samples of both Florida panthers and pumas from Idaho *Puma concolor hippolestes*. The expected heterozygosities in the Florida panthers and Idaho pumas were 0.147 and 0.348. In other words, for this much larger set of loci, contemporary Florida panthers had 0.422 of the variation observed in the Idaho population, a difference nearly as large as we observed between the contemporary and museum Florida panthers. Assuming that Florida panthers in the 1890s were part of the much larger population of North American pumas, then the higher heterozygosity observed in the Idaho pumas is a reflection of the heterozygosity found throughout North America, including Florida panthers, before extensive hunting by Europeans.

If we use the same approach as above to determine the bottleneck and population size necessary to result in the observed differences in heterozygosity in these samples, the results for a generation length of 5 years are given in Fig. 2. First, for a constant lower population size, N_e needs to be 9.6 instead of 7.4. For four generations of a bottleneck of four, N_e in the other generations needs to be 18.5 instead of 10.4. Finally, for two generations of a bottleneck of two, N_e in the other generation needs to be 25 instead of 13.2. Although these numbers are not quite as low as the estimates above, in general they strongly indicate that an extreme bottleneck is necessary to result in this large difference in heterozygosity.

mtDNA

The control region primers were designed specifically to amplify a very small product (111 bp) to allow for amplification of potentially degraded museum samples and six of the museum samples amplified. From this control region product, three haplotypes were found among the contemporary and museum samples. All of the contemporary Florida panthers examined had haplotype A. For the six museum

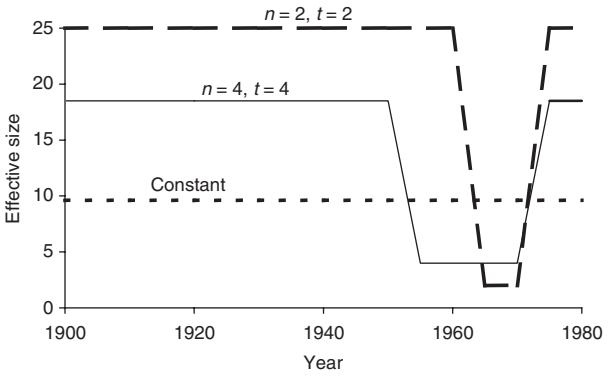

Figure 2 Three scenarios that can result in the predicted loss of microsatellite variation from Idaho to contemporary Florida panther *Puma concolor coryi* samples when the generation length is 5 years: a bottleneck of 2 for two generation in the late 1960s with $N_e = 25$ in other generation (long broken line), a bottleneck of 4 for four generation in the 1950s and 1960s with $N_e = 18.5$ in other generations (solid line), and a constant effective population size of 9.6 (short broken line).

samples, four samples, 777–780 (all from the 1890s), had haplotype C that is also found in Arizona (M. Culver, unpubl. data). Sample 785 (1898) had haplotype A, found in much of North America including contemporary Florida. Sample 792 (1922) had haplotype B, also found in historical samples from New York (M. Culver, unpubl. data). Overall then, the sample size corrected mtDNA haplotype diversities for the contemporary and museum samples are 0.000 and 0.600, respectively.

What effective population size in females can explain the reduction in mtDNA diversity observed from the museum to contemporary samples? A similar approach as used above for microsatellite loci cannot be used because expression (2b) is not defined when $H_t = 0$. As a result, a Monte Carlo simulation procedure was used to determine the probability of loss of mtDNA variation for different female effective population sizes. These simulations were initiated with the observed haplotype mtDNA frequencies and 5000 replicates of each combination of parameters were used.

The probability that $H_t = 0$ is given in Fig. 3 for a range of female effective population sizes that were run either for 13, 16 or 20 generations. For example, when $N_{e.f}$ is 10, then the population is homozygous (broken line in Fig. 3) *c*. 67, 75 and 84% of the time for $t = 13$, 16 and 20 generations, respectively). For lower population sizes, these values are even higher. In others words, for female effective sizes similar in magnitude to the total effective population size estimated for microsatellite loci in Table 2, the proportion of simulated populations fixed for a mtDNA haplotype is very high. Further, if there is a bottleneck and variation in the size of the population in different generations, then mtDNA diversity is likely to be reduced more quickly than nuclear variation. For example, only one generation of a bottleneck of size 2 (one female and one male) would result in a complete loss of mtDNA variation. As a result, the observed loss of variation for mtDNA haplotypes is consistent with that observed for microsatellite variation in that genetic drift effects of the same magnitude, for example, several generations of a severe bottleneck, could be responsible for both observations.

Estimation of N_e/N

Only limited published data exist on the number of lifetime offspring produced by individual Florida panther females and males (past population viability analyses of the Florida panther have used general estimates of Florida panther demographic data for input, see Maehr *et al.*, 2002). As a result, we used a dataset from northern Yellowstone pumas collected from 1987 to 1995 (Murphy, 1998) to estimate effective population size (and N_e/N) using a demographic approach. In this case, parentage of 70% of the litters was determined over a 9-year period using molecular genetic markers, a nearly complete reproductive history of a single generational cohort (Table 3). Noteworthy is that two males fathered 23 and 15 offspring, 72% of all the genotyped kittens. Also 15 males that were present on the study area did not have any offspring during this period.

From these data, we calculated the mean number and variance in number of offspring for females and males (Table 3). The variance in offspring in males is 11.8 times its mean reflecting the very unequal reproduction noted above. Using these values in expressions (3a) and (3b), then $N_{e.f} = 9.14$ and $N_{e.m} = 4.45$. We compared the effective population size with the census number of potentially breeding adults for each sex (note that the census number used here is a low estimate because a number of other animals were present on the study area at some time between 1987 and 1995). For females, this ratio is $N_{e.f}/N_f = 9.14/14 = 0.653$ and for males, it is $N_{e.m}/N_m = 4.45/24 = 0.185$. If there were random reproduction, these values should approach unity but here, particularly for males, it is much below unity.

The effective population sizes for each sex can then be used in expression (4) to obtain an estimate of the overall effective population size $N_e = 11.97$. The ratio of this N_e to overall census number of potentially breeding adults is $N_e/N = 11.97/38 = 0.315$. In other words, the total census

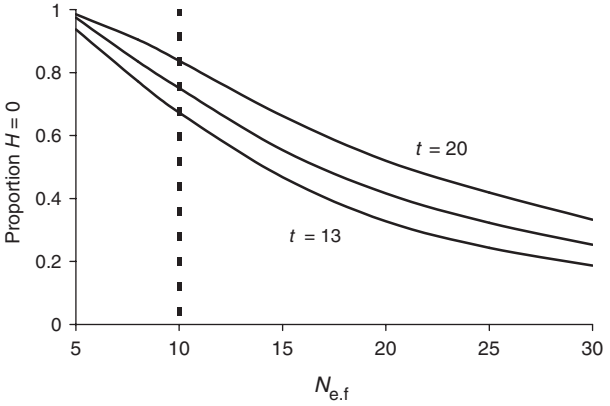

Figure 3 The proportion of 5000 simulations which are monomorphic for a mtDNA haplotype after 13, 16 or 20 generations for different female effective population sizes, $N_{e.f}$.

Table 3 The number of kittens for different females and males in northern Yellowstone for cougar litters born from 1987 to 1995 (Murphy, 1998)

Females		Males	
Number of kittens	Number of females	Number of kittens	Number of males
0	2	0	15
2	1	2	1
3	3	3	4
4	1	4	2
5	2	15	1
7	2	23	1
8	1		
9	1		
17	1		
$\bar{k}_f = 5.21$	$V_{k.f} = 19.10$	$\bar{k}_m = 2.50$	$V_{k.m} = 29.39$

number during the period for which we have been examining the loss of genetic variation may be approximately $N/N_e = 3.17$ larger overall. Using the same approach for the two sexes, for females, $N_f/N_{e.f} = 14/9.14 = 1.53$ and for males, $N_m/N_{e.m} = 24/4.45 = 5.39$.

Discussion

The amount of genetic variation in contemporary Florida panthers (1980s) is far less than observed in Florida panther museum samples from the 1890s for both microsatellite loci and mtDNA variation. The existence of extensive hunting activities in the 1890s, when the museum samples were collected, suggests a substantial population number during this period. On the other hand, the Florida panther was thought extinct in the early 1970s and the population census estimate during the late 1980s and early 1990s was only 30–50 individuals. In other words, the most likely scenario is that the population declined from its relatively high level in the 1890s, went through a bottleneck in Florida in the middle of the last century for at least a few generations, and then increased somewhat at the end of the last century. There is little information on the population size during much of the middle of the 20th century, and in the early 1970s there was no known population of Florida panthers (Nowak, 1993). Fortunately, even without good direct information on the population size during this period, the molecular data presented here allow us to obtain a general estimate of the extent and length of the bottleneck that would be necessary to cause the observed decline in genetic variation, thereby giving the first estimate of the size of the population bottleneck for this period.

From the nuclear data, the reduction in heterozygosity from 0.311 to 0.101 can be explained by an effective population size of 7.4 for this entire period or by an effective population size of 13.2 over the entire period except for two generations with an extreme bottleneck of two (for a generation length of 5 years). Using the Yellowstone data above, the effective population size is about 31.5% that of the census number. This translates into a census number of potentially breeding adults of *c*. 41 for all the generations, except in the case of the two extreme bottleneck generations where the estimated census number would be 6.2.

Using the data from Driscoll *et al.* (2002) comparing Idaho pumas to contemporary Florida panthers, the ratio of the heterozygosities is 0.422 (0.348 and 0.147, respectively). The bottleneck size necessary to explain this difference is nearly as severe as from our data. For example, given a bottleneck of two generations of two, then the effective size in the other generations needs only to be 25. Although it seems likely that the Idaho population is an appropriate sample of a population that has not recently gone through a bottleneck, and therefore reflects the ancestral (1890s) heterozygosity, it is possible that some factors may have reduced its genetic variation. If so, then the 0.348 heterozygosity estimate may be an underestimate of the ancestral heterozygosity and an overestimate of the bottleneck size necessary to explain the loss of genetic variation.

The mtDNA variation declined from 0.60 to 0.00, apparently more quickly than the nuclear variation. However, because mtDNA is maternally inherited and haploid, the effective population size is expected to be 1/2 $N_{e.f}$ or 1/4 N_e if the effective population sizes for the two sexes are equal (Hedrick, 2005), resulting in a faster loss of variation than for nuclear genes. Using a Monte Carlo simulation approach, a constant female effective population size of 10 is expected to result in a complete loss of mtDNA variation 75% of the time (generation length of 5 years). This is consistent with the effective size estimated above for nuclear genes, considering that the effective population size is about twice as large in females as males. A bottleneck of size 2 in only one generation could result in a complete loss of mtDNA variation.

Despite substantial effort, we were not very successful in determining the microsatellite genotypes for museum samples. However, we were more successful in obtaining mtDNA genotypes of museum samples and estimates of the bottleneck size necessary to produce the observed loss of microsatellite and mtDNA variation were similar, supporting our estimate of microsatellite variation in the museum samples. In general, a major problem in obtaining microsatellite genotypes from museum samples is allelic dropout (true heterozygotes appearing as homozygotes because one sequence does not amplify). Assuming this is a likely scenario, then microsatellite variation in the museum samples would actually be higher than we estimated (our estimate is a minimum) and the necessary bottleneck size to result in the observed loss of variation would be even more severe than we have determined.

Not only does the Florida panther have low genetic variation (Roelke, Martenson & O'Brien, 1993; Culver *et al.*, 2000), but it has also suffered from several detrimental traits, including a high proportion of chryptorchidism (undescended testicles), kinked tails, atrial septal defects and very poor sperm quality (Roelke *et al.*, 1993). Furthermore, it appears that the frequency of chryptorchidism increased over time in the 1970s and 1980s (Roelke *et al.*, 1993; Mansfield & Land, 2002). When the effective population size is low, then detrimental traits can increase because the effect of selection against them is weak compared with chance effects from genetic drift (e.g. Hedrick, 1994). For example, Kimura (1983) suggested that genetic drift is more important than selection when $s < 1/(2N_e)$ where s is the selection coefficient against homozygotes and N_e is the effective population size. If we assume that $N_e = 2$ as in the hypothesized bottleneck generations, then if $s < 0.25$, then genetic drift would be expected to dominate selection. Because several of the detrimental traits in Florida panthers are sex-specific, the maximum s could be is 0.5 and because chryptorchidism and other Florida panthers with poor sperm quality can still reproduce, then s may be < 0.25. In other words, the extreme bottlenecks we have proposed in the Florida panthers suggest that the very high frequency of these detrimental traits are likely to have occurred by chance.

To rectify the high frequency of detrimental traits and low genetic variation in Florida panthers, eight female pumas from Texas were released into Florida in 1995 (Seal, 1994; Hedrick, 1995). In 2000, the ancestry from the Texas animals was about 20% (Land & Lacy, 2000) and has increased somewhat since then (Maehr & Lacy, 2002; Land *et al*., 2005). The frequency of chryptorchidism and kinked tail in animals with Texas ancestry was low (Land *et al*., 2005); in other words, the infusion of animals from Texas appears to have started to overcome the increase in frequency in detrimental traits. Furthermore, this infusion should also help counteract the loss of neutral genetic variation in microsatellite loci and mtDNA we discussed above, and other variation throughout the genome, and increase the level of variation for these loci. In addition, analysis of life-history traits appear to show improvement in animals with Texas ancestry (Pimm, Dollar & Bass, 2006).

The conservation efforts on behalf of the Florida panther have been extraordinary in the last decade. The extensive monitoring of the population before and since the introduction of Texas pumas should provide detailed data on the process of genetic restoration of a severely endangered species. Our study complements these data by providing insight into the bottleneck that led to critical status of the Florida panther. Overall, we will be able to construct a classic example in the temporal change in numbers for an endangered species that includes the bottleneck that nearly resulted in extinction and the subsequent recovery because of protection and genetic restoration.

Acknowledgements

We would like to thank Laurie Wilkins at the Florida Museum of Natural History and Maria Rutsmozer at the Museum of Comparative Zoology for allowing us to collect samples from their respective museum collections. We also thank Melody Roelke for collecting samples from the contemporary Florida panthers. This work was supported for P.W.H. in part by funds from the Ullman Professorship.

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