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Long-term survival despite low genetic diversity in the critically endangered Madagascar fish-eagle

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Abstract

The critically endangered Madagascar fish-eagle (*Haliaeetus vociferoides*) is considered to be one of the rarest birds of prey globally and at significant risk of extinction. In the most recent census, only 222 adult individuals were recorded with an estimated total breeding population of no more than 100–120 pairs. Here, levels of Madagascar fish-eagle population genetic diversity based on 47 microsatellite loci were compared with its sister species, the African fish-eagle (*Haliaeetus vocifer*), and 16 of these loci were also characterized in the white-tailed eagle (*Haliaeetus albicilla*) and the bald eagle (*Haliaeetus leucocephalus*). Overall, extremely low genetic diversity was observed in the Madagascar fish-eagle compared to other surveyed *Haliaeetus* species. Determining whether this low diversity is the result of a recent bottleneck or a more historic event has important implications for their conservation. Using a Bayesian coalescent-based method, we show that Madagascar fish-eagles have maintained a small effective population size for hundreds to thousands of years and that its low level of neutral genetic diversity is not the result of a recent bottleneck. Therefore, efforts made to prevent Madagascar fish-eagle extinction should place high priority on maintenance of habitat requirements and reducing direct and indirect human persecution. Given the current rate of deforestation in Madagascar, we further recommend that the population be expanded to occupy a larger geographical distribution. This will help the population persist when exposed to stochastic factors (e.g. climate and disease) that may threaten a species consisting of only 200 adult individuals while inhabiting a rapidly changing landscape.

Keywords: conservation genetics, effective population size, genetic diversity, *Haliaeetus*, Madagascar, microsatellite DNA

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Introduction

The assessment of population genetic diversity plays an important role in informing the strategies for the conservation of threatened and endangered species although the importance of maintaining *high* levels of genetic diversity for the long-term persistence of populations is debatable (Arden & Lambert 1997; Lacy 1997; Lehman 1998; Jamieson 2007). While hereditary genetic diversity is crucial for adaptation and speciation, it is less clear how *low* neutral diversity, as measured by marker loci, impacts upon the ability of populations to persist through time (Willi *et al*. 2006; Pertoldi *et al*. 2007; Hughes *et al*. 2008). For example,

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many factors such as fluctuations in population size, mating system characteristics (e.g. polygyny or inbreeding), or metapopulation structure may influence the upper limit to genetic diversity in a population (Pimm *et al*. 1989; Hedrick 1996; Amos & Harwood 1998), and low levels of diversity may have existed for long periods of time without any obvious reduction in fitness (Brodie 2007). Alternatively, populations that have experienced a recent severe reduction in size may have lost significant levels of genetic diversity due to drift and the frequency of rare deleterious recessive alleles may increase resulting in reduced fitness through inbreeding depression (Keller & Waller 2002; Hale & Briskie 2007; Leberg & Firmin 2008). From a conservation perspective, it is important to recognize that how a population responds to a reduction in size, and the potential genetic problems that it may experience, will largely depend on its history. Populations experiencing a reduction in census size may not also experience a corresponding reduction in effective population size (N_e) if historic N_e has always been low.

A useful approach to ascertain whether a particular population of concern has recently experienced a population genetic bottleneck or has been historically limited to small numbers of individuals is to obtain levels of genetic diversity of samples collected before and after the proposed decline (Wandeler *et al*. 2007). This approach has been successfully employed to either confirm (Groombridge *et al*. 2000; Johnson *et al*. 2004, 2007; Nyström *et al*. 2006; Culver *et al*. 2008) or refute (Matocq & Villablanca 2001; Paxinos *et al*. 2002; Hadly *et al*. 2003; Brown *et al*. 2007; Taylor *et al*. 2007) the influence of recent population declines on observed low levels of contemporary population genetic diversity. However, it is not always possible (or practical) to obtain historic samples and where they do exist, the ages of the specimens may postdate the time period of interest. Therefore, for some species, alternative approaches are needed to determine the timing and potential influence of population size changes on contemporary levels of genetic diversity.

Recent advances in population genetics have produced methods to infer demographic and historic processes based on contemporary samples. For example, through coalescent-based modelling, we can estimate demographic parameters from patterns of genetic variation in contemporary populations to infer the extent and timing of historic demographic changes that have helped shape their current distribution (Beaumont 2004; Pearse & Crandall 2004). This approach is particularly useful with data collected from remote geographical areas where limited knowledge exists concerning the demographic history of a species.

The endemic Madagascar fish-eagle (*Haliaeetus vociferoides*) inhabits coastal and inland riparian habitat along the western side of Madagascar between Morombe to Antsiranana (Tingay 2005), and is found in areas with abundant fish for foraging and adequate nesting habitat (large trees or cliffs; Berkelman *et al*. 1999; Watson *et al*. 2000). The most recent published census reported a total of 222 adult individuals, including 63 known breeding pairs and an estimated total breeding population of no more than 100–120 pairs (Rabarosoa *et al*. 1997). Because of its current small population size and historic references citing a much larger abundance (Owen 1833; Schlegel & Pollen 1868), this species is listed as Critically Endangered by The World Conservation Union (IUCN 2007) and has been cited as one of the rarest bird species in the world (e.g. Krüger & Radford 2008). However, there is concern that the historic accounts of a larger geographical distribution and increased species abundance may be inaccurate and unsubstantiated (Tingay 2005). If this concern is correct, it is possible that the Madagascar fish-eagle exhibits historically persistent low population abundance (see also Tingay 2005), and other factors besides current population size (e.g. habitat fragmentation) may be of greater importance for improving population viability.

Although we acknowledge that the current population size is extremely small, it is not known whether the population has recently experienced a rapid decline in abundance within the past 50–100 years as others have suggested (Thiollay & Meyburg 1981) or if the population may actually have persisted at small size for a much longer period of time (Tingay 2005). The distinction between these two scenarios is crucial for determining the best approach to prevent this species' extinction. Here we determine contemporary levels of genetic diversity in the Madagascar fish-eagle population relative to other closely related *Haliaeetus* species, and investigate whether these levels of diversity are due to recent or more historic changes in population size. The results from this study have important implications for the conservation of the Madagascar fish-eagle and provide insight concerning the conservation of other island endemic species.

Materials and methods

Sampling and genotyping

Blood samples from four *Haliaeetus* species were obtained consisting of 44 Madagascar fish-eagles from the Antsalova region in western Madagascar (Tingay 2005; Tingay *et al*. 2007), 12 African fish-eagles (*Haliaeetus vocifer*) from Lake Naivasha, Kenya, eight bald eagles (*Haliaeetus leucocephalus*) from Florida, USA (Hillsborough, Manatee, Polk and Sarasota counties; Tingay *et al*. 2007), and 44 white-tailed eagles (*Haliaeetus albicilla*) from southern Sweden (Hailer *et al*. 2006). All individuals were presumed to be unrelated. With the exception of the Madagascar fish-eagle, the remaining three *Haliaeetus* species are labelled as Least Concern by the IUCN (2007) and not of immediate

Table 1 Estimates of microsatellite population genetic diversity for the Madagascar fish-eagle (f.e.) and its congeners grouped according to amplified loci in common. Samples size, *n*; number of alleles per locus, *A*; allelic richness, A_R; private alleles, A_{Priv}; expected (H_E) and observed (H_O) heterozygosities; F_{IS}, fixation index. Standard errors are given in parentheses. See Tables S1 and S2 for specific loci used per group

	n	*A*	A_R	A_{Priv}	H_E	H_O	F_{IS}
36 loci							
Madagascar f.e.	44	1.9 (0.2)	1.6 (0.1)	0.7 (0.1)	0.189 (0.036)	0.172 (0.034)	0.099 (0.042)*
African f.e.	12	3.3 (0.3)	3.0 (0.2)	2.1 (0.3)	0.484 (0.041)	0.506 (0.053)	–0.004 (0.068)
18 loci							
Madagascar f.e.	44	1.4 (0.1)	1.3 (0.1)	0.6 (0.1)	0.101 (0.039)	0.091 (0.036)	0.054 (0.044)
African f.e.	12	3.0 (0.4)	2.7 (0.4)	2.0 (0.4)	0.413 (0.070)	0.433 (0.086)	–0.004 (0.079)
White-tailed sea eagle	44	4.7 (0.4)	3.4 (0.3)	3.2 (0.3)	0.579 (0.050)	0.571 (0.050)	0.013 (0.029)
16 loci							
Madagascar f.e.	44	1.7 (0.2)	1.4 (0.1)	0.6 (0.2)	0.163 (0.051)	0.153 (0.053)	0.123 (0.083)
African f.e.	12	3.1 (0.4)	2.6 (0.2)	1.5 (0.3)	0.462 (0.058)	0.449 (0.075)	0.083 (0.114)
Bald eagle	8	2.2 (0.2)	2.0 (0.2)	1.4 (0.3)	0.349 (0.064)	0.315 (0.068)	0.079 (0.098)
8 loci							
Madagascar f.e.	44	1.6 (0.3)	1.4 (0.2)	0.8 (0.2)	0.163 (0.074)	0.136 (0.069)	0.139 (0.093)
African f.e.	12	3.5 (0.5)	2.9 (0.3)	2.0 (0.4)	0.554 (0.060)	0.524 (0.103)	0.116 (0.152)
White-tailed sea eagle	44	5.1 (0.4)	3.2 (0.4)	2.6 (0.4)	0.606 (0.076)	0.594 (0.074)	0.022 (0.055)
Bald eagle	8	2.6 (0.4)	2.4 (0.3)	1.6 (0.4)	0.464 (0.093)	0.444 (0.092)	–0.007 (0.107)

*significantly different from zero.

conservation concern given their current population size and overall geographical distribution. The African fish-eagle is distributed throughout much of central to southern Africa with current population size estimates from 100 000 to 200 000 pairs (Ferguson-Lees & Christie 2001). In contrast, both the bald eagle and the white-tailed eagle, which are largely Nearctic and Palearctic in distribution, respectively, experienced significant population declines and local extinction late in the 20th century (Buehler 2000; Hailer *et al*. 2006). Recently, however, both species have increased in population size to levels of less conservation concern (Ferguson-Lees & Christie 2001; IUCN 2007). Obtaining measures of genetic diversity of populations with known recent demographic history provide a useful approach for comparing diversity levels to that of species of unknown history, and can provide valuable insights to the consequences of rarity and are critical for conservation planning.

A total of 47 microsatellite loci were screened for polymorphism by polymerase chain reaction (PCR) for both Madagascar and African fish-eagle samples and 22 microsatellite loci were amplified in bald eagle and white-tailed sea eagle samples (see Table S1, Supporting information for details). To reduce the potential for ascertainment bias when comparing levels of diversity across species, the microsatellite loci used in this study were originally described from a total of six taxa within Accipitridae (32% of markers were from *Haliaeetus vociferoides*, Tingay *et al*. 2007; 15% from *H. leucocephalus*, Tingay *et al*. 2007; 17% from *H. albicilla*, Hailer *et al*. 2005; 30% from *Aquila adalberti*, Martinez-Cruz *et al*. 2002; 4% from *A. heliaca*, Busch *et al*. 2005; 2% from *Milvus milvus*, Peck 2000; see Table S1). Because not all loci were amplified in all four species, genetic diversity analyses were performed on subsets of loci and species (see Tables 1 and Table S2, Supporting information). For each subset of taxa genotyped for the same microsatellites, we only considered loci that were polymorphic in at least one of those species for diversity comparisons.

Statistical analyses

Microsatellite genotypes were tested for linkage equilibrium and departure from Hardy–Weinberg equilibrium within each population using the program GDA 1.1 (Lewis & Zaykin 2000), and sequential Bonferroni corrections were applied to correct for multiple simultaneous comparisons (Rice 1989). The program Micro-Checker (van Oosterhout *et al*. 2004) was used to test for null alleles and scoring errors due to large allele dropout and stutter peaks. Mean number of alleles per locus (*A*), expected (H_E) and observed (H_O) heterozygosities, and F_{IS} were calculated with GDA. Significance of F_{IS} was determined by bootstrapping over loci to estimate a 95% confidence interval based on 10 000 replications. To account for differences in sample sizes between species, measures of allelic richness (A_R) and number of private alleles (A_{Priv}) were obtained based on rarefaction using the program HP-Rare (Kalinowski 2005).

Differences in genetic diversity estimates between species were tested for significance using a Wilcoxon signed-rank test.

To obtain estimates of contemporary effective population size (N_e) for the Madagascar fish-eagle, we used two methods that are based on a single temporal population sample. The first method is based on gametic disequilibrium (Hill 1981) and includes a bias correction (Waples 2006) as implemented in the program LDNE (Waples & Do 2008). This method assumes selective neutrality of unlinked markers and a single closed population, and has been shown to perform well in non-ideal populations with skewed sex ratio or nonrandom variance in reproductive success (Waples 2006). A jackknife method was used to obtain 95% confidence intervals (CI) on loci, and estimates were calculated assuming random mating and excluded all alleles ≤ 0.05 (see Waples & Do 2008). The second method used to calculate contemporary N_e was based on summary statistics and an approximate Bayesian computation as implemented in the program ONeSAMP (Tallmon *et al.* 2008). This method also assumes that all loci are neutral and unlinked, and is based on simulations of a single, closed population. We used lower and upper bounds on the prior for N_e of two and 200, respectively, with upper bounds based on estimates of census size of approximately 200 adult individuals (Rabarisoa *et al.* 1997). Although estimates of N_e are typically much lower than census size (e.g. Frankham 1995), we also calculated N_e using upper bounds of 400 and 1000 to help further substantiate our results depending on the choice of prior used with this data set.

A significant change in population size (i.e. bottleneck) can produce distinctive genetic signals in population genotypic data (Cournuet & Luikart 1996; Luikart & Cornuet 1998; Beaumont 1999). Here we used two methods to ascertain whether historic changes in population size have occurred for the Madagascar fish-eagle population. The first method is based on the heterozygosity excess test implemented in the program Bottleneck version 1.2.02 (Piry *et al.* 1999). This approach follows the observation that recent population bottlenecks cause rare alleles to be lost more rapidly than common alleles, which can then lead to an expected heterozygosity excess when compared to a population at equilibrium with the same number of alleles (Cournet & Luikart 1996). With 22 polymorphic loci observed with the Madagascar fish-eagle (see below), this method should provide sufficient power to detect whether or not a population bottleneck had occurred within the recent past ($< 4N_e$ generations ago; see Cournet & Luikart 1996). Using the above method, equilibrium conditions were simulated using 1000 replications assuming an infinite allele model (IAM), a stepwise-mutation model (SMM), or a two-phase model of mutation (TPM, with 10, 20 or 30% multistep mutations), and significance was tested using Wilcoxon signed-rank tests.

The second method used to investigate changes in population size was a Bayesian coalescent-based approach as implemented in the program MSVAR 0.4b1 (Beaumont 1999). This method assumes an SMM and estimates the posterior distributions of the rate of population change ($r = N_0/N_1$, where N_0 = current effective number of chromosomes and N_1 = number of chromosomes at the time of population decline or expansion), the time in generations when the population started to expand or decline ($t_f = t_a/N_0$, where t_a = number of generations since the beginning of the expansion or decline), and the genetic parameter $\theta = 2N_o\mu$, where μ is the locus mutation rate. In a declining population, $\log_{10}(r)$ is smaller than 1, while in an expanding or a stable population, $\log_{10}(r) > 1$ or $= 1$, respectively. Rectangular priors were assumed for the parameters with limits of (–4, 2) for $\log_{10}(r)$ (–6, 2.5) for $\log_{10}(t_f)$, and (–0.5, 2.5) for $\log_{10}(\theta)$ with an exponential model of population change, while parameter limits of (–5, 3) (–5, 1), and (–2, 6), respectively, were used with a linear model. To check for stability of parameter estimates, we conducted five independent replications using Markov chain simulations for each model of population change using different parameter configurations and starting values. Each run consisted of 20 000 thinned updates and a thinning interval of 100 000 steps, producing 2×10^9 updates. The first 10% of updates were discarded as burn-in, and the remaining data were used to obtain the mode and 10, 50, and 90% highest posterior densities (HPD) of the posterior distributions for each parameter using the statistical package *R* (www.r-project.org) with the module Locfit 2.0 (http://locfit.herine.net). Because we do not know the degree of historic polymorphism for each locus amplified for the Madagascar fish-eagle, we used only polymorphic loci ($n = 22$) observed in the extant population for this method.

Results

Genetic diversity

Out of 47 microsatellite loci, only 22 loci were polymorphic in the Madagascar fish-eagle population. When compared to its sister taxon, the African fish-eagle (see Lerner & Mindell 2005), all estimates of genetic diversity (Table 1) based on the total number of loci polymorphic in at least one of the two species (36 loci) were significantly lower in the Madagascar fish-eagle (Wilcoxon signed-rank tests; P values < 0.001). Similarly, significantly lower ($P < 0.04$) estimates of genetic diversity were observed in most cases when we compared the Madagascar fish-eagle population with all other species, regardless of the particular set of loci used in the analyses (Table 1). The two exceptions consisted of estimates for mean A and A_{Priv} between the Madagascar fish-eagle and bald eagle populations, which was likely influenced by the small sample size ($n = 8$) for the bald eagle population used in this study.

All population/locus combinations were in Hardy–Weinberg equilibrium; however, there was evidence of a possible null allele in the Madagascar fish-eagle population for locus Hvo12 (see also Tingay *et al.* 2007). Removal of this locus from the analyses did not change the interpretation of our results. In all but one case, no evidence of linkage disequilibrium was observed after correcting for multiple comparisons. Significant linkage disequilibrium was observed between Hvo05 and Hvo06 (see also Tingay *et al.* 2007); however, this was observed only in the Madagascar fish-eagle population. Population F_{IS} estimates were not significantly different from zero (Table 1), with the exception of the Madagascar fish-eagle population when utilizing all 22 polymorphic loci ($F_{IS} = 0.099$). This result was largely due to two loci (Hvo012, $F_{IS} = 0.446$ and Hle05, $F_{IS} = 1.0$) each possessing two alleles and showing heterozygote deficiency. After re-analysing the Madagascar fish-eagle population excluding the above two loci, F_{IS} (= 0.083) was not significantly different from zero.

Contemporary effective population size

Estimates of contemporary effective population size (N_e) for the Madagascar fish-eagle was 23.6 (95% CI 11.8–59.5) based on the unbiased linkage disequilibrium method of Waples (2006). Using ONeSAMP, our median estimate of N_e was 24.0 with 95% credible limits (CL) of 18.9–34.8 breeding individuals. With upper priors on N_e of 400 and 1000, estimates of N_e were 29.0 (95% CL = 21.9–44.8) and 31.1 (95% CL = 22.1–51.2), respectively, thereby supporting that this method is relatively robust to changes in the prior (see also Tallmon *et al.* 2008). Both LDNe and ONeSAMP gave similar estimates for N_e. Therefore, we chose to use $N_e = 24.0$ (18.9–34.8) for the remainder of this study, particularly for the purpose of quantifying the timing of population decline (t_f) from MSVAR (see below).

Demographic history

None of the Wilcoxon signed-rank tests from the program Bottleneck were significant, regardless of mutation model or the percentage of multistep mutations used in the TPM. These results suggest that the Madagascar fish-eagle population has not experienced a recent and/or strong population bottleneck within the past 2–4N_e generations (Cournuet & Luikart 1996).

In contrast, our results from MSVAR strongly support the assertion that the Madagascar fish-eagle population experienced a reduction in population size with 90% HPD for $\log_{10}(r) < 1$ (Fig. 1). However, this reduction occurred many generations in the past given the positive posterior distributions for $\log_{10}(t_f)$ (see below). Five independent simulations for each model of population change (i.e. linear and exponential) showed concordant results, although wider posterior distributions were observed for the simulations using the linear model of population change (data not shown) than those from the exponential model (Fig. 1).

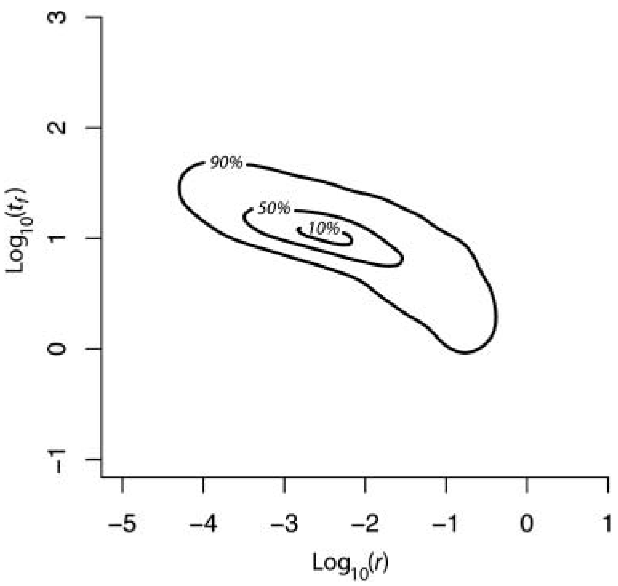

Fig. 1 Plot of the marginal posterior distribution of $\text{Log}_{10}(r)$ and $\text{Log}_{10}(t_f)$ for the Madagascar fish-eagle. $\text{Log}_{10}(r) < 1.0$ and > 1.0 indicate decline and expansion in population size, respectively, and increasing values of $\text{Log}_{10}(t_f)$ indicate change in population size occurring in the more distant past. The solid lines give the 0.9, 0.5, and 0.1 HPD limits estimated from the microsatellite data.

Based on these simulations and the average mode of –2.395 (90% HPD –3.825 to –0.831) for $\log_{10}(r)$, the contemporary population is roughly 0.4% (range 0.01–14.8%) of its historic size suggesting a strong decline in the Madagascar fish-eagle population. The average mode of $\log_{10}(t_f)$ was 1.069 (90% HPD 0.381–1.589), indicating that the population started to decline 11.7 N_o generations ago. Assuming that the contemporary N_e is 24 (corresponding to $N_o = 48$, measured as the number of chromosomes) and the earliest recorded age at first breeding for Madagascar fish-eagles is 5 years (Tingay 2005), the estimate of the population decline dates to approximately 2800 years ago, or 600–9000 years BP when considering the 90% HPD limits on $\log_{10}(t_f)$.

Because individual life expectancy has not been determined for this species, we do not have an accurate estimate of the average age of reproduction; however, it is probably more than 5 years, or the age at which breeding plumage is first obtained. Therefore, using a more biologically realistic value for the average age of reproduction, these dates would be even further back in time. For example, at a generation time of 10 years, the estimate of population decline would date to ~5600 years (or between 1200 and 19 000 years

BP based on the 90% HPD limits). Although, low precision was observed when considering the 90% HPD limits for each parameter, the Bayesian approach of Beaumont (1999) does provide strong general support for a long-term historic decline in the Madagascar fish-eagle population as opposed to a more recent decline within the past 100 years. This finding is further supported from the results obtained from the program Bottleneck as described above.

Discussion

The critically endangered Madagascar fish-eagle population possesses extremely low levels of neutral genetic diversity compared to its sister taxon, the African fish-eagle in Kenya, and other closely related congeners, the bald eagle from southeastern USA and the white-tailed eagle from Sweden. In the majority of pairwise comparisons between species, these differences in genetic diversity measures (Table 1) were significant ($P < 0.05$). However, the cause of the low level of genetic diversity in Madagascar fish-eagles appears not due to recent declines in population size (at least within the past 50–100 years), but rather to a much older bottleneck event dating back approximately 11.7 N_0 generations ago. Despite some uncertainty regarding details of microsatellite DNA evolution (see Ellegren 2004), which may then influence the accuracy of our estimated demographic parameters, the results from both MSVAR and Bottleneck do suggest, however, that the Madagascar fish-eagle population has been surviving for many generations with low levels of neutral genetic diversity. Therefore, this low estimate should not indicate that this species is of immediate conservation concern based exclusively on levels of genetic diversity alone (see also Amos & Harwood 1998; Brodie 2007; but see Frankham 2001, 2005). Thus, factors such as habitat availability, direct/indirect human persecution, or the introduction of pathogens are likely to be of more immediate concern for this species' long-term persistence (e.g. Watson *et al*. 1999; Watson & Rabarisoa 2000; Tingay 2005).

Only a few studies have documented long-term persistence despite low levels of neutral genetic diversity in wild vertebrate populations such as brown bears (*Ursus arctos*; Paetkau *et al*. 1998), kangaroo rats (*Dipodomys heermanni morroensis*; Matocq & Villablanca 2001), tuco-tucos (*Ctenomys sociabilis*; Hadly *et al*. 2003), snub-nosed golden monkeys (*Rhinopithecus roxellana*; Li *et al*. 2003), saddlebacks (*Philesturnus carunculatus*; Taylor *et al*. 2007), and wandering (*Diomedea exulans*) and Amsterdam (*Diomedea amsterdamensis*) albatrosses (Milot *et al*. 2007). Some of these populations are thought to have survived for thousands of generations with low levels of genetic diversity. For example, a population of the endemic colonial subterranean rodent the tuco-tuco (*C. sociabilis*) in northern Patagonia, Argentina, has persisted with low levels of genetic diversity for at least 2000 years as determined by temporal analyses using ancient DNA (Hadly *et al*. 2003; Chan *et al*. 2005) and coalescent simulations (Chan *et al*. 2006). Likewise, Milot *et al*. (2007) reported extreme genetic uniformity in amplified fragment length polymorphism (AFLP) data in two avian sister species, the wandering and Amsterdam albatrosses. They suggested that these two albatross species have possessed low genetic variability since diverging from their common ancestor approximately 840 000 years ago.

Ultimately, the amount of neutral genetic variation maintained in a population over time is due to a balance between gain (i.e. mutation and gene flow) and loss (i.e. genetic drift). In the case of the albatross as mentioned above, Milot *et al*. (2007) proposed multiple factors that could explain the continuance of such low diversity over time, such as the influence of low annual fecundity and/or small population size on the accumulation of nucleotide substitutions, or a reduced rate of mutation attributed to increased body size and slower metabolic rates (e.g. Gillooly *et al*. 2005). In relation to drift, fluctuating population size (Vucetich *et al*. 1997), structure (i.e. metapopulation; Whitlock & Barton 1997), and various life-history traits or demographic parameters can have a strong influence on N_e and the amount of genetic diversity maintained in a population (Anthony & Blumstein 2000). Traits such as overlapping generations, unequal sex ratios, and variance in both reproductive success and family size can also affect the overall effective size of a population (Caballero 1994), while those such as age at first breeding and life expectancy could be important for buffering the population against extinction when experiencing environmental and/or demographic stochasticity (Sæther *et al*. 2004, 2005) or increase the risk of extinction in declining species (Purvis *et al*. 2000).

Similar to other large-bodied birds of prey, many of the above factors have likely influenced the effective size of the Madagascar fish-eagle population over many generations. In general, large-bodied species at high trophic levels (e.g. top-predators) tend to have small population sizes and densities (Purvis *et al*. 2000; Krüger & Radford 2008; see also Lodé 2006), and often their continued presence is a good indicator of ecosystem health (Terborgh *et al*. 2001; Sergio *et al*. 2006). Conservatively, our results indicate that the Madagascar fish-eagle population has been maintained at small size for hundreds to thousands of years and its current low level of genetic diversity is the result of historic small population size rather than due to a recent decline within the past 100 years. The estimate of contemporary N_e based on genetic methods is only ~24 breeding individuals (range: 12–60 depending on the method and 95% CI). Although, it is recognized that island taxa typically possess smaller N_e compared to mainland taxa (Frankham 1997), such a low effective population size is a possible concern in light of new potential human-induced stressors that this species has not experienced in the past, particularly in context to its low genetic diversity and ability to persist in a rapidly changing environment (see Whiteman *et al*. 2006; Hale & Briskie 2007; Reed *et al*. 2007; Hannah *et al*. 2008).

Recent landscape changes through human habitat modifications and encroachment are likely to affect the breeding biology and population viability of the Madagascar fish-eagle (Tingay 2005). Direct persecution by humans includes destruction of nests and young (Thiollay & Meyburg 1981; Rabarisoa *et al*. 1997), theft of nestlings for pets and food (Watson *et al*. 1999), shooting and trapping of adults, and the use of eagle body parts in traditional medicine (Rabarisoa *et al*. 1997). Likewise, indirect effects are also of concern. Increased habitat alteration by cutting of trees used for nesting, the conversion of wetlands to rice paddies, and increased disturbance associated with fishing practices and tourism are examples likely to influence productivity and survivorship of this rare species (Rabarisoa *et al*. 1997; Watson & Rabarisoa 2000). After having been exposed to negative environmental perturbations, the Madagascar fish-eagle population would also be less likely to recover quickly due to low reproductive rates by producing only a single chick per reproductive cycle (Watson *et al*. 1999).

An important conclusion from this study is that it is unwise to assume that low neutral genetic diversity in endangered populations is necessarily due to a recent population bottleneck. Certainly, there are fitness-related concerns associated with populations of small size (Frankham 2005) and its ability to persist in a rapidly changing environment (Hannah *et al*. 2008), but whether low genetic diversity is the cause for small population size per se, or that small size is the cause of low genetic diversity, is an important distinction for informing the conservation management of an endangered species. Populations that have been at small size for many generations may be less likely to show signs associated with inbreeding depression because these populations may have been purged of deleterious recessive alleles early in the demographic history of the species (Crnokrak & Barrett 2002; Leberg & Firmin 2008).

Furthermore, our estimates of neutral genetic diversity may not correspond closely with actual levels of genetic diversity associated directly with population fitness traits or adaptive variation (Hedrick 2001; Moss *et al*. 2003). In a sufficiently small population, however, both deleterious and/or adaptive alleles can drift to fixation or be removed from the population based on neutral expectations (e.g. Florida panther, *Puma concolor coryi*, Culver *et al*. 2008; California condor, *Gymnogyps californianus*, Ralls *et al*. 2000). Numerous studies have highlighted the importance of phenotypic plasticity and its role in a population's ability to react to a changing environment (Pertoldi *et al*. 2007; Gienapp *et al*. 2008), and such a capacity may be particularly important in populations of small size with low neutral genetic diversity (e.g. Noel *et al*. 2007). Because we document extremely low levels of neutral genetic diversity of which the population has presumably maintained for hundreds of generations, any future population declines will likely go unnoticed without any further appreciable genetic loss (see Taylor & Jamieson 2008), at least with markers similar to those used in this study. We conclude that future strategies aimed at preventing the extinction of the Madagascar fish-eagle will benefit most from measures introduced to minimize human-induced perturbations. The Madagascar fish-eagle has extremely low levels of neutral genetic diversity, yet this alone does not indicate that it is unlikely to persist for future generations. As this study highlights, small inbred populations may not necessarily be at risk of extinction because of inbreeding depression or loss of evolutionary potential (see also Pertoldi *et al*. 2007). However, we must acknowledge that the contemporary rate of environmental change likely differs from that in the past, particularly in Madagascar (see Hannah *et al*. 2008). Therefore, we further recommend that the population be expanded to occupy a larger geographical distribution. An increase in the species' distribution will help prevent their extinction when exposed to stochastic factors such as climate change and disease that could seriously threaten a species with a population size of only 200 individuals.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Details for the 47 Accipitridae microsatellite loci tested for polymorphism in four *Haliaeetus* species.

Table S2 Variability of Accipitridae microsatellite loci in four *Haliaeetus* species. N, number of unrelated individuals; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.