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Combining genetic and ecological data to assess the conservation status of the endangered Ethiopian walia ibex

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Abstract

Knowledge about the phylogenetic history, genetic variation and ecological requirements of a species is important for its conservation and management. Unfortunately, for many species this information is lacking. Here we use multiple approaches (phylogenetics, population genetics and ecological modelling) to evaluate the evolutionary history and conservation status of *Capra walie*, an endangered flagship species of wild goat endemic to Ethiopia. The analysis of mitochondrial cytochrome *b* and Y-chromosome DNA sequences suggests that *C. walie* forms a monophyletic clade with *Capra nubiana*, but potentially has been isolated for up to 0.8 million years from this closely related species. Microsatellite DNA analyses show that *C. walie* has very low genetic variation (mean heterozygosity = 0.35) compared with other endangered mammals. This reduced variation likely derives from a prolonged demographic decline and small effective population size. Ecological niche modelling using the bioclimatic features of habitats occupied by *C. walie*, suggests ecological differences between *C. walie* and *C. nubiana*, and identifies the areas most suitable for future reintroductions of *C. walie*. The genetic and bioclimatic data suggest that *C. walie* is distinct and requires immediate conservation actions including genetic monitoring and reintroductions to establish independent populations. This study illustrates how combining noninvasive sampling along with genetic and ecological (bioclimatic) approaches can help assess conservation status of poorly known species.

Introduction

Ethiopia is renowned for its endemic fauna and flora, which arise from the vast extent and isolation of its highlands within the Afrotropical region (Stephens *et al*., 2001). These habitats host unique communities of species adapted to high altitudes, with remarkable levels of endemism (Kingdon, 1997). Over the last century, many of Ethiopia's endemic species have been decimated by habitat loss and fragmentation (Yalden *et al*., 1996; East, 1997).

The critically endangered walia ibex *Capra walie* is the most southerly distributed taxon of its genus, confined to a small area (95 km^2) in the Simen Mountains (Fig. 1). The species occupies a narrow habitat niche and is vulnerable to human disruptions such as habitat loss, illegal hunting, disease and competition from livestock (Nievergelt, 1981). Historical data on species abundance and distribution are scarce. Surveys conducted in the last four decades estimate a population size of 150–400 individuals (Blower, 1970; Nievergelt, 1981; Hurni, 1986). Currently, the estimated total population size is about 450 animals (Simen Mountains National Park Office, pers. comm.).

Endangered species often face multiple threats. Their conservation assessment and planning is therefore complex, and requires multiple approaches, including studies on demography, population genetics and ecological modelling. Unfortunately, this information is often hard to collect, and most studies focus only on a limited array of aspects. This lack of information is particularly relevant in tropical regions (Gardner, Barlow & Peres, 2007), where most threatened and endemic taxa are concentrated (Orme *et al*., 2005; Grenyer *et al*., 2006).

Capra walie is a remarkable and charismatic example of this situation. Like other endangered species, *C. walie* faces

Figure 1 Geographic distribution of *Capra walie* and *Capra nubiana*.

multiple threats including environmental change, small population size, demographic stochasticity and risk of inbreeding and loss of genetic diversity (e.g. Hoelzel *et al*., 1993; Paetkau *et al*., 1998; Flagstad *et al*., 2000). Inbreeding can reduce fitness-related traits such as fecundity and survival, and can therefore increase extinction risk (Frankham & Ralls, 1998; Saccheri *et al*., 1998; Hogg *et al*., 2006). Genetic variability may interact with demographic effects to produce the 'extinction vortex' of small populations (Gilpin & Soulé, 1986). Demographic and genetic processes often act synergistically (O'grady *et al*., 2006).

Moreover, the taxonomic status of *C. walie* is not clear. The traditional classification of the genus *Capra* is almost exclusively based on morphological characters, such as size, coloration, horn morphology and cranial proportion (Nievergelt, 1981). Few molecular studies have investigated the phylogeny of this genus (Manceau *et al*., 1999; Pidancier *et al*., 2006; Kazanskaya, Kuznetsova & Danilkin, 2007), and none of them included *C. walie*. As a consequence, the phylogenetic position of *C. walie* is still uncertain (Nievergelt, 1981; Yalden *et al*., 1996; Shackleton, 1997). Several authors have considered *C. walie* to be a subspecies of *Capra ibex* and/or *Capra nubiana*, while others consider it to be a separate species (Wilson & Reeder, 2005). The Ethiopian authorities and the IUCN usually treat *C. walie* as a distinct unit. The accurate identification and classification of organisms is an important prerequisite for conservation (May, 1990). It is therefore a priority to ascertain the taxonomic status of *C. walie*.

Here, we assess the conservation status of the *C. walie* using multiple approaches including molecular phylogenetics, population genetics and niche modelling. We evaluated the position of *C. walie* within the *Capra* genus by (1) analyzing mitochondrial DNA (mtDNA) and Y-chromosome sequences and (2) analyzing and comparing its ecological niche (using bioclimatic data) with that of the most closely related species, *C. nubiana*. We also used microsatellite loci to quantify genetic variation and test for recent population demographic declines. Our assessment of the conservation status of this species suggests an urgent need of management actions, including monitoring and translocations to help the survival of this critically endangered wild ungulate.

Materials and methods

Study area

The Simen Mountains National Park is a World Heritage Site, established in 1969 for the protection of *C. walie* (Fig. 1). It covers an area of 179 km^2 of the Simen Mountains watershed. Most of the Park is mountainous, with elevations ranging from 1900 to 4430 m a.s.l. Agriculture is the dominant land use in the area. Endemic species, such as

C. walie, the Ethiopian wolf *Canis simensis*, and the gelada baboon *Theropithecus gelada* are flagships for this area, and are threatened by extinction.

Molecular genetic analysis

We collected fecal pellets from 37 known individuals of *C. walie* during the rainy season. Most of the samples were collected fresh, kept in separate vials containing 20 g of silica gel and stored at 4 °C until extraction. For interspecific comparisons, we also included tissue samples from *C. nubiana* ($n = 8$), *Capra cylindricornis* ($n = 2$), *Capra caucasica* ($n = 2$), *Capra falconeri* ($n = 2$), *C. ibex* ($n = 1$) and *Capra pyrenaica* ($n = 1$) (Table 1).

Whole genomic DNA was extracted from fecal samples after a 20-min soak in washing buffer, using a DNeasy blood extraction kit (Qiagen GmbH, Hilden, Germany) with a 2-h protease incubation at 56 °C. DNA from tissue samples was extracted using the tissue extraction kit QIAamp Animal Tissue kit (Qiagen). We sequenced a part of the cytochrome (Cyt) *b* gene shown to be useful for inferring Bovidae phylogenies (Hassanin & Douzery, 1999) for 48 individuals: 32 *C. walie*, eight *C. nubiana*, two *C. cylindricornis*, two *C. caucasica*, two *C. falconeri*, one *C. ibex* and one *C. pyrenaica* (Table 1). A total of 600 base pairs (bp) of mitochondrial Cyt-*b* was amplified with the primers: CYTB_F (5′-CCCCACAAA ACCTATCACAAA-3′) and CYTB_IN_R (5′-CCTGTTTC GTGGAGGAAGAG-3′) (Pedrosa *et al.*, 2005). The PCR reactions were performed in a final volume of 25 μL containing 2 μL of DNA extract, 1 μM of each primer, 1 × PCR buffer, 200 μM of each dNTP, 1.5 mM $MgCl_2$ and 1 U of AmpliTag Gold polymerase (Applied Biosystems, Foster City, CA, USA). PCR was performed according to the following protocol: 10 min of initial denaturation at 95 °C followed by 35–40 cycles of: 30 s denaturation at 95 °C, 30 s annealing at 55 °C, 1 min extension at 72 °C; a final 7 min extension at 72 °C. The PCR products were purified using the Qiaquick kit (Qiagen).

The use of sequences from both the Y-chromosome and mtDNA is important because together they give a more comprehensive view of paternal and maternal phylogenetic history. Alone, mtDNA or any other single marker can give biased or incomplete information. We amplified the fifth exon region of the amelogenin gene, located on the Y-chromosome, for seven *C. walie* males using the primers CAPY1F: 5′-CCCAGCAGACTCCCCAGAATC-3′ and CAPY1R: 5′-CCAGAGGGAGGTCAGGAAGCA-3′ (Pidancier *et al.*, 2006), with 45 PCR cycles (95 °C for 10 min, 95 °C for 30 s, 58 °C for 40 s and 72 °C for 60 s). Two PCR products (320 and 400 bp) were obtained for males. The 320 bp Y-chromosome-linked band was purified using a QIAquick Gel Extraction kit (Qiagen).

Purified PCR products for Cyt-*b* and amelogenin genes were used as templates in 20 μL reactions using the BigDye Terminator Cycle Sequencing kit version 3.1 and analyzed on an ABI Prism 3130 automated sequencer. SeqScape 2.5 (ABI) was used to reconcile chromatograms of complementary fragments and to align sequences across taxa. All the sequences generated in this study were deposited in GenBank (Fig. 2). The sequences produced were analyzed together with sequences (31 Cyt-*b*, six amelogenin) from different wild goat species retrieved from GenBank (Fig. 2). For amelogenin, we retrieved one sequence per species. Sequences were aligned with Mega 3.1 (Kumar, Tamura & Nei, 2004), and then visually adjusted.

Eleven microsatellite loci previously analyzed in *C. ibex* (Maudet *et al.*, 2002) were genotyped in this study: BM4505, ETH10, HEL1, ILSTS030, OarAE54, OarFCB48, SR-CSRP24, SR-CSRP26, SR-CSRP6, SR-CSRP8 and SR-CSRP9. Loci were amplified individually in a total reaction volume of 25 μL containing 1.5 μL of DNA, 0.4 μM of each primer, 100 μM dNTP, 2.5 mM $MgCl_2$, PCR buffer, 5 ng of BSA and 1 U of AmpliTag Gold polymerase (ABI). Electrophoresis was performed on an ABI 3100 sequencer using the POP7 polymer. PCR amplification was performed applying the multi-tubes approach to reduce genotyping error (four replicates per each amplification; Taberlet *et al.*, 1996). For polymorphic loci, the rate of allelic dropout was 9.6%.

Table 1 Wild goat samples used for Cyt-*b* phylogeny

Taxon	Country	# of samples	# of haplotypes
Capra walie	Ethiopia	32	1
Capra nubiana	Israel	5	5
	Saudi Arabia	2	1
	Egypt	1	1
Capra cylindricornis	Russia	2	2
Capra caucasica	Russia	2	2
Capra falconeri	Pakistan	1	1
	Turkmenistan	1	1
Capra ibex	Italy	1	1
Capra pyrenaica	Spain	1	1
Total		48	16

GenBank accession numbers are in Fig. 2.

Phylogenetic reconstructions and divergence dates

Phylogenetic analyses and divergence dates were computed using Bayesian analyses (MrBayes V3.1.2, Huelsenbeck & Ronquist, 2001), maximum likelihood (ML), and neighbour-joining (NJ) methods. In MrBayes, the Markov Chain Monte Carlo (MCMC) search was run with 3×10^6 generations and repeated three times, sampling the Markov chain every 100 generations, with a burn-in of 3000 trees (as detected by plotting the log likelihood scores against generation number). The most appropriate likelihood model was determined using the Akaike Information Criterion implemented in MrAIC.pl 1.4.3 (Nylander, 2004). ML analyses were first performed with PHYML 2.4.4 (Guindon & Gascuel, 2003), using a HKY model of sequence evolution (Hasegawa, Kishino & Yano, 1985). Clade stability was estimated by nonparametric bootstrapping in 100 replicates with PHYML. NJ trees were constructed using MEGA v.3.1 (Kumar *et al.*, 2004). The distance matrix was constructed

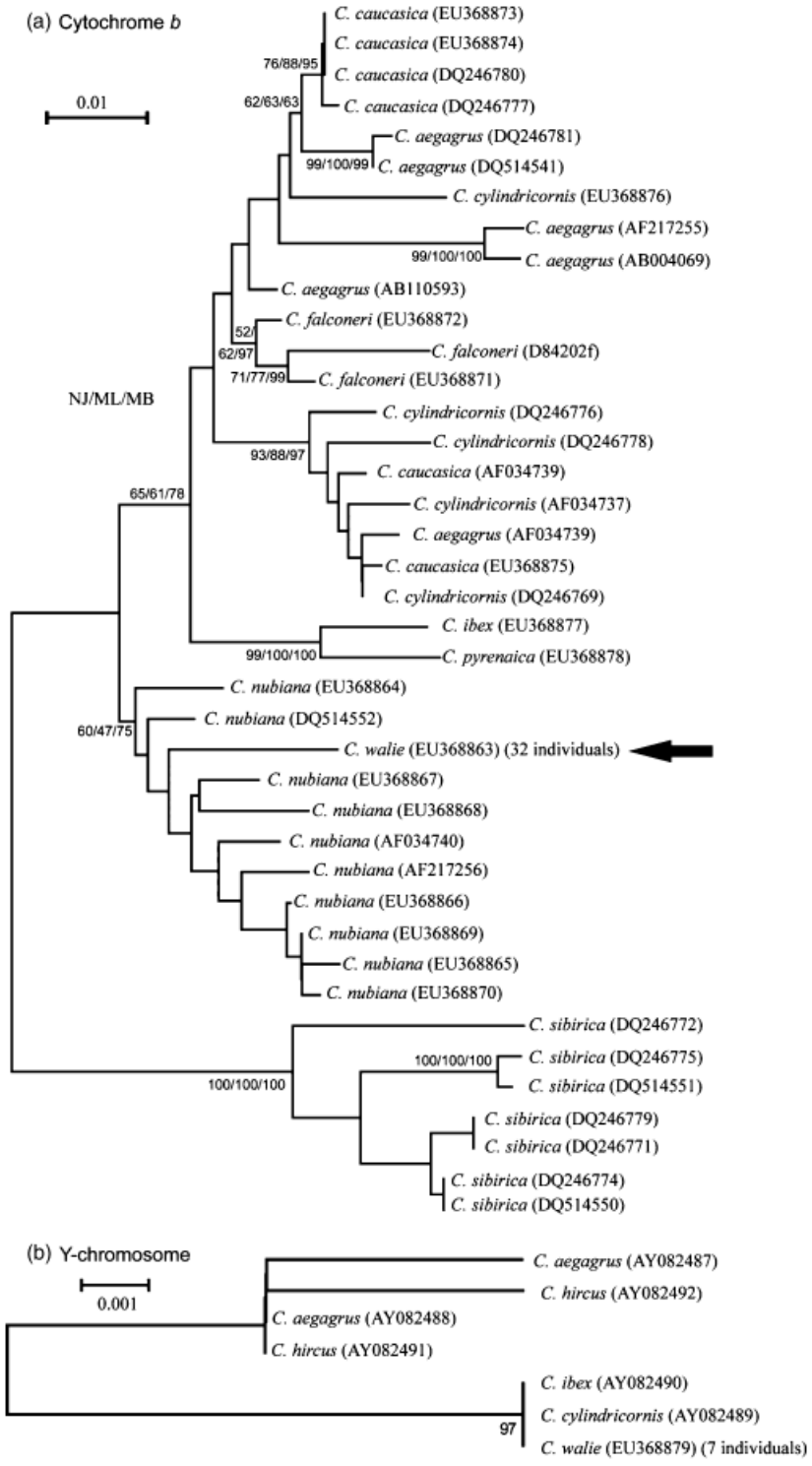

Figure 2 Position of *Capra walie* in the phylogeny of genus *Capra*. (a) Bayesian tree, based on mitochondrial DNA (Cyt-*b*); numbers are bootstrap values of neighbour-joining, maximum likelihood and Bayesian analyses. (b) Bayesian tree, based on Y-chromosome (amelogenin). GenBank accession numbers are shown in parentheses.

using Kimura's two-parameter distance (Kimura, 1980) and the robustness of each branch was determined by a non-parametric bootstrap test with 1000 replicates and a tree bisection-reconnection branch swapping algorithm. *Capra sibirica* (the most divergent *Capra* taxon) was used as out-group.

Because the likelihood ratio test rejected a global molecular clock for Cyt-*b* ($P<0.05$), estimates of divergence times were obtained with the Bayesian relaxed molecular clock approach with the Multidistribute program package, including Estbranches and Multidivtime (Thorne & Kishino, 2002). Estbranches was used to estimate the branch

lengths of the constrained topologies and the corresponding variance–covariance matrices. The F84 + γ model was used with ML parameters previously estimated by PAML (Yang, 1997). The variance–covariance matrices produced by Estbranches were used to run a MCMC analysis to estimate mean posterior divergence times on nodes with associated standard deviation and 95% credibility interval. The MCMC was sampled 10 000 times every 100 cycles and the burn-in stage was set to 100 000 cycles. The analysis was repeated three times. Priors were set according to the guidelines in Multidivtime's manual. To determine the time separating the in-group root from the present (rttm), this method needs to test different priors for the in-group age. The estimates of the age of the *Capra* in-group ranged from 5 to 7 million years ago (MYA) according to fossil records and previous molecular data (Hartl *et al.*, 1990; Hernandez-Fernandez & Vrba, 2005). Subsequently we used five input values for the mean in-group age (rttm = 5.0, 4.5, 3.7, 3.0 and 2.5 MYA), and the value giving the smallest standard deviations for the age of nodes was retained for further analyses. We used this conservative approach to avoid inflating the time of divergence.

Microsatellite data analysis

We used Genepop 3.4 (Raymond & Rousset, 1995) to test each locus for deviations from Hardy–Weinberg proportions and gametic disequilibrium. Genetix 4.05 (Belkir, 2004) was used to calculate the observed and the unbiased expected heterozygosity per each locus and across all loci. We also calculated F_{IS} as a measure of the level of inbreeding in a population (Wright, 1969); the significance of F_{IS} was estimated using 10 000 permutations. Bottleneck 1.2 was used to test for heterozygosity excess (bottleneck signature) using a Wilcoxon test (Cornuet & Luikart, 1996) and assuming a two-phase mutation model incorporating 5% multi-step mutations (variance of 12) and 95% stepwise mutation model, iterated 10 000 times. The test was also repeated assuming 100% infinite allele model and 100% stepwise mutation model. A Bayesian modelling approach implemented in MvSAR 1.3 was run to test for a past population decline (Beaumont, 1999; Storz & Beaumont, 2002). This method assumes a single stable population that was of size N_1 individuals t_a years ago and subsequently changed to the current population size N_0. MvSAR estimates the posterior probability distributions of the above demographic parameters using MCMC, and assuming a strict stepwise mutation model. We also calculated $r = N_0/N_1$ as a measure of demographic trend. All MCMC runs lasted 2×10^8 iterations on the basis of these parameters. The first 10% of runs of the chains were discarded as a burn-in. The remaining data yielded the median and the 90% highest posterior density (HPD) for each parameter. To ensure convergence and the stability of the model, it was run twice; all runs provided exactly the same results. We used an uninformative prior distribution (Goossens *et al.*, 2006), a generation time of 5 years (as in *C. ibex*: Maudet *et al.*, 2002), and a linear population growth (more suitable than exponential for modelling long-term population changes; Beaumont, 1999).

Bioclimatic niche assessment and potential distribution

To evaluate the realized bioclimatic niche of *C. walie* (Kearney, 2006) and test for differences in the realized niche between *C. walie* and *C. nubiana*, we assessed the observed geographic distribution of each species with a set of environmental variables. These variables included six bioclimatic variables and two topographic variables assessed at a resolution of 1 km^2. The bioclimatic variables (annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation of the warmest quarter and precipitation of the coldest quarter) were extracted from the Worldclim database (Hijmans *et al.*, 2005). The altitude and slope were extracted from the Hydro1k database (http://edc.usgs.gov/products/elevation/gtopo30/hydro/index.html).

The distribution for *C. nubiana* was extracted from the African Mammals Databank (AMD), an atlas of medium to large mammals (IEA, 1998). The distribution from the AMD was extracted in the form of extent of occurrence (EO), which defines the boundaries of a polygon in which observers have recorded occurrences of the species. The EO does not incorporate recorded absences, and does not provide information about how abundance varies across the range. In order to match the resolution of environmental data, species' range polygons were rasterized to a 1×1 km grid. Because the resolution of the AMD was too coarse for the restricted distribution of *C. walie*, we extracted its geographic distributions from data collected during multiple surveys performed from 1993 to 2006 (B. Gebremedhin, unpubl. data).

To analyze the realized niches of both *C. nubiana* and *C. walie*, and assess the potential distribution of *C. walie*, we developed boosted regression trees (BRT) (Ridgeway, 1999; Thuiller *et al.*, 2006) implemented in the Biomod framework (Thuiller, 2003). In a recent massive comparative analysis, BRT was proven to be one of the most powerful species distribution models (Elith *et al.*, 2006). We fitted BRT using 3000 relatively simple models (size = 2) whose predictions are combined to give more robust estimates of the response. Our algorithm used a boosted regression tree assuming a binomial distribution where each individual model consists of a simple classification tree, that is a rule-based classifier that consists of recursive partitions of the dimensional space defined by the predictors into groups that are as homogeneous as possible in terms of response. To estimate the relative importance of each predictor in the model, we used a permutation procedure, which randomly permutates each predictor independently and computes the associated reduction in predictive performance. We finally used BRT to produce the potential distribution of *C. walie* by projecting the realized niche of the species onto Ethiopia.

Results

DNA sequences

For mtDNA, all 32 *C. walie* individuals analyzed had the same haplotype (Table 1). The 47 samples collected from other *Capra* species yielded 38 different haplotypes. For the 39 mtDNA haplotypes, 111 nucleotide sites (nt) over the 600 bp used for the phylogenetic analyses were variable, and 81 nt were phylogenetically informative. The nucleotide frequencies were: 30.45% A, 27.72% C, 13.97% G and 27.86% T. The transition/transversion ratio (TS/TV) was 115/12 (9.58). Similarly, the seven *C. walie* males sequenced for Y-chromosome (amelogenin gene) in this study shared the same haplotype, while six haplotypes were found among all species (Fig. 2).

Phylogenetic analyses

The three independent Bayesian analyses (runs) converged on similar log-likelihood scores and became stationary before 2 000 000 generations. The consensus topologies of the three runs were identical (Fig. 2). The two other phylogenetics methods (ML and NJ) gave the same topology (data not shown, bootstrap values given in Fig. 2 on the consensus Bayesian tree), almost identical to that published by Manceau *et al.* (1999) and Pidancier *et al.* (2006). Several monophyletic groups supported by high bootstrap values are distinguished, such as the *C. walie*/*C. nubiana* clade, and the *C. ibex*/*C. pyrenaica* clade.

Using Multidivtime, the age of the in-group giving the smallest standard deviations for the age of nodes was 4.50 ± 0.5 MYA. This value was used to calibrate the Bayesian tree for estimating the divergence times under a relaxed molecular clock approach. The divergence of the *C. walie* and *C. nubiana* group from the *C. sibirica* group occurred about 2.14 ± 0.39 MYA. The divergence between *C. walie* and *C. nubiana* occurred 0.80 ± 0.44 MYA, while between *C. ibex* and *C. pyrenaica* occurred 1.23 ± 0.55 MYA.

The cladogram obtained from the analysis of the Y-chromosome grouped *C. walie* with *C. cylindricornis* and *C. ibex* (Fig. 2b). These three taxa shared nearly identical sequences.

Microsatellite polymorphism

All 24 *C. walie* individuals yielded clear genotype profiles at all loci. Six out of the 11 microsatellites were monomorphic (ETH10, SR8, SR9, SR24, SR26 and BM4505). No gametic disequilibrium was detected between pairs of loci (all $P > 0.05$ after Bonferroni's correction) and none deviated strongly from Hardy–Weinberg proportions ($P > 0.05$ in all tests). However, a significant heterozygote deficiency was detected across all loci ($P = 0.019$). The population level inbreeding coefficient F_{IS} was significantly greater than zero ($F_{IS} = 0.258$, $P = 0.006$). At the five polymorphic loci, we detected only two or three different alleles per locus. The total number of alleles across these polymorphic loci was only 11. The average observed heterozygosity among the 11 loci was 0.117 (range: 0–0.33) and the average expected heterozygosity was 0.157 (range: 0–0.494) (Table 2). When only polymorphic loci were considered, as in most microsatellite studies, the average observed heterozygosity was 0.258 and the average expected heterozygosity was 0.346.

Table 2 Genetic variability measured at five variable microsatellite loci

Locus	*K*	H_o	H_e
HEL7	3	0.292	0.423
FCB48	2	0.203	0.254
SR6	2	0.167	0.156
AE54	2	0.333	0.494
ILSTS030	2	0.292	0.403
Mean	2.2	0.256	0.346

Only polymorphic loci are shown here; 24 individuals were analyzed for all loci.

K, number of alleles per locus; H_o and H_e, observed and expected heterozygosity, respectively.

No signatures of recent population bottlenecks were detected using Bottleneck (one tailed tests, all $P > 0.07$). However, it should be noted that this test does not use the monomorphic loci, and that the power is low when using less than *c.* 10 polymorphic loci (Cornuet & Luikart, 1996). MsVAR detected a strong past decline of the population. Using only polymorphic loci, the demographic trend was estimated to be: $\mathrm{Log}_{10}(r) = -2.0$ (HPD $= -4.1/-0.1$), which approximately indicates a 100-fold population decline. The beginning of the decline t_a was estimated to occur about 10 000 years ago. However, the HPD intervals were very large (90–400 000 years). Because the five monomorphic loci were potentially polymorphic in the ancestral population, we repeated this analysis using the monomorphic loci (as in Beaumont, 1999). The analysis including all the 11 loci yielded similar results, but with a slightly more negative demographic trend [Log10 $(r) = -2.2$; HPD $= -4.2/-0.6$] and a longer period of decline (about 30 000 years, HPD = 500–1 200 000). The large HPD intervals of these analyses suggest that a larger number of variable microsatellites would be required for a more precise estimate of the inferred demographic decline.

Realized niches of *C. walie* and *C. nubiana*

For both *C. walie* and *C. nubiana*, one topographic (altitude) and two bioclimatic (mean temperature of the coldest month, precipitation of the warmest quarter) variables were selected as the best correlates of the species distributions, based on their predictive power (Fig. 3). The predictive accuracy of the models measured by the area under the receiver–operating curve (AUC) was 0.98 for *C. walie* and 0.96 for *C. nubiana*, showing a very good fit. The three-dimensional representation distinctly showed a strong niche differentiation between the two species. *Capra walie* occur at high altitude, in wet environments and at very extreme temperatures, while *C. nubiana*, also called the desert ibex, occurs at low altitude, over a large precipitation gradient

and not below *c*. 5 °C. For the three selected variables (Fig. 3) there is absolutely no overlap. Surprisingly, *C. nubiana* expresses a U-shaped relationship to temperature of the coldest month, possibly due to a nonequilibrium situation in some areas. The potential distribution of *C. walie* depicts relatively constrained suitable areas in Ethiopia (Fig. 4).

Discussion

Our study presents the first data on the phylogenetic history, genetic diversity and environmental variables for the critically endangered Ethiopian ibex. It also illustrates the usefulness of combining genetic and bioclimatic data to assess a species' status and develop management recommendations for conserving a species.

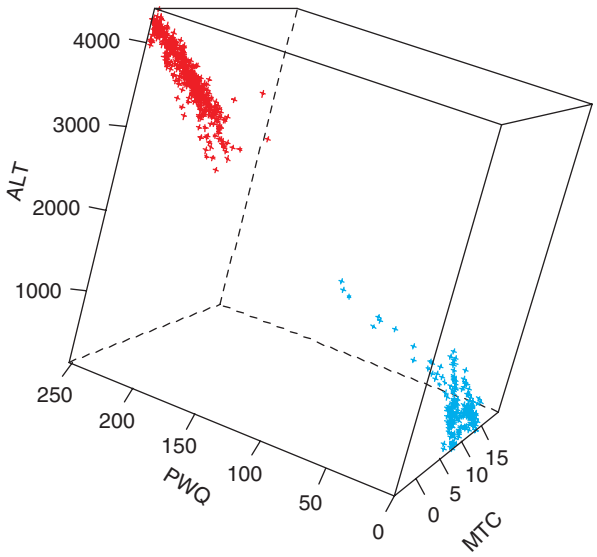

Figure 3 Three dimensional representation of the realized ecological niches of *Capra walie* (red) and of *Capra nubiana* (blue). The realized niches are expressed as a function of altitude (ALT), precipitation of the warmest quarter (PWQ) and temperature of the coldest month (MTC).

The large geographic range and the scattered distribution pattern of *C. nubiana* may have led to the observed high mtDNA diversity within this species, and consequently may have affected the apparently low support for the *nubiana*/*walie* clade. Despite relatively low bootstrap support, phylogenetic analysis suggested that *C. walie* and *C. nubiana* constitute a single clade (Fig. 2a), that *C. nubiana* is paraphyletic, and that *C. walie* potentially has been isolated from *C. nubiana* for up to 0.8 million years. This represents a relatively long period of reproductive isolation and would be sufficient for substantial adaptive differentiation, which can occur rapidly, even on contemporary time scales (Stockwell, Hendry & Kinnison, 2003). The genetic differentiation between *C. walie* and *C. nubiana* is similar to the differentiation observed between the alpine (*C. ibex*) and the Spanish ibex (*C. pyrenaica*), which are currently considered separate species. These results are consistent with the Shackleton (1997) classification, suggesting possible species status for *C. walie*.

Our phylogenetic data also strengthen the previous hypothesis (Pidancier *et al*., 2006) that there was a single wave of immigration of the *Capra* individuals towards Africa from ancestors in Central Asia. Phylogeographic studies of African mammals suggest that their evolutionary history was influenced by geological and climatic events of the Pleistocene, which resulted in repeated isolation of populations into refugia, and subsequent expansion of surviving individuals when environmental conditions became favorable (Arctander, Johansen & Coutellec-Vreto, 1999; Flagstad *et al*., 2001).

Genetic diversity measured by mtDNA, Y-chromosome and microsatellites were concordant and extremely low in *C. walie* relative to other mammals (Supporting Information Appendix S1). The single mtDNA haplotype (Fig. 2), low microsatellite heterozygosity and low allelic diversity are characteristic of small, isolated populations and populations

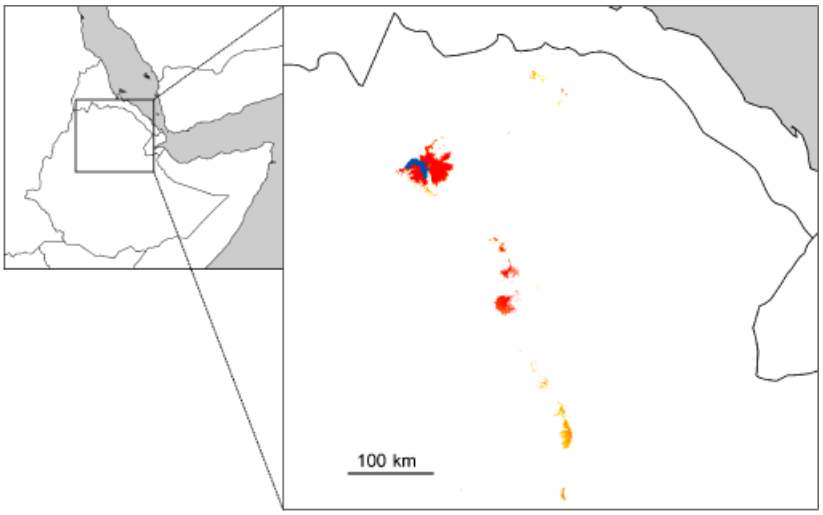

Figure 4 Modelled potential distribution of *Capra walie* in Ethiopia. The blue dots represent the current observed distribution of *C. walie* in Ethiopia, while the red shape represents the potential distribution of *C. walie* extrapolated from the species distribution model.

that have experienced extreme bottlenecks (Culver *et al.*, 2000; Alpers *et al.*, 2004; Jamieson, Wallis & Briskie, 2006). mtDNA has been proposed as a sensitive indicator of loss of genetic diversity (Avise, Ball & Arnold, 1988; Mulligan, Kitchen & Miyamoto, 2006). Moreover, the six monomorphic microsatellite loci are polymorphic in other species including several *Capra* spp. (Luikart *et al.*, 1999; Maudet, Luikart & Taberlet, 2001; Beja-Pereira *et al.*, 2004; Maudet *et al.*, 2004*a*). The high mean F_{IS} observed may be caused by population subdivision (Wahlund effect), or by high levels of mating between relatives.

Only a few critically endangered species and very small, isolated populations showed heterozygosity values as low as *C. walie* (Supporting Information Appendix S1). Only 5% of species of endangered birds reviewed by Jamieson *et al.* (2006), and only 14% of amphibians reviewed by Ficetola, Garner & De Bernardi (2007) showed values of expected heterozygosity lower than *C. walie*. These comparisons include only polymorphic loci because most publications use and report results from polymorphic microsatellites (Maudet *et al.*, 2002). Low genetic diversity is also evident if we include monomorphic loci for comparisons with other taxa for which monomorphic loci were reported. For example, with monomorphic loci, the expected heterozygosity was only 0.16 for *C. walie*, 0.14 for *Monachus monachus*, 0.20 for *C. ibex*, 0.25 for *Bison bonasus* and 0.27 for *Ursus arctos* of the Kodiak Island (see Supporting Information Appendix S1 for references). The low variation, including the lack of polymorphism at microsatellite loci that are usually highly variable in ungulates, suggests a severe loss of genetic diversity in *C. walie*. Future studies using microsatellites should report information also on monomorphic loci, as is standard practice for other markers, such as allozymes.

Genetic diversity is usually considered necessary for adaptation to changing environments (Reusch & Wood, 2007). The reduced genetic diversity in *C. walie* might increase immunological homogeneity, and decrease the species' potential to adapt to diseases or environmental modifications. Furthermore, populations with reduced heterozygosity and high levels of inbreeding often suffer inbreeding depression (Keller & Waller, 2002), which may reduce fitness and increases the risk of extinction (O'Grady *et al.*, 2006). Nevertheless, low diversity measured using neutral loci may provide incomplete indications, because selection can maintain higher diversity at fitness-related loci (Aguilar *et al.*, 2004). Future studies should evaluate whether *C. walie* suffers inbreeding depression, for example, by monitoring fitness and survival of individuals that are relatively inbred versus outbreed (Schwartz *et al.*, 2007). From a management perspective, it is urgent to conserve genetic diversity in *C. walie* by increasing population size and the number of populations. This can perhaps be accomplished by translocating unrelated individuals into different areas of the species' range.

The Bayesian modelling of microsatellite variation also suggested that the current small population size is the result of a prolonged and severe decline. Severe population declines are known to increase extinction risk by reducing efficiency of natural selection and increasing the frequency of deleterious alleles (e.g. Lande, 1994). Documenting the decline of a species is one criterion used to determine a species' conservation status (IUCN, 2007), yet it is difficult to ascertain past declines when historical information is lacking. Molecular genetics can aid in reconstructing past demography of a species or population, and can therefore play an important role in assessing conservation status.

Ecological models showed that *C. walie* and *C. nubiana* occupy clearly different bioclimatic niches. Only a few parameters (altitude, precipitation and temperature) were enough to clearly differentiate between these two closely related species, and no overlap was observed (Fig. 3). *Capra walie* lives in areas with colder temperature, higher elevation and more precipitation than *C. nubiana*. This suggests a complete isolation of these two species that might have developed unique ecological requirements related to the different habitat features within their respective range. The definition of the bioclimatic niche of *C. walie* allowed us to delineate the most suitable localities in Ethiopia (Fig. 4). The main suitable areas were located in the Simen highlands, where the last viable population of the species currently lives. The model also showed that there were a few potentially suitable areas both north and south of the current distribution ranges. If archaeological studies can confirm the past presence of *C. walie* in these mountains, they may in the future be used for the introduction of new populations.

Combining environmental and phylogenetic data together provide strong evidence that *C. walie* is distinct from *C. nubiana* and that *C. walie* warrants separate status as a conservation unit (or species). Such multidisciplinary approaches are too often not conducted even though they are highly advisable and often enlightening. For example, the brown bear (*U. arctos*) and the polar bear (*Ursus maritimus*) have striking differences in habitat, behavior and morphology, and are usually classified as distinct species. Nevertheless, genetic studies showed that *U. arctos* is paraphyletic with respect to *U. maritimus*, and the two taxa diverged only 0.3 MYA, probably in response to an adaptation to very different environments (Taberlet & Bouvet, 1992; Talbot & Shields, 1996). In such cases, the application of a strict phylogenetic species concept would merge the two bear taxa into a single species. It is therefore more appropriate to combine phylogenetic and ecological information, for the definition of evolutionary significant units that can be used as units for conservation. Following this approach, *C. walie* should be considered as a distinct unit for conservation. *C. walie* in this study represents an excellent example of the importance and usefulness of combining data from multiple sources when identifying conservation units, assessing a species status and developing management recommendations.

Conservation recommendations

The analysis of mtDNA suggests that *C. walie* is phylogenetically distinct and diverged thousands of years ago from its

closest relative, *C. nubiana*. The combination of ecological and genetic data implies that *C. walie* should be considered as a distinct conservation unit. The low genetic variation, the existence of only one small population of *C. walie*, along with continued risks of habitat loss, climate change and potential emerging diseases, call for urgent conservation actions, even though the population size is currently increasing slightly.

A similar situation has been observed in populations of the alpine ibex *C. ibex* in Europe (Maudet *et al.*, 2002). *Capra ibex* was reduced to fewer than 200 individuals in the 18th century, but reintroductions allowed a rapid rebound of the species, which now contains about 40 000 individuals. The ecological niche analysis showed that *C. walie* might have occupied larger areas, mainly around the Simen Mountains and probably in other highlands west of the Rift Valley (Fig. 4). Careful attempts to reintroduce *C. walie*, along with post-reintroduction monitoring, should help re-establish the species' historical range (IUCN, 1998).

Reintroduction into suitable habitats is probably the best option to rapidly counteract immediate extinction risks. Habitat protection and reintroductions have facilitated the successful recovery of other ungulates, such as *C. ibex* (Maudet *et al.*, 2002), the Arabian oryx *Oryx leucoryx* (IUCN, 1998), and bighorn sheep *Ovis canidensis* (Singer, Papouchis & Symonds, 2000). Eventually, managing wildlife corridors connecting the different mountain ranges (that are currently fragmented by human activities) might help the species expand into a network of metapopulations, as long as devastating diseases are not transmitted through these corridors. However, additional detailed ecological studies should precede translocations, as this study focused on bioclimatic features and altitude.

Our study illustrates the importance and usefulness of combining environmental and genetic studies in a relatively holistic approach to identifying units for conservation, assessing demographic and genetic status and providing management information such as potential reintroduction locations where ecological conditions appear most appropriate. This multidisciplinary approach can be particularly useful for poorly known species, or for species facing multiple threats.

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References

Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D. & Wayne, R.K. (2004). High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proc. Natl. Acad. Sci. USA* **101**, 3490–3494.

Alpers, D.L., Van Vuuren, B.J., Arctander, P. & Robinson, T.J. (2004). Population genetics of the Roan antelope (*Hippotragus equinus*) with suggestions for conservation. *Mol. Ecol.* **13**, 1771–1784.

Amills, M., Jimenez, N., Jordana, J., Riccardi, A., Fernandez-Arias, A., Guiral, J., Bouzat, J.L., Folch, J. & Sanchez, A. (2004). Low diversity in the major histocompatibility complex class ii drb1 gene of the Spanish ibex, *Capra pyrenaica*. *Heredity* **93**, 266–272.

Arctander, P., Johansen, C. & Coutellec-Vreto, M.A. (1999). Phylogeography of three closely related African bovids (tribe Alcelaphini). *Mol. Biol. Evol.* **16**, 1724–1739.

Avise, J.C., Ball, R.M. & Arnold, J. (1988). Current versus historical population sizes in vertebrate species with high gene flow – a comparison based on mitochondrial-DNA lineages and inbreeding theory for neutral mutations. *Mol. Biol. Evol.* **5**, 331–344.

Beaumont, L.J. (1999). Detecting population expansion and decline using microsatellites. *Genetics* **153**, 2013–2029.

Beja-Pereira, A., Zeyl, E., Ouragh, L., Nagash, H., Ferrand, N., Taberlet, P. & Luikart, G. (2004). Twenty polymorphic microsatellites in two of North Africa's most threatened ungulates: *Gazella dorcas* and *Ammotragus lervia* (Bovidae; Artiodactyla). *Mol. Ecol. Notes* **4**, 452–455.

Belkir, K. (2004). *Genetix, logiciel sous WindowsTM pour la génétique des populations*. Monpellier: Université de Montpellier II.

Blower, J.H. (1970). The Simien – Ethiopia's new National Park. *Oryx* **10**, 314–316.

Chappell, D.E., Van Den Bussche, R.A., Krizan, J. & Patterson, B. (2004). Contrasting levels of genetic differentiation among populations of Wolverines (*Gulo gulo*) from Northern Canada revealed by nuclear and mitochondrial loci. *Conserv. Gen.* **5**, 759–767.

Cornuet, J.M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001–2014.

Culver, M., Johnson, W.E., Pecon-Slattery, J. & O'Brien, S.J. (2000). Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* **91**, 186–197.

Dalén, L., Kvaloy, K., Linnell, J.D.C., Elmhagen, B., Strand, O., Tannerfeldt, M., Henttonen, H., Fuglei, E., Landa, A. & Angerbjorn, A. (2006). Population structure in a critically endangered arctic fox population: does genetics matter? *Mol. Ecol.* **15**, 2809–2819.

Driscoll, C.A., Menotti-Raymond, M., Nelson, G., Goldstein, D. & O'brien, S.J. (2002). Genomic microsatellites as evolutionary chronometers: a test in wild cats. *Genome Res.* **12**, 414–423.

East, R. (1997). Ethiopia. *Antelope Survey Update* **6**, 23–49.

Elith, J., Graham, C.H., Anderson, R.P., Dudık, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A.,

Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J.M., Peterson, A.T., Phillips, S.J., Richardson, K.S., Scachetti-Pereira, R., Schapire, R.E., Soberon, J., Williams, S., Wisz, M.S. & Zimmermann, N.E. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**, 129–151.

Ficetola, G.F., Garner, T.W.J. & De Bernardi, F. (2007). Genetic diversity, but not hatching success, is jointly affected by post glacial colonization and isolation in the threatened frog, *Rana latastei*. *Mol. Ecol.* **16**, 1787–1797.

Flagstad, Ø., Syvertsen, P.O., Stenseth, N.C. & Jakobsen, K.S. (2001). Environmental change and rates of evolution: the phylogeographic pattern within the Hartebeest complex as related to climatic variation. *Proc. Roy. Soc. Lond. Ser. B – Biol. Sci.* **268**, 667–677.

Flagstad, Ø., Syvertsen, P.O., Stenseth, N.C., Stacy, J.E., Olsaker, I., Roed, K.H. & Jakobsen, K.S. (2000). Genetic variability in Swayne's Hartebeest, an endangered antelope of Ethiopia. *Conserv. Biol.* **14**, 254–264.

Frankham, R. & Ralls, K. (1998). Conservation biology – inbreeding leads to extinction. *Nature* **392**, 441–442.

Fredsted, T., Pertoldi, C., Schierup, M.H. & Kappeler, P.M. (2005). Microsatellite analyses reveal fine-scale genetic structure in grey mouse lemurs (*Microcebus murinus*). *Mol. Ecol.* **14**, 2363–2372.

Gardner, T.A., Barlow, J. & Peres, C.A. (2007). Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biol. Conserv.* **138**, 166–179.

Garner, A., Rachlow, J.L. & Waits, L.P. (2005). Genetic diversity and population divergence in fragmented habitats: conservation of Idaho ground squirrels. *Conserv. Gen.* **6**, 759–774.

Garnier, J.N., Bruford, M.W. & Goossnens, B. (2001). Mating system and reproductive skew in the Black Rhinoceros. *Mol. Ecol.* **10**, 2031–2041.

Gilpin, M.E. & Soulé, M.E. (1986). Minimum viable populations: processes of extinction. In *Conservation biology: the science of scarcity and diversity*. 19–34. Soulé, M.E. (Ed.). Sunderland, MA: Sinauer Associates.

Goossens, B., Chikhi, L., Ancrenaz, M., Lackman-Ancrenaz, I., Andau, P. & Bruford, M.W. (2006). Genetic signature of anthropogenic population collapse in Orang-utans. *PLoS Biol.* **4**, 285–291.

Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., Jones, K.E., Olson, V.A., Ridgely, R.S., Rasmussen, P.C., Ding, T.-S., Bennett, P.M., Blackburn, T.M., Gaston, K.J., Gittleman, J.L. & Owens, I.P.F. (2006). Global distribution and conservation of rare and threatened vertebrates. *Nature* **444**, 93–96.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**, 696–704.

Hartl, G.B., Burger, H., Willing, R. & Suchentrunk, F. (1990). On the biochemical systematics of the Caprini and the Rupicaprini. *Biochem. Syst. Ecol.* **18**, 175–182.

Hasegawa, M., Kishino, H. & Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**, 160–174.

Hassanin, A. & Douzery, E.J.P. (1999). The tribal radiation of the family bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome *b* gene. *Mol. Phylogen. Evol.* **13**, 227–243.

Hernandez-Fernandez, M. & Vrba, E.S. (2005). Body size, biomic specialization and range size of African large mammals. *J. Biogeogr.* **32**, 1243–1256.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). High resolution interpolated climate surfaces for global land areas. *Int. J. Clim.* **25**, 1965–1978.

Hoelzel, A.R., Halley, J.M., O'brien, S.J., Campagna, C., Ambom, T., Le Boeuf, B., Ralls, K. & Dover, A.G. (1993). Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. *J. Hered.* **84**, 443–449.

Hogg, J.T., Forbes, S.H., Steele, B.M. & Luikart, G. (2006). Genetic rescue of an insular population of large mammals. *Proc. Roy. Soc. Lond. Ser. B – Biol. Sci.* **273**, 1491–1499.

Huelsenbeck, J.P. & Ronquist, F. (2001). Mrbayes. Bayesian inference of phyologenetic trees. *Bioinformatics* **17**, 754–755.

Hurni, H. (1986). Management plan: Simen Mountains National Park and surrounding rural area. UNESCO World Heritage Committee and Wildlife Conserv. Org., Ethiopia. 122pp. Printed in Switzerland.

IEA. (1998). *AMD African mammals databank – a databank for the conservation and management of the African mammals Bruxelles*. Report to the Directorate-General for Development (DGVIII/A/1) of the European Commission.

IUCN. (1998). *IUCN guidelines for re-introductions*. Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group.

IUCN. (2007). 2007 IUCN red list of threatened species. http://www.iucnredlist.org (last accessed on 15 February 2008).

Jakob, S.S. & Mammen, K. (2006). Eight new polymorphic microsatellite loci for genetic analyses in the endangered common hamster (*Cricetus cricetus* l.). *Mol. Ecol. Notes* **6**, 511–513.

Jamieson, I.G., Wallis, G.P. & Briskie, J.V. (2006). Inbreeding and endangered species management: is New Zealand out of step with the rest of the world? *Conserv. Biol.* **20**, 38–47.

Kazanskaya, E.Y., Kuznetsova, M.V. & Danilkin, A.A. (2007). Phylogenetic reconstructions in the genus *Capra* (Bovidae, Artiodactyla) based on the mitochondrial DNA analysis. *Rus. J. Gen.* **43**, 181–189.

Kearney, M. (2006). Habitat, environment and niche: what are we modelling? *Oikos* **115**, 186–191.

Keller, L.F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Kim, K.S., Min, M.S., An, J.H. & Lee, H. (2004). Cross-species amplification of bovidae microsatellites and low diversity of the endangered Korean goral. *J. Heredity* **95**, 521–525.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Kingdon, J. (1997). *The Kingdon field guide to African mammals*. London: Academic Press.

Kumar, S., Tamura, K. & Nei, M. (2004). Mega3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinf.* **5**, 150–163.

Lande, R. (1994). Risk of population extinction from fixation of new deleterious mutations. *Evolution* **48**, 1460–1469.

Loew, S.S., Williams, D.F., Ralls, K., Pilgrim, K. & Fleischer, R.C. (2005). Population structure and genetic variation in the endangered Giant Kangaroo rat (*Dipodomys ingens*). *Conserv. Gen.* **6**, 495–510.

Lorenzini, R. (2006). A panel of polymorphic microsatellites in the threatened alpine chamois (*Rupicapra pyrenaica ornata*). *Mol. Ecol. Notes* **5**, 372–374.

Luenser, K., Fickel, J., Lehnen, A., Speck, S. & Ludwig, A. (2005). Low level of genetic variability in European bisons (*Bison bonasus*) from the Bialowieza National Park in Poland. *Eur. J. Wildl. Res.* **51**, 84–87.

Luikart, G., Biju-Duval, M.P., Ertugrul, O., Zagdsuren, Y., Maudet, C. & Taberlet, P. (1999). Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). *Anim. Genet.* **30**, 431–438.

Maldonado, J.E., Leonard, J., Miranda, G.A., Ortega, J., Wayne, R.K. & Aguilera, R.J. (2006). Ten polymorphic microsatellite loci for the endangered Buena vista lake shrew (*Sorex ornatus relictus*). *Mol. Ecol. Notes* **6**, 349–352.

Manceau, V., Despres, L., Bouvet, J. & Taberlet, P. (1999). Systematics of the genus *Capra* inferred from mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* **13**, 504–510.

Maudet, C., Beja-Pereira, A., Zeyl, E., Nagash, H., Kence, A., Ozut, D., Biju-Duval, M.P., Boolormaa, S., Coltman, D.W., Taberlet, P. & Luikart, G. (2004*a*). A standard set of polymorphic microsatellites for threatened mountain ungulates (Caprini, Artiodactyla). *Mol. Ecol. Notes* **4**, 49–55.

Maudet, C., Luikart, G., Dubray, D., Von Hardenberg, A. & Taberlet, P. (2004*b*). Low genotyping error rates in ungulate feces sampled in winter. *Mol. Ecol. Notes* **4**, 772–775.

Maudet, C., Luikart, G. & Taberlet, P. (2001). Development of microsatellite multiplexes for wild goats using primers designed from domestic Bovidae. *Gen. Sel. Evol.* **33**, S193–S203.

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Wursten, C., Gauthier, D., Obexer-Ruff, G., Michallet, J., Taberlet, P. & Luikart, G. (2002). Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in alpine ibex [*Capra ibex* (ibex)]. *Mol. Ecol.* **11**, 421–436.

May, R.M. (1990). Taxonomy as destiny. *Nature* **347**, 129–130.

Michaux, J.R., Libois, R., Davison, A., Chevret, P. & Rosoux, R. (2004). Is the western population of the European mink (*Mustela lutreola*), a distinct management unit for conservation? *Biol. Conserv.* **115**, 357–367.

Mitrovski, P., Hainze, D.A., Broome, L., Hoffmann, A.A. & Weeks, A.R. (2007). High levels of variation despite genetic fragmentation in populations of the endangered Mountain Pygmy-possum, *Burramys parvus*, in alpine Australia. *Mol. Ecol.* **16**, 75–87.

Moodley, Y. & Harley, E.H. (2005). Population structuring in Mountain zebras (*Equus zebra*): the molecular consequences of divergent demographic histories. *Conserv. Genet.* **6**, 953–968.

Mulligan, C.J., Kitchen, A. & Miyamoto, M.M. (2006). Comment on "Population size does not influence mitochondrial genetic diversity in animals". *Science* **314**, 1390a.

Nievergelt, B. (1981). *Ibexes in an African environment: ecology and social system of the walia ibex in the Simen mountains, Ethiopia*. Berlin: Springer Verlag.

Nyakaana, S., Okello, J.B.A., Muwanika, V. & Siegismund, H.R. (2005). Six new polymorphic microsatellite loci isolated and characterized from the African savannah elephant genome. *Mol. Ecol. Notes* **5**, 223–225.

Nylander, J.A.A. (2004). *Bayesian phylogenetics and the evolution of Gall wasps*. Uppsala: Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology.

O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W. & Frankham, R. (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biol. Conserv.* **133**, 42–51.

Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J., Ding, T.-S., Rasmussen, P.C., Ridgely, R.S., Stattersfield, A.J., Bennett, P.M., Blackburn, T.M., Gaston, K.J. & Owens, I.P.F. (2005). Global hotspots of species richness are not congruent with endemism or threat. *Nature* **426**, 1016–1019.

Paetkau, D., Waits, L.P., Clarkson, P.L., Craighead, L., Vyse, E., Ward, R. & Strobeck, C. (1998). Variation in genetic diversity across the range of North American brown bears. *Conserv. Biol.* **12**, 418–429.

Pastor, T., Garza, J.C., Allen, P., Amos, W. & Aguilar, A. (2004). Low genetic variability in the highly endangered Mediterranean Monk seal. *J. Hered.* **95**, 291–3000.

Pedrosa, S., Uzun, M., Arranz, J.J., Gutierrez-Gill, B., Primitivo, F.S. & Bayon, Y. (2005). Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. *Proc. Roy. Soc. Lond. Ser. B – Biol. Sci.* **272**, 2211–2217.

Pidancier, N., Jordan, S., Luikart, G. & Taberlet, P. (2006). Evolutionary history of the genus *Capra* (Mammalia, Artiodactyla): discordance between mitochondrial DNA and y-chromosome phylogenies. *Mol. Phylogenet. Evol.* **40**, 739–749.

Ramey, R.R., Liu, H.P., Epps, C.W., Carpenter, L.M. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's Meadow Jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of z-hudsonius as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Raymond, M. & Rousset, F. (1995). Genepop version 1.2: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Reusch, T.B.H. & Wood, T.E. (2007). Molecular ecology of global change. *Mol. Ecol.* **16**, 3973–3992.

Ridgeway, G. (1999). The state of boosting. *Comp. Sci. Stat.* **31**, 172–181.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.

Schwartz, M.K., Luikart, G. & Waples, R.S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends Ecol. Evol.* **22**, 25–33.

Shackleton, D.M. (Ed.) (1997). *Wild sheep and goats and their relatives: Status survey and conservation action plan*. Gland, Switzerland: IUCN.

Sinclair, E.A., Costello, B., Courtenay, J.M. & Crandall, K.A. (2002). Detecting a genetic bottleneck in Gilbert's potoroo (*Potorous gilbertii*) (Marsupialia: Potoroidae), inferred from microsatellite and mitochondrial DNA sequence data. *Conserv. Genet.* **3**, 191–196.

Singer, F.J., Papouchis, C.M. & Symonds, K.K. (2000). Translocations as a tool for restoring populations of bighorn sheep. *Restor. Ecol.* **8**, 6–13.

Stephens, P.A., D'sa, C.A., Sillero-Zubiri, C. & Leader-Williams, N. (2001). Impact of livestock and settlement on the large mammalian wildlife of Bale Mountains National Park, Southern Ethiopia. *Biol. Conserv.* **100**, 307–322.

Stockwell, C.A., Hendry, A.P. & Kinnison, M.T. (2003). Contemporary evolution meets conservation biology. *Trends Ecol. Evol.* **18**, 94–101.

Storz, J.F. & Beaumont, L.J. (2002). Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. *Evolution* **56**, 154–166.

Taberlet, P. & Bouvet, J. (1992). Génétique de l'ours brun des pyrénées (*Ursus arctos*): premiers resultats. *C. R. Acad. Sci. Ser. III – Sci. Vie-Life Sci.* **314**, 15–21.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P. & Bouvet, J. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acid Res.* **24**, 3189–3194.

Talbot, S.L. & Shields, G.F. (1996). Phylogeography of brown bears *(Ursus arctos)* of Alaska and paraphyly within the ursidae. *Mol. Phylogenet. Evol.* **5**, 477–494.

Thevenon, S., Thuy, L.T., Ly, L.V., Maudet, F., Bonnet, A., Jarne, P. & Maillard, J.C. (2004). Microsatellite analysis of genetic diversity of the Vietnamese Sika deer (*Cervus nippon pseudaxis*). *J. Hered.* **95**, 11–18.

Thorne, J.L. & Kishino, H. (2002). Divergence time and evolutionary rate estimation with multilocus data. *Syst. Biol.* **51**, 689–702.

Thuiller, W. (2003). Biomod – optimizing predictions of species distributions and projecting potential future shifts under global change. *Global Change Biol.* **9**, 1353–1362.

Thuiller, W., Midgley, G.F., Rouget, M. & Cowling, R.M. (2006). Predicting patterns of plant species richness in megadiverse South Africa. *Ecography* **29**, 733–744.

Vidya, T.N.C., Fernando, P., Melnick, D.J. & Sukumar, R. (2005). Population genetic structure and conservation of Asian elephants (*Elephas maximus*) across India. *Anim. Conserv.* **8**, 377–388.

Wang, Y.Q., Williams, D.A. & Gaines, M.S. (2005). Evidence for a recent genetic bottleneck in the endangered Florida keys silver rice rat (*Oryzomys argentatus)* revealed by microsatellite DNA analyses. *Conserv. Genet.* **6**, 575–585.

Wilson, D.E. & Reeder, D.M. (2005). *Mammal species of the world. A taxonomic and geographic reference*. Baltimore: Johns Hopkins University Press.

Wright, S. (1969). *Evolution and the genetic of populations. Vol. 2: the theory of gene frequencies*. Chicago: University of Chicago Press.

Xia, S., Zou, F.D. & Yue, B.S. (2006). Six microsatellite loci in forest Musk deer, *Moschus berezovskii*. *Mol. Ecol. Notes* **6**, 113–115.

Yalden, D.W., Largen, M.J., Kock, D. & Hillman, J.C. (1996). Catalogue of the mammals of Ethiopia and Eritrea .7. Revised checklist, zoogeography and conservation. *Trop. Zool.* **9**, 73–164.

Yang, Z. (1997). PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* **13**, 555–556.

Zenger, K.R., Eldridge, M.D.B. & Johnston, P.G. (2005). Phylogenetics, population structure and genetic diversity of the endangered Southern Brown bandicoot (*Isoodon obesulus*) in south-eastern Australia. *Conserv. Genet.* **6**, 93–204.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Comparison of microsatellite studies in mammals. Here we show studies analysing at least 20 individuals with ≥ 5 polymorphic microsatellites.

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