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Phylogeography and conservation genetics of Eld's deer (*Cervus eldi*)

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Abstract

Eld's deer (*Cervus eldi*) is a highly endangered cervid, distributed historically throughout much of South Asia and Indochina. We analysed variation in the mitochondrial DNA (mtDNA) control region for representatives of all three Eld's deer subspecies to gain a better understanding of the genetic population structure and evolutionary history of this species. A phylogeny of mtDNA haplotypes indicates that the critically endangered and ecologically divergent *C. eldi eldi* is related more closely to *C. e. thamin* than to *C. e. siamensis*, a result that is consistent with biogeographic considerations. The results also suggest a strong degree of phylogeographic structure both between subspecies and among populations within subspecies, suggesting that dispersal of individuals between populations has been very limited historically. Haplotype diversity was relatively high for two of the three subspecies (*thamin* and *siamensis*), indicating that recent population declines have not yet substantially eroded genetic diversity. In contrast, we found no haplotype variation within *C. eldi eldi* or the Hainan Island population of *C. eldi siamensis*, two populations which are known to have suffered severe population bottlenecks. We also compared levels of haplotype and nucleotide diversity in an unmanaged captive population, a managed captive population and a relatively healthy wild population. Diversity indices were higher in the latter two, suggesting the efficacy of well-designed breeding programmes for maintaining genetic diversity in captivity. Based on significant genetic differentiation among Eld's deer subspecies, we recommend the continued management of this species in three distinct evolutionarily significant units (ESUs). Where possible, it may be advisable to translocate individuals between isolated populations within a subspecies to maintain levels of genetic variation in remaining Eld's deer populations.

Keywords: *Cervus eldi*, conservation genetics, control region, endangered species, mitochondrial DNA, phylogeography

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Introduction

Eld's deer, or brow-antlered deer (*Cervus eldi*), is a highly endangered Southeast Asian cervid. Eld's deer were once distributed throughout much of Asia, their range extending from Manipur in eastern India to Indochina and southern China (Fig. 1). Due largely to hunting and habitat degradation, these deer have been extirpated from much of their historical range and now persist only in small, fragmented populations (Wemmer 1998; McShea *et al.* 1999). The species is currently listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) and is considered endangered by the World Conservation Union (IUCN).

Eld's deer belongs to the subgenus *Rucervus* which also includes the swamp deer or barasinga (*C. duvauceli*) and the extinct Shomburk's deer (*C. schomburgki*) (Geist 1998). Although the relationships within this group have not been assessed using molecular data, a recent phylogenetic

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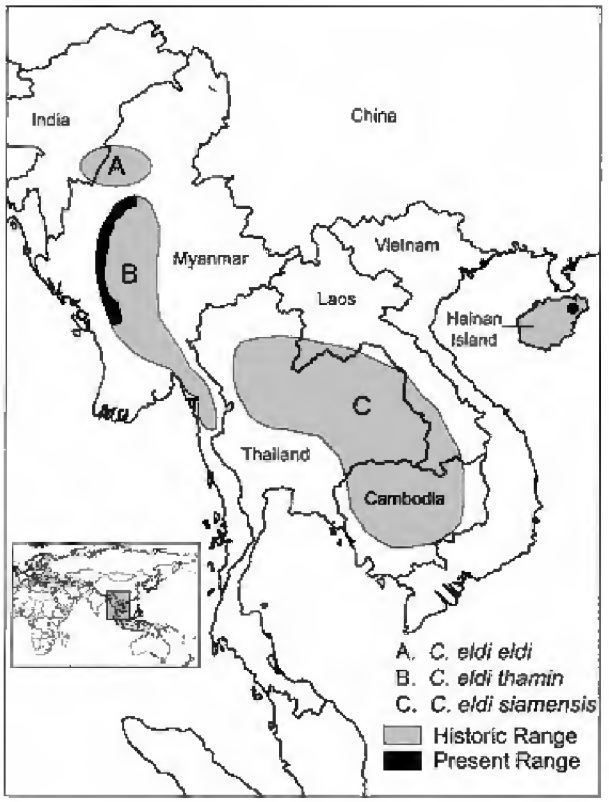

Fig. 1 Historical and present ranges for the three subspecies of Eld's deer. (A) *C. eldi eldi*, (B) *C. eldi thamin* and (C) *C. eldi siamensis*.

study placed Eld's deer as the sister taxon to another swamp-adapted species, Pere David's deer (*Elaphurus davidianus*), and these two species comprised the basal lineage in the *Cervus* clade (Randi *et al.* 2001).

Traditional taxonomy divides Eld's deer into three subspecies (Whitehead 1972). The most abundant of these is *C. e. thamin*, which is now found only in Myanmar. A survey conducted by the Wildlife Department of Myanmar in 1992 indicated that approximately 2200 deer remained in the country (Aung 1994), but more recent results suggest that fewer than half this number may remain in viable populations, most notably within Chatthin Wildlife Sanctuary (CWS) (McShea *et al.* 1999). Surveys have indicated that the CWS population declined by 40% between 1983 and 1995 (McShea *et al.* 1999), but intensive conservation efforts at this sanctuary have led to an increase in *C. e. thamin* numbers since 1996 (McShea & Aung 2001). In captivity, inbred *C. e. thamin* suffered greatly increased juvenile mortality (Ralls *et al.* 1979), suggesting that they may be highly susceptible to inbreeding depression.

The Indian and Indochinese subspecies, *C. e. eldi* and *C. e. siamensis*, respectively, have been pushed to the brink of extinction. Indeed, the Indian subspecies was considered extinct until a small population was rediscovered in the early 1950s (Ranjitsinh 1978). The population of *C. e. eldi* has since increased to approximately 150 individuals restricted to Indian zoos and Keibul Lamjao National Park in Manipur. The current status of *C. e. siamensis* in Indochina is largely unknown, although it is thought that small scattered herds may still remain (W. J. McShea, pers. comm.). A captive population of *C. e. siamensis* maintained at the Paris Zoo since 1937 was founded by only five individuals and has never been supplemented with animals from other populations. Ninety per cent neonatal mortality in this population is probably the consequence of severe inbreeding depression (Mauget *et al.* 2001). A population of approximately 800 *C. e. siamensis* also persists on Hainan Island in China (Wang *et al.* 2001). This island population has recovered from a severe bottleneck during which the population was reduced to 26 individuals (Wang 1976; Xu 1976). Some authors consider the Hainan population to be a distinct subspecies, *C. eldi hainanus* (Decoux 1993; Wang *et al.* 2001).

The three subspecies of Eld's deer show some variability in habitat preferences. *C. e. eldi* inhabit low-lying swamps (Lekagul & McNeely 1977) and live on floating mats of dense vegetation, known as 'phum' or 'phumdi'. This in an extremely scarce habitat, extending less than 15 km^2 in total area (Geist 1998). In contrast, *C. e. thamin* and *C. e. siamensis* are found most often in dry, deciduous dipterocarp forests with an open understorey (Salter & Sayer 1986). Lekagul & McNeely (1977) proposed that these animals originally inhabited swampy areas, but were forced recently into drier habitats due to pressures imposed by hunting and the expansion of agricultural areas. As a group, Eld's deer are primarily grazers, but will browse opportunistically and consume wild fruit and cultivated crops, particularly rice (Lekagul & McNeely 1977).

The three subspecies also show modest morphological differentiation. *C. e. eldi* possesses a modified foot, with splaying hooves and cornified skin on the back of its digits (Geist 1998). These modifications have been viewed as adaptations that may enable the deer to walk more easily on moist ground (Whitehead 1972). *C. e. eldi* also has the smallest antlers of the three subspecies (Geist 1998). Slight differences in pelage colour have also been noted. *C. e. siamensis* displays a more rufous colour than the other subspecies, whereas the Hainan Island deer reportedly bear white spots on the flanks (Geist 1998). It is not clear whether any of these morphological differences represent phenotypic plasticity or evolved responses to different habitats. Similar variation in foot morphology among barasinga subspecies (Geist 1998) suggests that this character may be relatively plastic over evolutionary time.

Phlyogeographic studies have demonstrated that traditional taxonomic boundaries are not necessarily concordant with the pattern of historical population structure revealed by genetic analysis (e.g. Mathee & Robinson 1999; Eizirik *et al.* 2001). In the past this has led to management

of subspecies as distinct populations in cases in which there was limited evidence for the subspecies designation (Ryder 1986). Alternatively, hybridization between genetically distinct lineages can lead to a loss of adaptive diversity and/or outbreeding depression (reviewed in Rhymer & Simberloff 1996).

Numerous strategies have been proposed to reconcile conflicts between taxonomic status and conservation objectives (Ryder 1986; Moritz 1994; Vogler & DeSalle 1994; Crandall *et al.* 2000; Fraser & Bernatchez 2001). The dominant paradigm for the identification of units for conservation is the evolutionarily significant unit (ESU). Under Moritz's (1994) framework, populations that are differentiated for mtDNA haplotypes or at nuclear loci are designated as management units (MUs), while populations that are reciprocally monophyletic for mtDNA haplotypes and show significant differentiation at nuclear loci are given ESU status. In cases where molecular data reflect modest levels of gene-flow, or recent historical connections, limited interbreeding between subspecies may help to prevent some of the deleterious effects of inbreeding. On the other hand, if populations are highly structured genetically, and meaningful adaptive variation is found to exist between taxonomic units, breeding between distantly related populations may lead to out-breeding depression and the subsequent loss of this adaptive variation. Although Moritz s paradigm provides a relatively straightforward method of assigning conservation status, this approach has been criticized for a number of weaknesses (Crandall *et al.* 2000; Fraser & Bernatchez 2001).

We used mtDNA control region sequences to examine the genetic structure of Eld's deer populations and to test whether current intraspecific taxonomy is congruent with evolutionary history. In particular, is the recognition of *C. e. thamin* and *C. e. siamensis* as distinct subspecies warranted and does the Hainan Island population deserve unique subspecies designation? We also assessed the distribution of genetic diversity among extant populations of Eld's deer by analysing individuals from a number of populations. Finally, we assessed the efficacy of strategies for maintaining genetic diversity in captive populations by comparing haplotype diversity in wild *C. e. thamin* with two captive populations, including one that is carefully managed and one that is not. Taken together, these data may assist conservationists and animal managers in developing genetic management strategies that help to ensure the long-term survival of Eld's deer.

Materials and methods

Sample collection

Hair or blood samples were collected from *C. e. eldi*, *C. e. siamensis* and *C. e. thamin* (Table 1). Most of the samples collected at CWS and the two samples from Hainan Island were collected opportunistically from the carcasses of dead animals. A few of the CWS samples were collected from deer that were live-trapped and released. The remaining samples were collected from animals in captivity (Table 1).

Laboratory methodology

Whole genomic DNA was isolated from 50 μL of whole blood, or 3–4 hairs using a QIAamp Tissue Kit (Qiagen). For hair samples we added 30 μL of 100 mg/mL dithiothreitol (DTT) to the tissue digestion buffer. DNA was amplified in a 50-μL reaction containing 0.25 mM $MgCl_2$, 1 μM of each primer, 0.25 mM of each dNTP and 1 unit of *Taq* DNA polymerase. Thermal cycling consisted of a denaturation step at 94° for 1 min, 30 cycles of denaturation (94°, 20 s), annealing (52°, 20 s) and extension (72°, 60 s) and a final extension step of 10 min at 72°. PCR products were gel-purified in 1% agarose, excised from the gel, and purified with a QIAquick Gel Extraction Kit (Qiagen).

Table 1 Sample names, sources and types. Samples from Myanmar and Hainan Island are from wild populations, whereas the rest were sampled in captivity

Subspecies	Sample	*n*	Source	Type
C. e. eldi	CEE01-05	5	Nehru Zoological Park, India	Hair
C. e. siamensis	CEH01,02	2	Hainan Island, China	Hair
	CES01-03	3	Dusit Zoo, Thailand	Blood
	CES04	1	Forest Department, Thailand	Blood
	CES05*	1	Paris Zoo	
	CEE06*	1	India	
C. e. thamin	CET01,02	2	Khao Kheow Open Zoo, Thailand	Blood
	CRC31-49	9	Conservation & Research Center, USA	Blood
	CWS01-14	12	Chatthin Wildlife Sanctuary, Myanmar	Hair
	YZM1-6	6	Yangon Zoo, Myanmar	Hair
	YZF1-6	6	Yangon Zoo, Myanmar	Hair

*Sequences published by Randi *et al.* (2001) (GenBank accession nos AF291892 and AF291893).

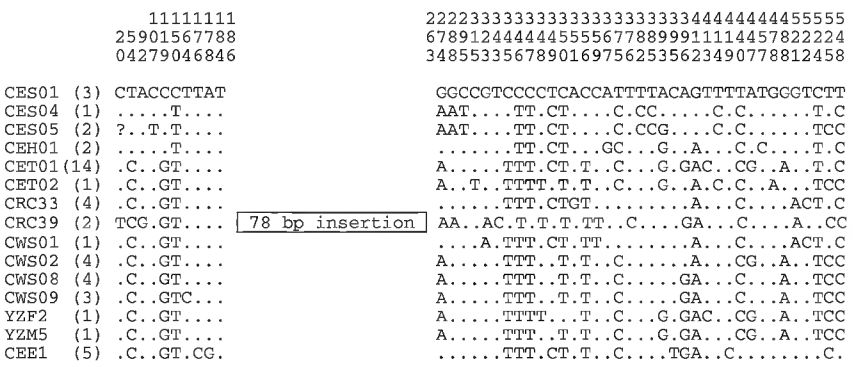

Fig. 2 Alignment of sequences used in this study. Only variable positions are shown. Numbers in parentheses indicate the number of individuals sharing each haplotype. The 78 bp insertion in the CRC 39 haplotype is a duplication of the following 78 bases. Except for the CET 01 haplotype, which was also recorded in individuals from CRC and Yangon Zoo, each unique haplotype was found in only one of the sampled populations.

Based on published sequences, deer specific primers were designed for the 5 hypervariable portion of the mtDNA control region with primers located in transfer RNA Proline and the central domain of the control region, respectively (Cerv.tPro: 5 -CCACYATCAACACCCAAAGC-3 , CervCRH: 5 -GCCCTGAARAAAGAACCAGATG-3). Double-stranded PCR products were sequenced directly in cycle sequencing reactions using *Taq* DNA Polymerase FS (PE Applied Biosystems). After the removal of unincorporated dNTPs using a Sephadex (G-50 Fine) spin column, sequencing reaction products were run on an ABI 377 automated DNA sequencer. Sequence data have been submitted to GenBank (accession nos AY137080–AY137125).

Data analysis

Control region sequences were aligned by eye in Se-Al (Rambaut 1996). Single base indels were present only in comparing the outgroup and ingroup taxa. Gaps were treated as a fifth character state in the phylogenetic analysis. No length variation was found among Eld's deer sequences except for a 78 base pair (bp) duplication that was present in two individuals from the Conservation and Research Center population (Fig. 2). This duplication was recoded as a single character in the phylogenetic analysis. Sequences obtained from GenBank, including CES05, CES06 and the outgroup *Elaphurus davidianus* (GenBank accession nos AF291892–AF291894; Randi *et al.* 2001), were lacking 27 bases at the 5 end of the fragment we sequenced. This region was coded as missing for these three taxa.

Phylogenetic analyses were conducted in PAUP* (version 4.0b8, Swofford 2001) using the parsimony optimality criterion. Based on a recent phylogenetic analysis of the Cervinae (Randi *et al.* 2001), *E. davidianus* was chosen as the outgroup. We conducted a branch and bound search using one representative of each unique haplotype. Bootstrap values (1000 replicates) were calculated to assess the relative support for branches in the most parsimonious trees.

Nucleotide diversity (Tajima 1983; Nei 1987) was calculated using ARLEQUIN V2.000 (Schneider *et al.* 2000). Haplotype diversity was calculated according to Nei (1987). We also conducted an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) to assess the distribution of genetic variation among the putative subspecies. For the AMOVA, populations were defined based on sampling location and were nested within groups corresponding to subspecies.

The two *C. eldi* sequences acquired from GenBank were originally attributed to *C. e. siamensis* and *C. e. eldi*, respectively (Randi *et al.* 2001). These animals, however, share identical sequences, a result that is inconsistent with the distribution of haplotypes in our data set (see Results). The possible explanations for this are: (1) the *C. e. eldi* individual in question was actually of hybrid origin, or (2) human error occurred during the cataloguing or sequencing of these specimens. A third possibility that seems remote in light of our data is that an ancient haplotype has persisted in both the *C. e. eldi* and *C. e. siamensis* populations. For the purposes of our population genetic analysis, we classified both sequences as *C. e. siamensis*.

We also analysed published sequences of Japanese sika deer, *Cervus nippon*, another broadly distributed deer species (GenBank accession nos AB012364–AB012385, Nagata *et al.* 1999). Only the portion of the control region homologous to the fragment that we amplified in Eld's deer was utilized. Diversity indices were calculated for both the northern and southern lineages of Japanese sika deer, which are differentiated genetically (Nagata *et al.* 1999). Estimates of nucleotide diversity based on control region sequences were also available for the endangered Pampas deer (*Ozotoceros bezoarticus*) (Gonzalez *et al.* 1998) and the much more abundant roe deer (*Capreolus capreolus*) (Wiehler & Tiedemann 1998). We also calculated haplotype diversity for these species. Although sampling regimes varied among studies, data from these other cervid species provide a relative assessment of the genetic diversity harboured in Eld's deer.

Results

Control region characteristics

The control region fragment we amplified in *Cervus eldi* consisted of 478 bp in all but two individuals. These two

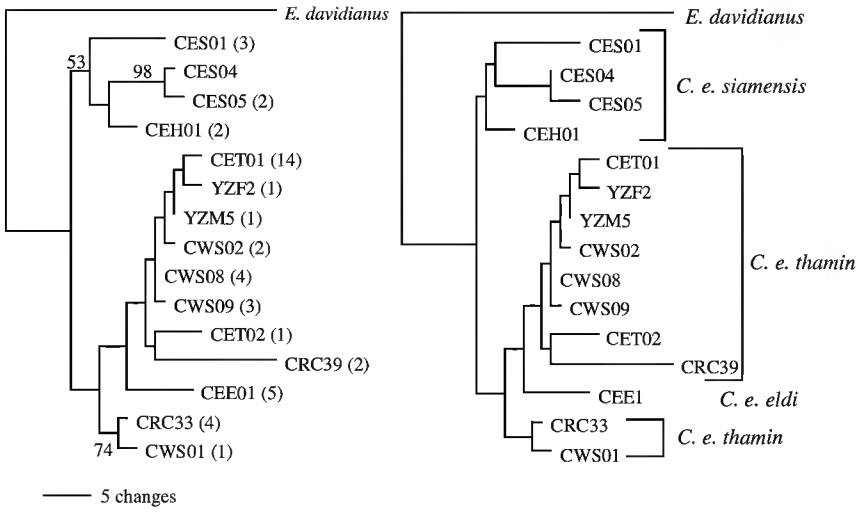

Fig. 3 Two equally parsimonious trees of length 113 (CI = 0.70) found in an analysis of unique *C. eldi* mtDNA haplotypes. Numbers above branches indicate bootstrap support indices. Numbers in parentheses indicate the number of individuals that share each haplotype.

individuals, both from CRC, possessed a 78 bp duplication that appears to be derived uniquely in *C. e. thamin* (Fig. 2). The final alignment, including the outgroup but excluding the duplication, consisted of 480 characters. This alignment was generated by the insertion of a single gap in the ingroup sequences, and another in the outgroup. Of these 480 characters, 402 were constant and 47 were parsimony informative.

Among the 48 individuals analysed in this study, 15 unique haplotypes were identified. Four of these haplotypes were within *C. e siamensis* and 10 were within *C. e thamin*, whereas all five *C. e eldi* had the same haplotype. Among the ingroup taxa, a single C → G transversion was observed, which diagnosed and was unique to the *C. e. eldi* plus *C e. thamin* clade. All other changes were transitions.

Phylogenetic relationships

A branch and bound tree search revealed two most parsimonious trees of 113 steps (consistency index (CI) = 0.70) (Fig. 3). The trees were essentially identical, differing only in the placement of the two *C. e. siamensis* individuals sampled from Hainan Island. Both topologies support the reciprocal monophyly of the *C. e. siamensis* clade and a clade comprising *C. e. thamin* and *C. e. eldi*. The two topologies differ in that one places the two Hainan samples within the *C. e. siamensis* clade, whereas the other shows the Hainan individuals as the sister group to the rest of the siamensis clade. Both topologies nest *C. e. eldi* within the *C. e. thamin* clade. A heuristic search in which each of the three subspecies was constrained to be monophlyletic resulted in seven topologies that were only one step longer than the unconstrained trees. These seven trees were not significantly worse than the unconstrained trees [$P > 0.5$, test described by Templeton (1983) and implemented in PAUP* (Swofford 2001)].

Phylogeographic structure

An analysis of molecular variance (AMOVA) indicated a high degree of substructure both among subspecies and among populations within subspecies. A large proportion of genetic variation was attributable to differences between the three subspecies ($F_{CT} = 0.46$) and to differences among populations within subspecies ($F_{SC} = 0.41$). This is not surprising, given that the three subspecies do not share any mtDNA haplotypes (Fig. 4). We also note that only one haplotype was recorded from more than one population (the most common haplotype in *C. e. thamin*). With this one exception, different haplotypes were found in each location or population sampled. These results indicate strong genetic substructure both between and within subspecies.

Genetic diversity

Both *C. e. thamin* and *C. e. siamensis* still harbour a substantial amount of genetic diversity (Table 2). Haplotype diversity for *C. e. siamensis* and *C. e. thamin* was 0.82 and 0.81 and nucleotide diversity was 0.024 and 0.014, respectively. Although Eld's deer is the most highly endangered of the deer species for which estimates of nucleotide diversity were available, mtDNA diversity in Eld's deer is comparable to that observed in other species, including *Capreolus capreolus*, a much more abundant species (Table 2).

Of the three *C. e. thamin* populations that were sampled in this study, the Yangon Zoo population had the lowest gene and nucleotide diversity. The nucleotide diversity of the Yangon Zoo population was approximately an order of magnitude lower than that of the CRC population. Diversity measures in the Yangon Zoo population were also lower than those of the wild population sampled at CWS (Table 2).

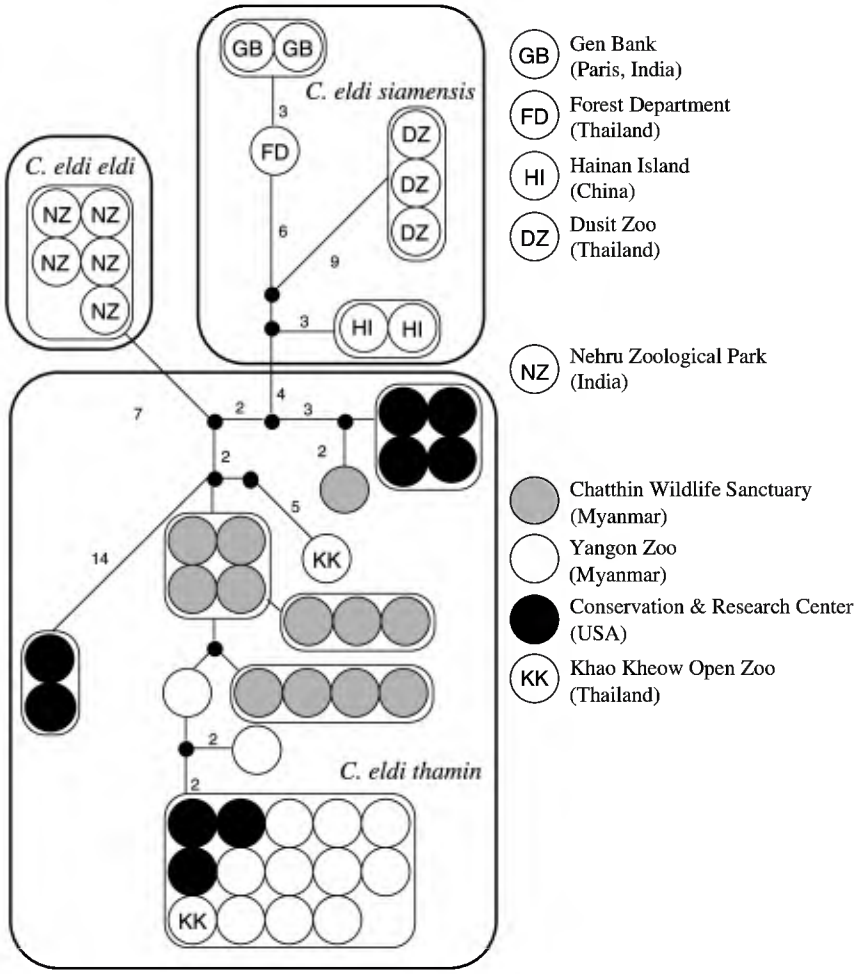

Fig. 4 Unrooted parsimony network of *C. eldi* mitochondrial control region haplotypes (length = 73 steps, CI = 0.66). Each large circle represents an individual animal coded according to source. Individuals within a box have identical haplotypes. Numbers indicate branch lengths.

Table 2 Control region characteristics for the three subspecies and seven populations in this study, haplotype diversity and nucleotide diversity. Also included are diversity estimates from three other deer species. The range of values for nucleotide diversity indicate diversity estimates for various subpopulations

Population	*n*	Haplotypes	Haplotyp diversity	π, Nucleotide diversity
Cervus eldi	48	15	0.89	0.022
C. eldi eldi	5	1	0	0
C. eldi siamensis	8	4	0.82	0.024
CES	4	2	0.50	0.016
CEH	2	1	0	0
C. eldi thamin	35	10	0.81	0.014
CET	2	2	1.0	0.021
CRC	9	3	0.72	0.022
CWS	12	4	0.77	0.006
YZ	12	3	0.32	0.002
Cervus nippon	21	18	0.98	0.014–0.022
Ozotoceros bezoarticus†	54	45	0.99	0.011–0.025
Capreolus capreolus‡	40	19	0.97	0.0097

*Nagata *et al.* (1999).
†Gonzalez *et al.* (1998).
‡Wiehler & Tiedemann (1998).

Discussion

Genetic variation and population history

Eld's deer has suffered dramatic population reduction throughout its range. Nevertheless, both *C. e. thamin* and *C. e. siamensis* have retained a substantial amount of genetic variation. Measures of nucleotide diversity in these subspecies is comparable to that found in the Pampas deer, which is also considered endangered. High nucleotide diversity suggests that these species probably had large effective population sizes in their recent history. Furthermore, these values were comparable to values obtained for control region sequences in the more abundant roe and sika deer and in a number of bovid species (Arctander *et al.* 1996). Assuming that the dynamics of control region sequence evolution are comparable between bovids and cervids, this suggests that in recent history *C. eldi* may have had population sizes comparable to those of the aforementioned bovid species (> 1 000 000 individuals). The relatively high diversity found in *C. e. thamin* and *C. e. siamensis* is not necessarily a surprising result, as theory suggests that many generations of reduced population size are required to substantially erode genetic variability (Nei *et al.* 1975).

Much of the current haplotype diversity in *C. eldi* is probably a consequence of strong phylogeographic structure within subspecies as well as between subspecies. For example, a single and different mtDNA haplotype was obtained from each of the different sources of *C. e. siamensis* that were available for our study. Similarly, although each of the *C. e. thamin* populations includes multiple haplotypes, only one common haplotype was shared among the three captive sources. We also note that none of the four mtDNA haplotypes in the wild CWS population are represented among captive *C. e. thamin*. This pattern suggests a strong degree of spatial genetic structure within *C. e. thamin* and limited dispersal historically of individuals between local populations.

In contrast to *C. e. thamin* and *C. e. siamensis*, the genetic data for *C. e. eldi* are consistent with a substantial loss of genetic diversity. This subspecies, unlike the other two, is known to have suffered a severe bottleneck during which the population was reduced to fewer than 20 individuals. While the sample size in this study is small and nonrandom (all five individuals were sampled from captivity in a single zoo), the lack of variation among these individuals may be a cause for concern for the subspecies as a whole. The two individuals from Hainan Island also shared identical haplotypes. Similar results were obtained in a recent study in which 55 Hainan Island deer were found to share an identical control region haplotype (J. Pang *et al.* pers. comm.). This population is also known to have suffered a severe bottleneck and the current population was founded by approximately 26 individuals (Wang 1976; Xu 1976).

The three main *C. e. thamin* populations sampled in this study present an interesting opportunity for analysis of the efficacy of captive breeding practices. The CWS population represents the largest, most stable population of Eld's deer in existence, while the captive CRC population, founded from a mixture of individuals from the Yangon Zoo and from the wild, is carefully managed to maintain genetic diversity. Lastly, the Yangon Zoo population consists of a captive herd in which reproduction has not been managed to maintain genetic diversity. A particularly interesting result of our study was the limited degree to which haplotypes were shared among these populations. In fact, none of the haplotypes found in the CWS population are represented in the captive populations, and only a single haplotype is shared between Yangon Zoo and the CRC. Of the 12 individuals sampled from Yangon Zoo, 10 shared an identical haplotype. This asymmetrical distribution of haplotype frequencies was reflected in dramatically lower haplotype diversity (0.32) in the Yangon Zoo population and may be related to unmanaged breeding. In contrast, haplotype diversity was markedly higher in the CRC population (0.72), where reproduction is managed through the use of a regional studbook.

The results of our study provide strong evidence of historical population structure in Eld's deer. The relatively large genetic distance between *C. e. siamensis* and the clade containing *C. e. thamin* and *C. e. eldi* corresponds to a historical range disjunction at the Dawna Ridge on the boundary between Myanmar and Thailand, which has probably presented a long-term barrier to gene flow between these populations. The closer association between *C. e. thamin* and *C. e. eldi* in our phylogenetic analysis corresponds to a smaller geographic distance between the historical ranges of these two subspecies and indicates a more recent common ancestry. Interestingly, while *C. e. siamansis* and *C.e. thamin* are more distantly related, they are nearly identical morphologically, whereas *C. e. eldi* has unique morphological characters.

One caveat is that mtDNA has certain limitations as a genetic marker. If variance in reproductive success is similar for males and females, the effective population size for mtDNA is four times smaller than that of nuclear markers, making it much more sensitive to population bottlenecks (Nei *et al.* 1975). In addition, because it is maternally inherited, mtDNA reflects only the phylogeographic history of female lineages. To the extent that dispersal is male-biased, both in the wild and in transfers of animals between captive populations, mtDNA may exhibit greater differentiation among populations than nuclear markers and may underestimate the genetic diversity present within populations. Countering this effect is the high variance in male reproductive success in polygynous species such as Eld's

deer, which reduces the difference in effective population size for mtDNA and nuclear genes. We suspect that natural long-distance dispersal is very rare in this species, and find no evidence of sex bias in the transfer of animals between captive populations (M. Rodden pers. comm.). Nevertheless, analyses of nuclear markers would help to further characterize the distribution of genetic variation in Eld's deer.

Conservation implications

From a practical perspective, the effective genetic management of extant Eld's deer populations as distinct units will be difficult to achieve. This is due in part to the great disparity in population sizes of the three subspecies (i.e. *C. e. thamin* are relatively abundant compared to the rare *C. e. eldi* and *C. e. siamensis*). Given our results, one prescription would be to maintain the three subspecies as ESUs. The critical question, however, is whether small, isolated populations (i.e. *C. e. eldi* and *C. e. siamensis* on Hainan) can remain viable without the introduction of new genetic material. Although Eld's deer subspecies are genetically differentiated, interbreeding individuals across subspecies boundaries may help to ensure the long-term viability of regional populations and the species as a whole. Incorporating genetic material from a more abundant and/or genetically diverse subspecies may alleviate negative consequences of inbreeding in a more homogeneous subspecies or population. In a study of the Mariana crow, two populations were found to be reciprocally monophyletic but the less numerous and less stable population was found to harbour greater genetic diversity relative to the more abundant population (Tarr & Fleischer 1999). The authors therefore recommended translocations from the more diverse to the more homogeneous population, despite the genetic divergence between the two groups. A number of studies have demonstrated that artificially restoring gene flow between isolated populations can counteract the effects of inbreeding depression (e.g. Hedrick 1995; Madsen *et al.* 1999). Despite these successes, translocations are fraught with potential dangers. These include the introduction of exotic pathogens and a host of difficulties associated with acclimating captive raised animals to natural environments (Snyder *et al.* 1996). Furthermore, interbreeding between isolated populations can eventually result in a loss of overall genetic diversity (Lacy 1987).

Situations such as the Mariana crow, and perhaps Eld's deer, exemplify a critical flaw in basing ESU designation solely on molecular data. A recent approach proposed by Crandall *et al.* (2000), in which the authors espouse Templeton's (1998, 1989) species designation criteria of ecological and genetic exchangeability may provide a more appropriate means of identifying units for conservation. The main advantage of this approach is that it encourages consideration of both historical and ecological factors and promotes the maintenance of meaningful adaptive diversity, rather than only historically isolated populations. Moritz's criteria of reciprocal monophyly of mtDNA alleles and/or significant divergence in nuclear allele frequencies can be used to test the hypothesis of genetic exchangeability. Determining whether or not organisms are ecologically exchangeable, however, will be more difficult in many cases.

Although the Hainan Island population appears to be the largest and most stable population of *C. e. siamanesis*, it is highly inbred (J. Pang *et al.* pers. comm.). Our data do not necessarily support the designation of this population as a distinct subspecies (Fig. 3), but it is characterized by a unique and relatively divergent mtDNA haplotype, suggesting some duration of historical isolation. Indeed, genetic differentiation between Hainan deer and other *C. e. siamensis* may approach a level inconsistent with genetic exchangeability. Ecological exchangeability, however, is perhaps the more important criterion and there is no evidence that these populations exhibit any adaptive divergence. Supplementing the Hainan population with individuals from mainland or captive populations could enhance its genetic diversity and reduce inbreeding. In turn, a genetically diverse and stable population of *C. e. siamensis* on Hainan Island could serve as a valuable reservoir for supplementing captive populations and perhaps for founding new wild populations on the mainland.

This logic could be extended in support of managed interbreeding between Eld's deer subspecies, although issues of ecological exchangeability may mitigate against this approach. Based on mtDNA haplotypes, *C. e. siamensis* is reciprocally monophyletic to a clade comprising *C. e. thamin* and *C. e. eldi*. A single additional step yields a haplotype tree in which all three subspecies are monophyletic and would therefore qualify as genetically distinct MUs under Moritz's criteria. Although *C. e. thamin* shares a more recent common ancestor with *C. e. eldi*, it still may be ecologically exchangeable with *C. e. siamensis*. Karyotypic comparisons showed no major chromosomal distinctions separating the two subspecies (Thévenon *et al.* 2000), which reduces the likelihood of hybrid incompatibilities should they interbreed. In fact, a fertile hybrid *C. e. thamin* × *C. e. siamensis* cross was observed in captivity (Decoux 1994). Based on these similarities, some have discussed the possibility of augmenting the size and genetic diversity of the highly inbred *C. e. siamensis* population in the Paris Zoo through interbreeding with the more abundant *C. e. thamin* (Mauget *et al.* 2001). If the condition of captive and wild *C. e. siamensis* continues to decline, this may become an acceptable management strategy, particularly if evidence suggests that declining viability of the population is due to inbreeding depression. At present, however, we argue that because (1) relatively large, and genetically diverse populations of *C. e. thamin* still exist in both the wild and captivity, (2) *C. e. thamin* are

genetically distinct from *C. e. siamensis* and (3) the status of *C. e. siamensis* in the wild is not well known, interbreeding of these two subspecies is not warranted at this time. If scattered herds still persist in Vietnam, Laos and/or Cambodia, it is possible that *C. e. siamensis* may still possess substantial genetic diversity. Indeed, the *C. e. siamensis* sampled in the present study exhibited a level of genetic variability comparable to that of *C. e. thamin*. If viable wild populations of *C. e. siamensis* can be identified, new founders (or their genetic material) could be introduced to Hainan Island and also to captive populations. In summary, until better information about the abundance and genetic diversity of *C. e. siamensis* becomes available, we recommend maintaining *C. e. siamensis* and *C. e. thamin* as distinct units for conservation.

C. e. eldi presents the most difficult, and perhaps most controversial, conservation management challenge. This subspecies appears to have a markedly different ecology and may also be genetically distinct from the other two subspecies. Like Hainan Island deer, *C. e. eldi* is highly inbred and would benefit from the incorporation of new genetic material. For the near future, however, we recommend that this population be given ESU status in an effort to maintain its distinctive ecological adaptations.

To summarize, our results indicate long-term historical isolation of Eld's deer populations and support the recognition of the three currently recognized subspecies as ESUs for conservation management purposes. Within a subspecies, transfer of individuals between populations is recommended to reduce the degree of inbreeding. This recommendation should be implemented now to bolster the *C. e. siamensis* population on Hainan Island and, if sufficient genetic variation is present, the critically endangered *C. e. eldi* of India. In both instances, the addition of new founder genes to the captive populations, either by the introduction of founder individuals or their genetic material (e.g. Monfort *et al.* 1993) would provide a hedge against extinction. If prospects for one or more of the subspecies deteriorate further, asymmetrical interbreeding may become warranted to introduce new genetic material from *C. e. thamin* into one or both of the less numerous subspecies. For *in situ* populations, a crucial next step is to conduct field surveys to identify and quantify wild populations of *C. e. siamensis* and to continue efforts to sustain the last remaining strongholds for *C. e. eldi* and *C. e. thamin* in India and Myanmar, respectively.

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