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RESEARCH ARTICLE

Phylogeographic analysis of nuclear and mtDNA supports subspecies designations in the ostrich (*Struthio camelus*)

Joshua M. Miller · Sara Hallager · Steven L. Monfort · John Newby · Kelley Bishop · Scott A. Tidmus · Peter Black · Bill Houston · Conrad A. Matthee · Robert C. Fleischer

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Abstract We investigated the phylogeography and subspecies classification of the ostrich (*Struthio camelus*) by assessing patterns of variation in mitochondrial DNA control region (mtDNA-CR) sequence and across fourteen nuclear microsatellite loci. The current consensus taxonomy of *S. camelus* names five subspecies based on morphology, geographic range, mtDNA restriction fragment length polymorphism and mtDNA-CR sequence analysis: *S. c. camelus*, *S. c. syriacus*, *S. c. molybdephanes*, *S. c. massaicus* and *S. c. australis*. We expanded a previous mtDNA dataset from 18 individual mtDNA-CR sequences to 123 sequences, including sequences from all five subspecies. Importantly, these additional sequences included 43 novel sequences of the red-necked ostrich, *S. c. camelus*, obtained from birds from Niger. Phylogeographic reconstruction of these sequences matches previous results, with three well-supported clades containing *S. c. camelus/syriacus*, *S. c. molybdophanes*, and *S. c. massaicus/australis*, respectively. The 14 microsatellite loci assessed for 119 individuals of four subspecies (all but *S. c. syriacus*) showed considerable variation, with an average of 13.4 (±2.0) alleles per locus and a mean observed heterozygosity of 55.7 (±5.3)%. These data revealed high levels of variation within most subspecies, and a structure analysis revealed strong separation between each of the four subspecies. The level of divergence across both marker types suggests the consideration of separate species status for *S. c. molybdophanes*, and perhaps also for *S. c. camelus/syriacus*. Both the mtDNA-CR and microsatellite analyzes also suggest that there has been no recent hybridization between the subspecies. These findings are of importance for management of the highly endangered red-necked subspecies (*S. c. camelus*) and may warrant its placement onto the IUCN red list of threatened animals.

Keywords *Struthio camelus* · Ostrich · Africa · Mitochondrial DNA control region · Phylogeography · Microsatellites

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J. M. Miller · R. C. Fleischer (✉)
Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, National Zoological Park, PO BOX 37012 MRC 5503, Washington, DC 20013-7012, USA
e-mail: fleischerr@si.edu

S. Hallager
Animal Care Directorate, Smithsonian National Zoological Park, PO BOX 37012 MRC 5507, Washington, DC 20013-7012, USA

S. L. Monfort
Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630, USA

J. Newby · K. Bishop
Sahara Conservation Fund, Rue des Tigneuses 2, 1148 L'Isle, Switzerland

S. A. Tidmus
Animal Programs and Environmental Initiatives, Disney's Animal Kingdom, Lake Buena Vista, FL 32830, USA

P. Black · B. Houston
Saint Louis Zoo, One Government Drive, St Louis, MO 63110, USA

C. A. Matthee
Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa

Introduction

Historically, the ostrich, *Struthio camelus*, was widely distributed throughout Africa and the Arabian Peninsula, but recently has been in decline, especially in the northern extent of its range. A number of subspecies have been described by different authors, but currently five subspecies are recognized based on morphology and geographic range (del Hoyo et al. 1992): three in northern and eastern Africa: *S. c. camelus*, *S. c. massaicus*, and *S. c. molybdophanes*; one in southern Africa: *S. c. australis*; and lastly, the Arabian ostrich: *S. c. syriacus*. However, human expansion and exploitation, as well as local extinctions have reduced this distribution significantly on the African continent and led to the recent, cir. 1940 (Seddon and Soorae 1999), extinction of *S. c. syriacus*. Of particular concern today is the Saharan red-necked subspecies (*S. c. camelus*), which has declined to critically low numbers (Ostrowski et al. 2001).

Previous studies elucidated the genetic relationships between the five subspecies and showed significant structuring among them (Freitag and Robinson 1993; Robinson and Matthee 1999). These studies, based on variation in mitochondrial restriction fragment length (RFLP) and mitochondrial DNA control region (mtDNA-CR) sequence, showed *S. c. camelus* and *S. c. syriacus* to fall into one clade, *S. c. massaicus* and *S. c. australis* into a second, and *S. c. molybdophanes* to form a third clade that was highly divergent from either of the others.

Here we present an expanded phylogeny and population genetic assessment of all extant subspecies, with special emphasis on the critically endangered *S. c camelus*. Our study includes 43 novel samples of *S.c camelus* from Niger, and also a greatly increased sample size of the other subspecies from what was included in previous work (Robinson and Matthee 1999). In addition, we assess variability and relationships using both mtDNA-CR sequences and fourteen nuclear microsatellite loci. The inclusion of nuclear microsatellites can give greater resolution to population genetic studies as they are bi-parentally inherited, evolve rapidly (Goldstein and Pollock 1997), and are often able to detect structuring at a finer scale than other markers (Coltman et al. 2003; Freeman-Gallant 1996). Inclusion of nuclear microsatellites also helps to address any incorrect inferences that can arise when relying solely on mtDNA for phylogenetic analysis (Ballard and Whitlock 2004).

Mitochondrial DNA sequences showed much the same pattern as those previously published, with the presence of three distinct clades. The microsatellite data revealed higher levels of population structure, showing a distinction between *S. c. massaicus* and *S.c. australis*, corresponding to the established subspecies classification system. Notably, all individuals thought to represent *S. c. camelus* in Niger show no evidence of mixed ancestry. This is important not only for proposed recovery plans within northern Africa but also for potential use of *S. c. camelus* to reintroduce ostriches back to the Arabian Peninsula (Seddon and Soorae 1999). Our results also lend credence to the suggestion of separate species status for *S. c. molybdophanes*, and perhaps for *S. c. camelus/syriacus* and *S. c. massaicus/australis* as well.

Methods and materials

Sample collection and DNA extraction

Samples were taken from 43 red-necked birds (*S. c. camelus*) in private collections at ten localities throughout Niger. These included 31 blood samples, eight dung samples, and four feathers. An additional sample was derived from an egg found in the Tin Toumma desert of Eastern Niger at N 16.20 E 12.00 on 21 February 2004, and estimated to be ~50 years old. The egg is presumed to be representative of *S. c. camelus* as it falls within the historical distribution of the subspecies.

Total genomic DNA was extracted from Niger ostriches using various protocols based on sample type at the Center for Conservation and Evolutionary Genetics lab at the National Zoological Park. Blood samples were extracted using the Qiagen Biosprint 96 DNA Extraction Workstation, which utilizes magnetic particles to separate DNA. Fecal DNA was extracted using the QIAamp DNA Stool Mini Kit following manufacturer's protocol. For feather samples the extraction protocol described by Horváth et al. (2005) was used. Additionally, DNA was extracted from the ~50 years old egg following a procedure outlined for ancient samples (Fleischer et al. 2000).

In addition, 76 samples representing the three other extant ostrich subspecies were extracted at Pretoria University using standard phenol–chloroform isolation methods (Robinson and Mathee 1999). These samples represent 23 localities from throughout most of the ostrich's range in Africa (Table 1) and correspond to those used in a previous RFLP study (Freitag and Robinson 1993).

Mitochondrial DNA amplification

A 445 base pair (bp) segment of the mitochondrial control region was amplified, corresponding to previously published sequences for the ostrich (Robinson and Matthee 1999). However, we were unable to optimize the previously published primer set because of the presence of a large mononucleotide repeat near the end of the reverse primer. Therefore, a new primer set was developed and used, OSTPCRF (5′-CGGATATTACCCGAGACCTACA-3′) and

Table 1 Geographic origin of samples used in the present study

Subspecies	Country	Locality	Number of samples
S. c. camelus	Niger		43
S. c. australis	Botswana		
		Mabuasehube game reserve	1
		Makgadikgadi pans	10
	Namibia		
		Escourt game farm	3
		Gorrasis game farm	2
		Ongombeanavita farm	1
	South Africa		
		De hoop nature reserve	1
		Doornkraal, de rust	3
		Joubertina	2
		Kalahari gemsbok national park	1
		Kleinsee nature reserve	1
		Kruger national park	3
		Ladismith	2
		Langjan nature reserve	3
		Matjiesrivier	2
		Oude muragie, de rust	3
		Oudtshoorn proefplaas	2
		Prince Albert	4
		Oudtshoorn	3
	Zimbabwe		
		Centeral estate	5
		Hwange national park	5
		Northern matabeleland	3
S. c. massaicus	Kenya		
		Kajiado	7
	Tanzania		
		Arusha	3
S. c. molybdophanes	Kenya		
		Mt Kenya	6

OSTPCRR (5′-TAGGCTCTCTGGGGTTCACT-3′) that extends beyond each of the previous primers and amplifies a product of 691 bp.

Amplification reactions were conducted in 10 µl volumes consisting of 1.0 µl genomic DNA extract, 1.0 µl 5× PCR buffer, 1.0 µl 2 µM dNTPs, 0.6 µl $MgCl_2$, 0.5 µl of each primer diluted to 10 µM, 0.1 µl AmpliTaq DNA polymerase, and 5.3 µl DNAse/RNAse free water. The thermocyler profile began with 95°C for 2 min followed by thirty cycles of 95°C for 30 s, 59°C for 30 s, 72°C for 30 s and a final extension step of 72°C for 2 min.

In cases where DNA quality was poor, such as with the egg, scat, and feather extracts, a set of nested internal primers, OSTINTF (5′CTAGGACTACAACTGTCACAA TCTCT-3′) and OSTINTR (5′-CGCATACTGGGTATGT GGTG-3′), were used to obtain the entire 445 bp region in two overlapping products. The primer sets were OS-TPCRF/OSTINTR (445 bp) and OSTINTF/OSTPCRR (368 bp) and amplification reactions were conducted in 20 µl volumes consisting of 2.0 µl DNA extract, 2.0 µl 5× PCR buffer gold, 2.0 µl 2 µM dNTPs, 2.0 µl $MgCl_2$, 0.8 µl of each primer, 2.0 µl BSA, 0.2 µl AmpliTaq DNA polymerase gold, and 8.2 µl DNAse/RNAse free water.

In all cases PCR reactions were purified using ExoSAP-IT (Amersham Biosciences) and then subjected to cyclesequencing with ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. Sequences were resolved on an ABI 3130xl genetic analyzer, initially aligned in Sequencher v4.8 (Gene Codes), exported as Nexus files, and finally aligned by hand in MacClade v4.06 OS X (Maddison and Maddison 2001). Additionally, eighteen previously published sequences, including two representing *S. c. syriacus*, were obtained from GenBank (accession numbers AF073001–AF073018), used as references, and included in subsequent analyzes.

Sequence analysis

A maximum likelihood (ML) tree with 500 bootstrap replicates was generated using RAxML Black Box (Stamatkis et al. 2008) under a GTR+I+G model, and imported into PAUP (Swofford 2003) for visualization. Relationships among haplotypes were also analyzed through a median-joining haplotype network created using Arlequin version 3.0 (Excoffier et al. 2005).

Genetic diversity was further analyzed using Arlequin version 3.0 (Excoffier et al. 2005) through calculations of F_{st} and AMOVAs. Initially, groupings corresponded to subspecies classification, regardless of sampling locality. Subsequently, nested AMOVAs were generated to look at partitioning of variance within subspecies, based on sampling localities. In cases where a sampling locality has only one or two individuals, several localities were pooled based on geographic proximity. AMOVAs were generated using each of the four available distance methods in Arlequin, with no difference among the results.

Microsatellite amplification/genotyping

Fourteen microsatellite loci designed for the ostrich (Tang et al. 2003) were used in this study. Only six of these loci were mapped on a microsatellite based genetic map by Huang et al. (2008), but each of the six were assigned to different linkage groups (of 13 linkage groups: CAU17 on 1, CAU69 on 11, CAU97 on 4, CAU84 on 9, CAU3 on 2,

and CAU14 on 6). The loci were arranged into five multiplexes based on common annealing temperature (T_a) and size ranges (Table 2). PCR reactions were carried out with dye labeled primers (the forward primer for each primer set) in 10 µl volumes with 1.0 µl DNA extract, 1.0 µl PCR buffer, 1.0 µl 20 µM dNTPs, 1.0 µl BSA, and 0.1 µl AmpliTaq DNA polymerase. Thermocycler profile began with 94°C for 5 min, followed by thirty-five cycles of 94°C for 40 s, T_a for 1 min, and 72°C for 1 min. A final extension step of 72°C for 30 min completed the reactions. Samples were then run on an ABI 3130xl genetic analyzer using ABI ROX 500 as an internal size standard. Genotypes were assigned using Genemapper v4.0 (Applied Biosystems). Note that DNA samples were not available for *S. c. syriacus*, and thus it was not represented in the microsatellite analysis.

Microsatellite analysis

We used Genepop (Raymond and Rousset 1995) to estimate heterozygosity, test for linkage disequilibrium, and conduct Hardy–Weinberg exact tests using the default parameters. Arlequin version 3.0 (Excoffier et al. 2005) was used to calculate F_{st} and R_{st} (pair-wise differences). As with the mtDNA analysis, initially the data were partitioned by subspecies alone then into subgroups based on sampling locality. A second nested AMOVA was calculated to investigate the effect of domestic stock designation on genetic structure in *S. c. australis*. In this case, samples were organized into three groups: domestic stocks, game reserves, and nature reserves.

The Bayesian clustering program Structure v2.2 was run to assess genetic structure among the subspecies (Pritchard et al. 2000). We evaluated K = 1–7 possible population groupings, using a burn in of 50,000 iterations followed by 500,000 iterations for each K. We allowed for admixture within individuals, allowed for independent allele frequencies between populations, and initiated each run with popinfo. Each K value was run with four replicates to assess stability.

In addition, clustering of individuals was assessed using the program Populations v 1.2.19 (Langella 1999). Populations generated a distance matrix, based on the shared allele distance DAS (Jin and Chakraborty 1994), which was used to generate a neighbor-joining network with 1,000 bootstrap replicates that was visualized in TreeView v1.6.6 (Page 1996).

Table 2 Summary statistics for microsatellite data for entire sample

Locus	Number of alleles	Expected heterozygosity	Observed heterozygosity
CAU28	4	0.64	0.56
CAU17	22	0.892	0.667
CAU36	2	0.19	0.144
CAU69	13	0.801	0.576
CAU97	10	0.83	0.706
CAU76	27	0.906	0.788
CAU5	11	0.859	0.709
CAU23	20	0.856	0.607
CAU84	11	0.746	0.667
CAU68	13	0.832	0.627
CAU92	6	0.414	0.259
CAU46	23	0.874	0.278
CAU3	9	0.689	0.485
CAU14	16	0.875	0.729

Primers and other specifics for the loci are available in Tang et al. (2003)

Results

Analysis of the mtDNA sequences

We successfully amplified the entire 445 bp control region segment for 106 individuals: 43 Niger birds and 63 of the samples representing the other extant subspecies. Additionally, we were able to amplify a 76 bp region from the degraded DNA isolated from the old egg using the internal primers (OSTINTF/OSTINTR). Combined with the 18 sequences from Genbank (Robinson and Matthee 1999), we had sequences for 125 individuals. We detected 80 haplotypes, characterized by 73 polymorphic sites. The haplotypes generally segregated according to subspecies, with only two haplotypes shared between subspecies: one between *S.c camelus* and *S. c. syriacus* and the second between *S. c. australis* and *S. c. massaicus*. As expected the egg fell within the *S. c. camelus/S. c. syriacus* clade. (Fig. 1).

Phylogenetic relationships Based on mtDNA

Three well-defined clades were found with the control region data from both ML analysis (Fig. 2) and construction of a minimum spanning network (Fig. 3). These correspond to those found in previous studies with *S. c. camelus* and *S. c. syriacus* in one clade, *S. c. massaicus* and *S. c. australis* in another, and *S. c. molybdophanes* monophyletic.

Pair-wise F_{st} comparisons between all subspecies indicated substantial divergences between subspecies, except *S. c. camelus* and *S. c. syriacus* (Table 3). For all F_{st} calculations, *S. c. massaicus* was found to be distinct from the other subspecies, including *S. c. australis*, despite the tree topology.

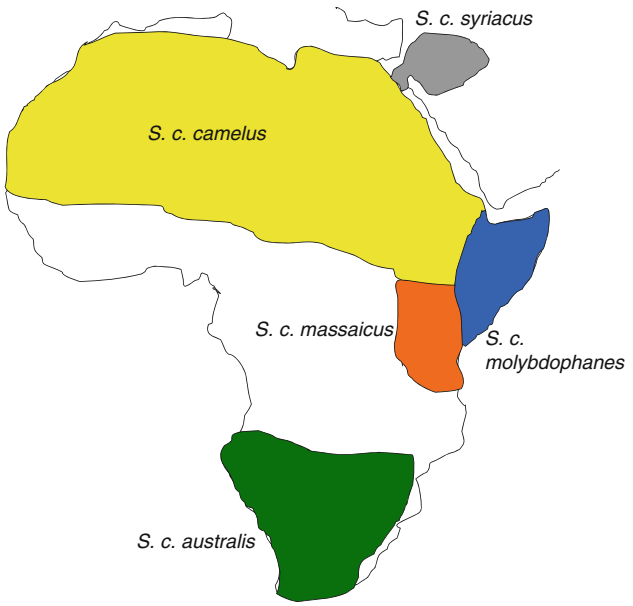

Fig. 1 Map of the historic range of the ostrich (*Struthio camelus*) in Africa, with each of the estimated subspecies ranges delineated by color (adapted from Robinson and Matthee 1999)

When samples were grouped by locality, nested AMOVA showed that over 64% of genetic variation was due to differences among subspecies groups, 5% was due to populations within groups, and 30% was due to variation within populations. In most cases, pair-wise F_{st} comparisons showed no significant differences between sampling localities within a subspecies.

Microsatellite amplification and diversity

All 119 birds were genotyped, with 97% of all microsatellite genotypes attempted attained. The number of alleles across subspecies ranged from 2 to 27 per locus, with a mean of 13.4 (SD = 2.0) alleles per locus and average observed heterozygosity of 55.7 (SD = 5.3) % (Table 2; Table S1, online supplementary information). Several loci (CAU 28, 36, 69, 97, 5, and 92) were observed to be fixed within *S. c. molybdophanes* (the subspecies with the smallest sample size), while in the other subspecies allele number ranged from one (CAU 36 in *S. c. camelus*) to twenty-two (CAU 76 in *S. c. australis*). No linkage disequilibrium was detected after performing sequential Bonferroni corrections ($P > 0.05$). Pooling all subspecies results in a strong heterozygote deficiency across all the loci (Table 2). However, all loci conformed to Hardy–Weinberg equilibrium within subspecies after sequential Bonferroni correction except CAU 46 ($P < 0.05$, heterozygote deficit in three subspecies), CAU23 and CAU3 ($P < 0.05$ heterozygote deficit in one subspecies each).

Phylogenetic relationships based on microsatellites

As with mtDNA analysis, pair-wise distance analysis comparing subspecies found significant differences between the subspecies, as measured both by F_{st} and R_{st} (Table 4). Each subspecies was found to be distinct in a neighbor-joining network based on DAS (not shown), as well as though Bayesian clustering (Fig. 4). While we supposed that the optimal number of populations, K, would be four we calculated ΔK, a measure of the second order rate of change in the likelihood of K (Evanno et al. 2005) to test this assumption. These calculations gave high weight to the presence of four populations, but also to three or five populations. When K = 3, *S. c. molybdophanes* and *S. c. massaicus* are clustered in a single population, while when K = 5, each subspecies is recovered with additional substructure in *S. c. australis*. In all cases the individuals held in Niger were assigned to a single cluster, presumably representing *S. c. camelus*; they show no evidence of genetic introgression from other subspecies or substructuring.

Discussion

Subspecies classification and taxonomy implications

Our analysis of mtDNA recovered three major clades consistent with those found in pervious studies (Freitag and Robinson 1993; Robinson and Matthee 1999) and largely consistent with current subspecies taxonomy. Though *S. c australis* and *S. c. massaicus* are nested together in the tree and share a haplotype, they were found to be significantly differentiated from one another as measured by F_{st} (Table 3). Original analysis of mitochondrial RFLPs (Freitag and Robinson 1993) found very limited diversity within and between subspecies of ostriches, save for *S. c. molybdophanes*, and it was postulated that a recent population bottleneck might have been the cause. The high degree of diversity seen in the results of Robinson and Matthee (1999) and our mitochondrial tree provides no evidence for such a bottleneck.

Nuclear microsatellite variation recovered finer levels of structure than mtDNA, showing each subspecies to be distinct. Tree building, AMOVAs, and Bayesian cluster analysis showed a clear distinction between *S. c australis* and *S. c. massaicus* (Fig. 3) not seen in mtDNA. This distinction suggests that the current subspecies classification system for each of these should be upheld. Though there appears to be strong heterozygote deficiency across loci (Table 2) this is most likely due to a Wahlund effect from comparing across differentiated subspecies.

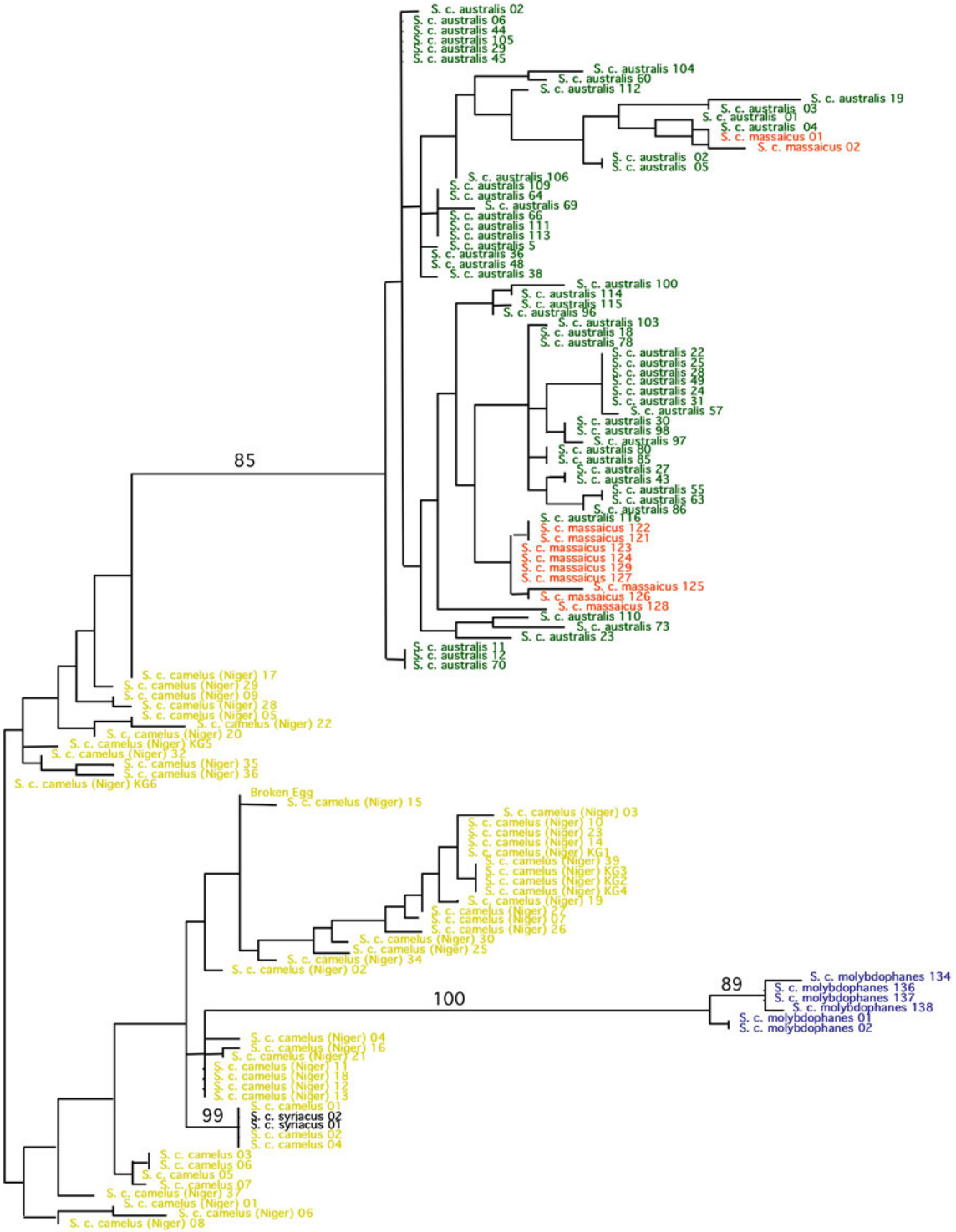

Fig. 2 Maximum likelihood tree based on 445 base pairs of mtDNA-CR sequence from 119 ostriches (this study) and 18 reference sequences (Robinson and Matthee 1999). Values above the bars are bootstrap support values (500 replicates). Taxa include *S. c. camelus, S. c. syriacus, S. c. massaicus, S. c. australis,* and *S. c. molybdophanes*

While calculation of ΔK gives high weight to K = 3, which groups *S. c. molybdophanes* and *S. c. massaicus* together, this makes little biological sense as it goes against the large genetic distances seen in mtDNA and microsatellites, as well as our DAS neighbor-joining tree and a principal components analysis (not shown). On the other hand,

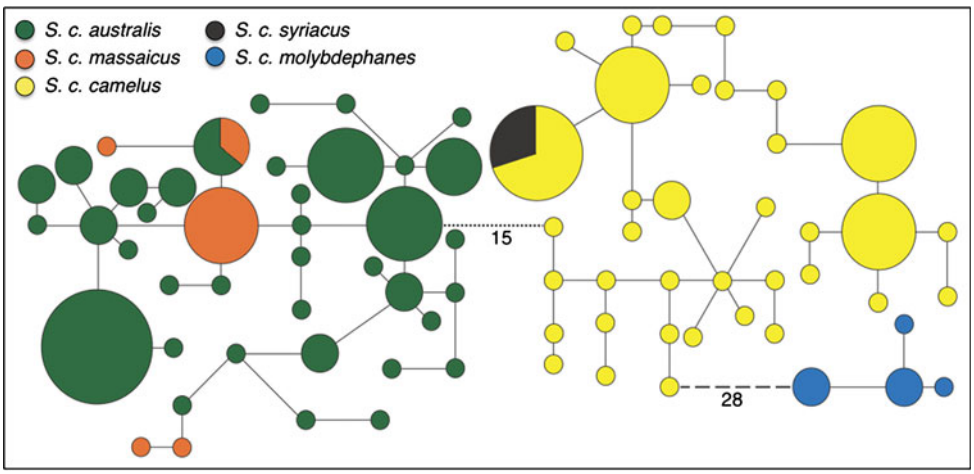

Fig. 3 Median-joining haplotype network of mtDNA-CR sequences from 119 ostriches (this study) plus 18 sequences from Robinson and Matthee (1999). *Circles* represent haplotypes, and their diameter is proportional to number of individuals with that haplotype. Branch lengths are proportional to the number of mutations, except for the *dashed* or *dotted lines*, where the number of mutations is noted below the line. Same color scheme as in Fig. 2

Table 3 Subspecies pairwise F_{st} based on mtDNA-CR sequence

	S. c. camelus	*S. c. australis*	*S. c. molybdophanes*	*S. c. syriacus*	*S. c. massaicus*
S. c. camelus	0				
S. c. australis	0.47828	0			
S. c. molybdophanes	0.53198	0.78684	0		
S. c. syriacus	**−0.00176**	0.71151	0.88444	0	
S. c. massaicus	0.39993	0.2139	0.74462	0.68119	0

Value in *bold* is not significant ($P > 0.05$); all others significantly different from zero at $P < 0.05$

Table 4 Subspecies pair-wise divergence based on microsatellites

	S. c. australis	*S. c. massaicus*	*S. c. molybdophanes*	*S. c. camelus*
S. c. australis	0	0.160	0.330	0.108
S. c. massaicus	0.127	0	0.398	0.208
S. c. molybdophanes	0.432	0.563	0	0.353
S. c. camelus	0.306	0.304	0.637	0

Values above the diagonal are calculated as traditional F_{st}; values below the line are calculated as R_{st}. All values are significantly different from zero ($P < 0.05$)

our STRUCTURE results for K = 4 and 5 both reflect the accepted subspecies classification, with the latter K showing additional structure within *S. c. australis*. This substructure does not appear to be the result of inclusion of domestic stocks in our analysis, nor does it correspond to specific geographic regions. Thus, we are unsure of the exact nature or cause of this substructure.

Previous publications have suggested that *S. c. molybdophanes* might be a distinct species (Freitag and Robinson 1993; Lewis and Pomeroy 1989; Brown et al. 1982). The high levels of differentiation between *S. c. molybdophanes* and the other subspecies, in both mtDNA and microsatellite analysis, support this assertion. In addition, the high level of differentiation between *S. c. camelus* and the other subspecies (except *S. c. syriacus*) suggests this clade may also represent a species level divergence.

Historical causes of diversification

The topology of the mtDNA tree roughly groups subspecies into a southern clade (*S. c. australis and S. c. massaicus*), a northern clade (*S. c. camelus* and *S. c. syriacus*), and a highly divergent eastern clade (*S. c. molybdophanes*). This type of eastern/southern split is also seen in many mammal species, including carnivores (Rohland et al. 2005; Freeman et al. 2001; Dubach et al. 2005; Barnett et al. 2006), and large ungulates (Flagstad et al. 2001; Moodley and Bruford 2007; Muwanika et al. 2003; Arctander et al. 1999; Brown et al. 2007).

In most cases the split is attributed to glacial refugia present in eastern, western and southern Africa separating populations during periods of climate change and glaciation. These refugia were then sources from which most

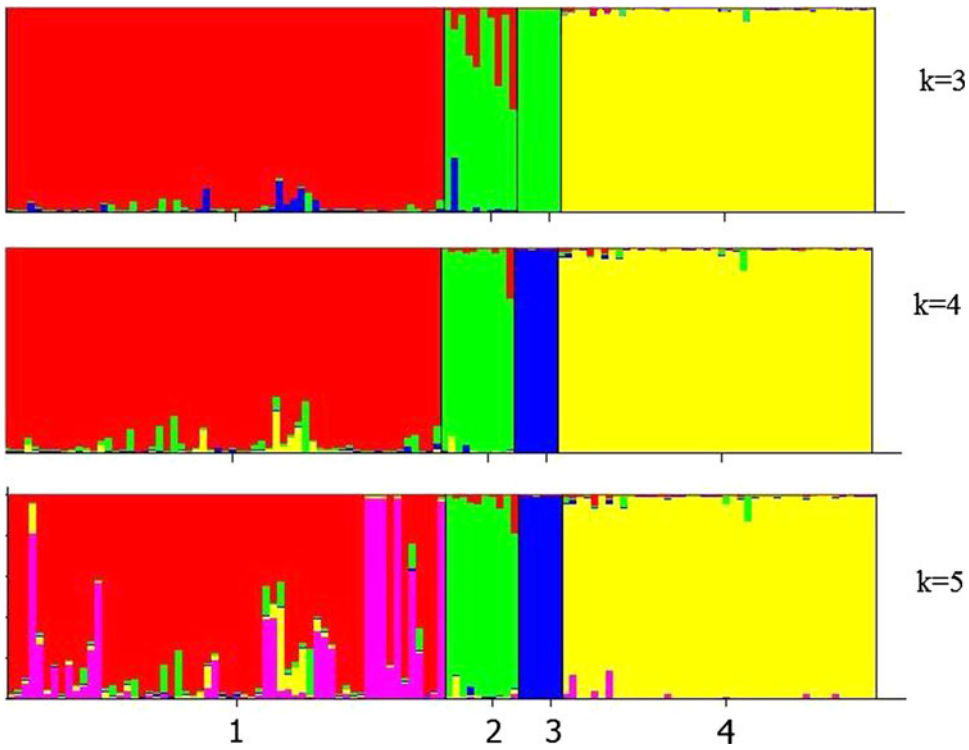

Fig. 4 Genetic structure of ostriches based on a Bayesian cluster analysis of 14 microsatellite loci using structure v2.2. (Pritchard et al. 2000). We assessed values of K from 1 to 7, and show results for K = 3, 4, and 5. Numbers below the axis correspond to subspecies, *1 S. c. australis, 2 S. c. massaicus, 3 S. c. molybdophanes,* and *4 S. c. camelus*

populations expanded their range and subsequently diversified (Hewitt 2004). Tectonic activity associated with the rift valley system has also been posed as a diversification mechanism (Faulkes et al. 2004; Partridge 1995). We did not attempt to date diversification among the ostrich clades because of the uncertainty of rate estimates for mtDNA-CR (Ruokonen and Kvist, 2002), and no appropriate calibration point being available for ostriches.

The abrupt disjunction in range boundary and large genetic divergence between *S. c. molybdophanes* and *S. c. massaicus* is seen between subspecies of other vertebrates from the same area (Faulkes et al. 2004; Brown et al. 2007). Such disjunctions are attributed to past paleoclimatic events initially separating the populations, thus leading to the evolution of differences in behavior, habitat preference, or other isolating mechanisms. These differences could continue to drive speciation even in zones of secondary contact. Reports of difficulties in mating between subspecies of ostrich, particularly *S. c. molybdophanes* and *S. c. massaicus* (Lewis and Pomeroy 1989), provide some evidence for the existence of such isolating mechanisms.

Conservation implications

The red-necked subspecies *S. c. camelus* is of critical conservation concern: very few individuals are currently seen in the wild (Ostrowski et al. 2001) and all 46 of the samples in this study come from birds held in private collections (with the exception of the old egg). While the original provenance of the 46 *S. c. camelus* individuals of our study is uncertain (probably arising from Chad), the old egg found in the Tin Toumma desert of Eastern Niger is very likely from a wild, resident Niger ostrich, and its clustering in the mtDNA-CR analysis with our Niger samples (and with the previous samples from Robinson and Matthee 1999) suggest that these are also birds from the region and *S. c. camelus* subspecies. In addition, the Bayesian clustering analysis of 14 microsatellites showed no indication of genetic structuring within the captive *S. c. camelus* populations or introgression from other subspecies. Thus these 46 individuals should make a good founding population for captive propagation efforts currently underway. The large numbers of haplotypes in mtDNA and alleles in microsatellites suggest no major loss of genetic diversity despite reports of drastic population decline.

Our analyzes of mtDNA and nuclear microsatellites support the designation of at least four described subspecies (four of the five subspecies previously based on morphology, range and other characteristics). The data also would perhaps support species status for three taxa: *S. c. molybdophanes*, *S. c. camelus/syriacus*, and *S. c. australis/massaicus*. Regardless of taxonomic status, we believe that the level of genetic divergence of *S. c. camelus* from other extant taxa, coupled with its current rarity and vulnerability, should

warrant its placement on the IUCN Red List of threatened species.

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