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Introduction or reintroduction? Last resorts for the latest bird to become extinct in Europe, the Andalusian hemipode *Turnix sylvatica sylvatica*

CINO PERTOLDI[1,2], JUAN J. NEGRO[3,*], JOAQUÍN MUÑOZ[1], FILIPPO BARBANERA[4] and HÉCTOR GARRIDO[3]

*[1]Laboratory of Molecular Ecology, Estación Biológica Doñana, CSIC, Pabellón del Perú, Avda. María Luisa, s/n. 41013 Seville, Spain; [2]Current addresses: Department of Ecology and Genetics, University of Aarhus, Building 540, Ny Munkegade, DK-8000 Aarhus C, Denmark, and Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, Kalø Grenåvej 14, DK-8410 Rønde, Denmark; [3]Department of Applied Biology, Estación Biológica Doñana, CSIC, Pabellón del Perú, Avda. María Luisa, s/n. 41013 Seville, Spain; [4]Department of Ethology, Ecology and Evolution, University of Pisa, Via A, Volta 6, 56126 Pisa, Italy; *Author for correspondence (e-mail: negro@ebd.csic.es)*

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Abstract. The Andalusian hemipode (*Turnix sylvatica sylvatica*) (Order: Turniciformes, Family: Turnicidae), formerly distributed in several Mediterranean countries, is a critically endangered bird, if not already extinct. Subspecies of the *T. sylvatica* complex, in turn composed by nine subspecies are widely distributed in Africa and southern Asia. The last free-ranging Andalusian hemipodes were shot by hunters near Doñana National Park (Spain) in 1981. Therefore, this species could be the last bird species getting extinct in Iberia and Europe in the XXth century. This investigation deals with the phylogenetic relationships of the Andalusian hemipode with the supposedly congeneric *T. varia*, *T. tanki*, *T. suscitator* and *T. pyrrhothorax*, and with the supposedly conspecific *T. sylvatica lepurana*, which is the geographically nearest buttonquail population (occurring in central and southern Africa). A 606 bp long fragment of the cytochrome *b* gene (approx. 1140 bp) of the mitochondrial DNA was sequenced, using both museum skins (the only available source for *T. s. sylvatica*) or blood/tissue samples from contemporary individuals (remaining species and subspecies). Seven haplotypes were found: two each for *T. varia* and *T. s. lepurana*, and one each for *T. tanki*, *T. suscitator*, *T. pyrrhothorax*, and *T. s. sylvatica*. Sequence divergence values obtained from pairwise distances between the *T. sylvatica* group haplotypes and the other species, ranged from 19.4 to 25.9%. The low genetic divergence between *T. s. sylvatica* and *T. s. lepurana* (0.00–0.01%) confirmed that the current classification based on morphological characters is correct, and that these two taxa may should be considered as subspecies. This close relationship would permit an introduction *T. sylvatica* where the species was last seen in Spain (i.e., Doñana National Park). This area is now strictly protected and human persecution is no longer a problem.

Introduction

The buttonquails (Aves, Turniciformes), also called hemipodes for missing the rear toe, are extremely secretive birds historically inhabiting Africa, southern

Europe, south-east Asia and Australia (Watkins 1857; Saunders 1871, 1877; Chapman 1884; Arévalo Baca 1887; Irby 1895). The population of south-western Europe and North Africa, known as the Andalusian hemipode (*T. sylvatica sylvatica)* (AH), strongly declined since the end of the XIXth century. It has disappeared from Italy (no later than 1920, Violani and Massa 1994), Portugal (in the first half of the XXth century), Libya (last seen in 1967) and Algeria (last record in 1976). Such a declining trend seems to have accelerated in the last 40 years both in Andalusia (southern Spain: cf., Bernis 1966) and North Africa. The reasons for the demise of the species are unknown. In southern Spain, the AH was typically found in dwarf palm (*Chamaerops humilis*), scrub and grasslands which have been greatly reduced due to the development of modern, intensive agriculture.

The last seen Andalusian hemipodes were shot in 1981 by hunters who flushed the birds with dogs in hunting states currently included within the limits of Doñana National Park (Spain). Five of these birds, killed between October 1978 and December 1981 were savaged and are now deposited in the collection of Doñana Biological Station.

In North Africa the species has not been recorded dead or alive by ornithologists in the last 20 years. However, the bird is still known to some peasants and farmers in Marocco, who are also aware of the presence of the common quail *Coturnix coturnix*, a very similar species, Some relic populations of *T. sylvatica* may thus persist in Marocco (H: Garrido, unpublished data).

The Andalusian hemipode is considered as a critically endangered species and is listed in the Annex I of the Birds Directive (Council Directive on the conservation of wild birds, 79/409/EEC). Although not yet declared as extinct officially, the Andalusian hemipode could be the last avian species extirpated from Iberia and Europe in the XXth century.

Sixteen species belonging to the genus *Turnix* are currently recognised, *T. sylvatica* being the species with the widest distribution. The range of the latter covers the sub-saharian Africa, southern-east Asia, and the Indian subcontinent. For *T. sylvatica*, nine subspecies have been described (see Table 1). The Kurrichane buttonquail (*T. s. lepurana)* is still common and widespread in southern African (Colahan 1997), but the population status of the remaining

Table 1. The nine subspecies for the species *T. sylvatica* are listed along with their distribution.

T. s. sylvatica	Andalucía (Spain) and north-western África
T. s. lepurana	Sub-saharian África
T. s. dussumier	India
T. s. davidi	Indochina and southern China
T. s. whiteheadi	Luzón isle (Philippines)
T. s. nigrorum	Negros isle (Philippines)
T. s. celestinoi	Bohol and Mindanao Isles (Philippines)
T. s. suluensis	Sulu isle (Philippines)
T. s. bartelsorum	Java and Bali isles (Indonesia)
T. pyrrhothorax	Australia

subspecies is unknown. Although specimens of the major subspecies collected before 1950 are well represented in museum collections worldwide, to our knowledge there are no *T. sylvatica* kept alive in zoos at present and this has made it difficult to get fresh samples for analysis.

Both the low dispersal capability attributed to the AH (e.g., they were not known to be migratory, Violani and Massa 1994), and the long geographic distances to other conspecific subspecies, could have kept isolated the AH populations for a long period. Such a genetic isolation might have led to independent evolution and formation of a distinct species.

The phylogenetic position of the *Turnicidae* is unresolved (Sibley and Ahlquist 1990). Historically, this family has successively been classified as a member of the orders Struthioniformes, Galliformes, Charadriiformes, Columbiformes and the Gruiformes. However, most of these studies followed phenetic methodology, using both morphological similarity of taxa and plesiomorphic characters. Recent phylogenetic studies dealing with morphological character analyses (Cracraft 1988), or comparisons based on DNA–DNA hybridization (Sibley and Ahlquist 1990), failed to clarify the phylogenesis of *Turnicidae* and suggested a position as '*incertae sedis*', either among the Gruiformes or as the parvclass 'Turnicae' as a sistergroup to the 'Neoaves' (Sibley and Ahlquist 1990). A study conducted by (Rotthowe and Starck 1998), based on skeletal and myological material, supports the hypothesis that *Turnix* is a member of the Gruiformes with close relationships to the Rallidae. Lastly, in a recent study, Paton et al. (2003) from the sequencing of a nuclear gene (RAG-1) suggested that *Turnix* is embedded within the *Charadriiformies*. Given this confusion at the higher taxonomical levels, the phylogenetic relationships at the genus, species and subspecies levels are even more uncertain and need to be clarified by means of molecular markers.

The aim of this investigation was to resolve the phylogenetic relationship between the AH the congeneric and the conspecific members of the *Turnix* genus and to coarsely quantify the degree of genetic divergence among the members of this genus, and, in particular, between the AH and the supposedly conspecific *T. s. lepurana*, which is also the geographically closest species. We also sought samples of the Asian *T. s. dussumier*, but we failed to amplify DNA from the museum skin snips available.

We have sequenced a fragment of the cytochrome *b* gene (cyt-*b*) of the mitochondrial DNA (mtDNA), as this gene is the most widely used gene for phylogenetic work. Although use of cyt-*b* has some pitfalls, Moore and DeFilippis (1997) argued that it could nevertheless be the best choice for resolving relatively recent evolutionary history, The cyt-*b* sequences have successfully been used to identify taxonomic groups even at subspecies level, for example in bluethroats (*Luscinia svecica svecica* and *L. s. namnetum*; Questiau et al. 1998) and in common guillemots (*Uria aalge*; Friesen et al. 1996).

The quantification of the degree of genetic divergences and the resolution of phylogenetic relationships within the *Turnix* genus will allow the planning of a

management strategy for *T. sylvatica*. Furthermore, it will make it possible to answer specific questions concerning the suitability of an introduction or reintroduction in southern Europe using supposedly conspecific or congeneric populations from Africa or elsewhere.

Materials and methods

Samples

A total of 11 specimens of five of the species within the *Turnix* genus (covering a major portion of the current and past distribution range of the species) have been used for phylogenetic reconstruction (see Table 2). The only available DNA source for the extinct populations of AH were skin samples collected in 1981 in Andalusia (southern Spain). DNA from *T. varia* and *T. pyrrhothorax was also* sequenced from dried skin snips or muscle tissues preserved in ethanol. An additional *T. varia* sequence was downloaded from Genbank: GenBank accession number AF168104). Contemporary living or dead individuals of the remaining species were sampled by collecting blood (one *T. tanki* and one *T. suscitator* imported from unknown locations and kept in a private aviary in Sevilla in 2000–2002) or muscular tissue (four *T. s. lepurana* collected in Namibia in 2004 by J.J. Negro and H. Garrido, and one *T. varia* donated by the Museum of Southern Australia). Blood and tissue samples were stored in ethanol or modified lysis buffer (Seutin et al. 1991).

In addition to the above mentioned samples, we also processed, but failed to get usable DNA from, ancient (>50 years old) specimens of: (a) four *T. s. sylvatica* collected in Algeria and southern Spain (Royal Museum of Scotland, Edinburgh, UK), and four specimens from Italy (Museum of Natural History and Land, University of Pisa, Italy), (b) two *T. maculosa salomonsis* from Guadalcanal Island (Field Museum of Natural History, Chicago, USA) and (c)

Table 2. List of the *Turnix* species analysed.

Species	Sample name	Origin	Acc. no.	Hap. name
T. varia	TvAF168104	see GenBank	AF168104	TvHap1
	Tv3004	?	AY703824	TvHap2
T. tanki	Tt2F	?	AY703823	TtHap1
T. suscitator	Tsu1M	?	AY703822	TsuHap1
T. sylvatica sylvatica	TssT009	Andalucía (Spain)	AY703821	TssHap1
	TssTO10	Andalucía (Spain)	AY703821	TssHap1
T. sylvatica lepurana	Ts11X	Namibia (Africa)	AY703819	TssHap2
	Ts12X	Namibia (Africa)	AY703821	TssHap1
	Tsl1Fr	South Africa	AY703821	TssHap1
	Tsl2Fr	South Africa	AY703819	TssHap2
T. pyrrhothorax	Tp2819	?	AY703820	TpHap1

Locality of origin (when known), GenBank accession number and the haplotype designation are reported.

Table 3. Matrix of the Pairwise Genetic Distances corrected for the selected evolutionary model.

	BbAF380305	GgX52392	TvHap1	TvHap2	TtHap1	TsusHap1	TssHap1	TssHap2	TpHap1
BbAF380305	–								
GgX52392	45.13	–							
TvHap1	50.40	50.91	–						
TvHap2	50.70	52.43	0.068	–					
TtHap1	49.18	58.91	27.09	25.97	–				
TsusHap1	47.64	52.28	24.51	23.46	05.88	–			
TssHap1	56.12	51.03	24.70	23.64	26.16	25.84	–		
TssHap2	56.72	51.60	25.06	24.00	25.77	25.45	0.016	–	
TpHap1	49.74	47.96	14.62	13.82	24.42	23.08	19.31	19.64	–

Differences are expressed as percentage in % between the different haplotypes within the *Turnix* genus (see Table 2 for abbreviations).

two *T. s. dussumier* from Raipur, India (Oxford University Museum of Natural History, Oxford, UK).

DNA isolation, PCR and sequencing

Blood and tissue samples were processed with a salting-out (Gemmell and Akayama 1996) and a standard phenol-chloroform method, respectively. DNA from museum samples was extracted with DNeasy Tissue Kits (Qiagen) following manufacturer instructions. However, washes in excess of TE9 (0.05 M Tris–HC1, 0.01 M NaCl, 0.02 M EDTA, pH 9.0), were included into the standard protocol to remove possible protease or PCR inhibitors (Hall et al. 1997). Contamination with PCR products or high molecular weight DNA was prevented by performing all museum samples extractions in an isolated laboratory, kept free of good-quality DNA and PCR products, and was monitored by including two extraction blanks in every extraction round.

The amplification conducted on all the *Turnix* samples using the primers (MT-AB/MT-FB) seemed to be problematic, as the chromatograms showed a double sequence that could indicate the presence of a 'Numt' with a different mutation rate (Lopez et a1. 1994; Bensasson et a1. 2001). One new primer was specifically designed in an internal region of the cyt-*b*, The 3′-fragment of the cytochrome *b*, was used to infer the phylogenetic relationship between the different species analysed. The MT-FB (5′-GAG TCT TCA GTT TTT GGT TTA CAA GAC-3′) and S5′-Cytb-R (5′-CAG TTG ACA ACC CAA CAC TAA CC-3′) primers pair were used to amplify a fragment of 691 bp. The last primer was designed in our lab. This fragment did not show spurious bands. Therefore, after an optimization process conducted using a temperature gradient PCR, a region of 606 bp in length of the cyt-*b* (approx. 1140 bp) has been successfully sequenced. All the sequences gave coding fragment without stop codon. Only one stop codon was found at the end of the sequences (TAA/UAA).

DNA amplification reactions contained at final concentration PCR Buffer, 1× (Bioline Taq Kit), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM of each primer, 1 U of *Taq* polymerase, and either 50–100 ng of total DNA or 5 μl of museum DNA extracts as template. Amplification reactions were performed in a MJ Research thermocycler, Model PTC-100 programmed for an initial denaturation cycle of 94 °C for 2 min, followed by 35 cycles of denaturation at 92 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. All reactions ended with a final extension at 72 °C for 5 min. To control the performance of the process and monitor for contamination, positive (diluted blood DNA) and negative (water) DNA controls, respectively, were included with each set of PCR reactions. Additionally, negative extraction controls (mock extractions with no starting material) were included in all of the amplifications of museum extracts. Amplification products were separated by electrophoresis on 1.5% agarose gel in TBE 1× buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA) in the presence of 0.33 mg/l EtBr, Gels were visualised under UV and

photographed with a digital image system (Kodak 1D3.5.4. software, Eastman Kodak Company), PCR products were cleaned through Montage-PCR (Millipore Corp.) and sequenced on an automated DNA sequencer (ABI-3100, Applied Biosystems) using the BigDye Terminator Cycle Sequencing Kit version 2.0 (Applied Biosystems, Inc.). The primers S5′-Cytb-R/MT-FB, used for the amplification, were also used for the sequence reaction. Sequences were aligned by using the program Sequencer^{TM} 4.1.2 (Gene Codes Corporation) and tested with Gblocks software ver. 0.91b. (Castresana 2002).

Genetic distances and phylogenetic methods

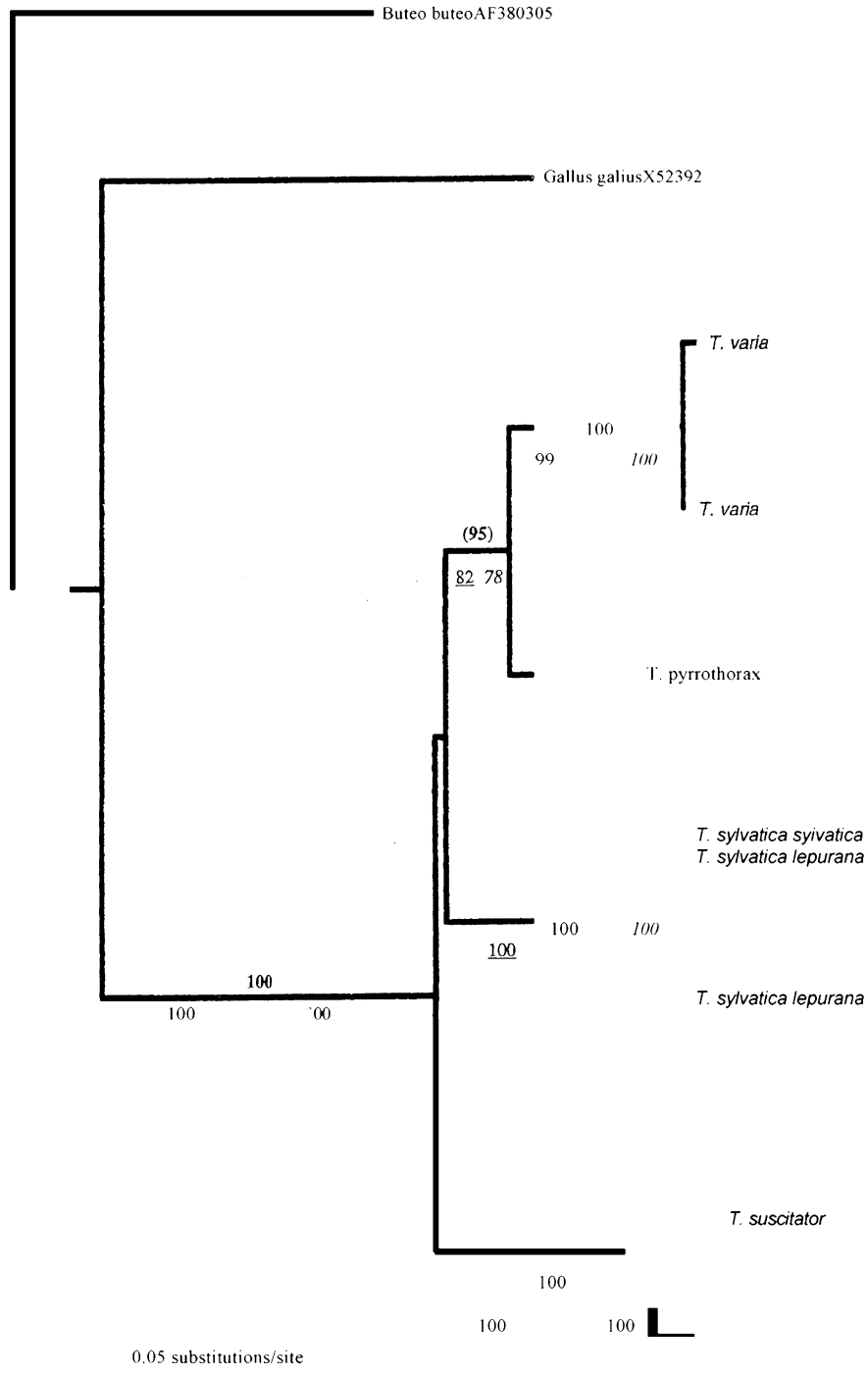
The model of DNA substitution that best fitted the data was selected with the program MODELTEST ver. 3.06 (Posada and Crandall 1998). The evolutionary model for our data selected by the Akaike Information Criterion (AIC) was GTR + G (G = 0.3115) which averaged a−nL = 2263.63712 and base frequencies A = 28.50%, C = 38.43%, G = 09.53% and T = 23.54%.

The phylogenetic relationships among haplotypes were analysed by distance and maximum likelihood in PAUP* v. 4.0b10 software (Swofford 1998), and by Bayesian inference in MrBayes v. 3.0b4 software (Huelsenbeck and Ronquist 2001), through a consensus tree with bootstrap values of Maximum Likelihood-Distance (minimum evolution tree with 2000 replicates), Maximum Likelihood-Heuristic Search (with 2000 replicates), and *a posteriori* probability values (with 100,000 generations and three independent chains).

The bootstrap values over 75% for the Maximum Likelihood-Heuristic search and over 99% for the Bayesian inference were assumed as significant support and they are indicated in the tree. Domestic fowl (*Gallus gallus*, GenBank accession number X52392) and the common buzzard (*Buteo buteo*, GenBank accession number AF380305) sequences were used as outgroups (Figure 1).

Results

Aligned sequences from the cyt-*b* included in this study totaled 606 bp and had a transition/transversion *ratio* of 129/38 = 3.3947. The base composition of these concatenated sequences reflected primarily the known under-representation of G in vertebrate mtDNA (A = 28.50%, C = 38.43%, G = 09.53%, T = 23.54%). The risk of contamination is due to the fact that despite that DNA extracted from museum specimens is degraded and only minute amounts are preserved, the PCR techniques are extremely sensitive and can easily pick up small amounts of contaminant DNA (O'Rourke et al. 2000). The great difficulty in the amplification of degraded DNA is due to physical and chemical degradation of DNA templates (Hofreiter et al. 2001). Ancient DNA can only be extracted in minute amounts, with small fragments and is often associated with PCR inhibitors. Several of the tissues analysed in this investigation failed


Buteo buteoAF380305
Gallus galiusX52392
T. varia
100
99
100
T. varia
(95)
82 78
T. pyrrothorax
T. sylvatica syivatica
T. sylvatica lepurana
100
100
100
100
T. sylvatica lepurana
100
00
T. suscitator
100
100
100
0.05 substitutions/site

◄

Figure 1. Neighbor-Joining tree of haplotypes. The haplotype names that are indicated in the tree are described in Table 1. Bootstraps over 75% were considered as good support in this tree, and over 99% for Bayesian's results. Numbers in bold letters above the branches were obtained from Bayesian inference (100,000 generations and three independent chains). Underlined numbers on the left and below the branches were obtained from Maximum Likelihood (ML)-Heuristic Search (with 2000 replicates). Italic numbers on the right and below the branches were obtained from ML-Distances (minimum evolution tree with 2000 replicates). An approximated value to the considered Bayesian inference is reported between brackets. The other values of this branch are close to the supported values for bootstrap.

to show a PCR products, indicating that the DNA was degraded and/or that PCR inhibitors were present in the tissues.

The nucleotide diversity ± SD = was 0.1271 ± 0.0659. Seven haplotypes were found: two haplotypes for *T. varia* (Tv Hap 1 and Tv Hap2), one for *T. tanki* (TtHap 1), one for *T. suscitator* (TsuHap 1), one for the AH (TssHap 1), two for *T. sylvatica lepurana* (TssHap 1 and TssHap2), and one haplotype for *T. pyrrhothorax* (TpHapl). The numbers of variable sites were 222 (36.63%) for all of the positions. Sequences have been deposited in GenBank (see Table 2).

Sequence divergence was evaluated from pairwise distances using the selected evolutionary model (GTR + G). Estimation between the *T. sylvatica* sp. group haplotypes (TssHap 1 and TssHap2) and the other species, ranged from 19.47% (TvHap2) to 25.96% (TtHapl). The overall average of corrected *p*-distances between the different species groups (*T. varia*, *T. tanki*, *T. suscitator*, *T. sylvatica* sp. and *T. pyrrhothorax)* was 20.16%, whereas the distances between conspecific haplotypes of *T. sylvatica lepurana* and *T. varia* were 0.16 and 0.68%, respectively. The smallest genetic differentiation between species was found between *T. suscitator* and *T. tanki* (5.88%), whereas, the largest differentiation between species was found between *T. varia* and *T. tanki* (26.53%).

Discussion

Genetic divergence within the Turnix genus

The phylogenetic relationships among haplotypes which were analysed by distance, maximum likelihood, and by Bayesian inference revealed three well supported branches corresponding (1) to the Australian endemics (*T. varia* and *T. pyrrhothorax*), (2) two south-Asian species (*T. tanki* and *T. suscitator*) and (3) two subspecies of the widely distributed *T. sylvatica* complex (*T. s. sylvatica* and *T. s. lepurana*). This also confirmed the current subdivision at the species level for the taxa involved. Indeed, the amount of genetic divergence found within the *Turnix* was relatively large (20.16%), suggesting a quite heterogeneous genus. However, given the relatively small mtDNA sequence utilised for this investigation, the genetic divergence value have to be considered as very coarse esti-

mates. The small genetic differentiation found between *T. suscitator* and *T. tanki* (5.8%) is in good agreement with the observation that these two species can hybridize in captivity (*sensu* Short 1969; H. Garrido and J.J. Negro, pers. comm.). The small differentiation found between the AH (*T. s. sylvatica*) and *T. s. lepurana* (0.00–0.16%) confirmed the extremely low level of genetic divergence in terms of cyt-*b* sequence between these two supposedly conspecific subspecies, which could even be considered too small to justify a subdivision. Such a small genetic divergence seems also to be too small even for the separation at the Evolutionary Significant Units level (ESU: cf., Ryder 1986).

In birds, mtDNA sequence divergence between ESUs has so far been shown to vary between 1 and 8% (Zink et al. 2000). However, a cautious approach should be undertaken as the use of genetic distances for the ESU definition, has been shown to be dubious when populations are compared, because there are indistinct boundaries between the levels of divergence observed within and among different taxonomic units in many groups. Furthermore, the ESU definition has sparked much discussion on the merits and practical approaches of identifying them (cf., Waples 1998). A variety of methods have been proposed based on ecological, biogeographical, and phenotypic data (Vogler and DeSalle 1994). Although divergences in such characters are recognised as important parameters, it has been suggested that the period of evolutionary time that populations have been isolated should also be considered, and that their identification should be at least partially based on molecular genetic data (Avise and Ball 1990).

Even if the genetic differentiation that we are reporting may not warrant subspecies separation, the two populations that we compared differ markedly in terms of biometry. The Mediterranean form (*T. s. sylvatica*) was significantly heavier (60–70 g, mean adult body mass) than African or Asian forms (35–50 g) and larger (85–92 mm for wing-chord, compared to 67–73 mm of *T. s. dussumier* and 76–83 mm of *T, s. lepurana*: Violani and Massa (1994) suggested that AH might be considered as an example of gigantism by isolation. Conversely, we may argue that individuals of the *Turnix* population inhabiting highly seasonal areas in the Mediterranean area, thus suffering from harsh winters compared to their African or Asian counterparts, may have been selected for larger sizes following Bergmann's rule (1847).

What molecular markers can tell about reintroductions

No conservation task is more daunting, both politically and scientifically, than the reintroduction of species to areas from which they have been eliminated or reduced in abundance. Restoring the former distributions or abundances of such species requires overcoming biological, economic, logistical, sociological and legal hurdles. The need for criteria for the evaluation of the feasibility or not of a plan of reintroduction, repopulation or translocation is therefore urgent, and the molecular analyses provides invaluable tool. In fact, DNA

sequences provide a wealth of new characters to help resolve phylogenetic relationships that have proven intractable with other types of data. Phylogenetic studies using gene sequences should utilise a large amount of sequence data (Cao et al. 1994; Cummings et al. 1995; Harlid et al. 1997), relatively dense taxon sampling including relevant outgroups (Graybeal 1998), and appropriate models of sequence evolution (Huelsenbeck and Crandall 1997). When any of these critical elements are lacking, phylogenetic relationships of the ingroup taxa may be estimated inaccurately.

The contemporary use of different genetic markers (i.e., STR or microsatellites) could increase the power of the conclusions which can be drawn from our analyses, Unfortunately, the limited sample size of our specimens (in particular of *T. s. sylvatica*) allows no further recovery of useful information. Given the relatively short period of radiation of the different species and subspecies within the *Turnix* genus, as stressed by the relatively short length of the branches of the phylogenetic tree, the above mentioned issues should not affect our interpretation of the results.

How to bring back T. sylvatica to Europe

This investigation allowed us to exclude a large genetic distance between *T. s. sylvatica* and *T. s. lepurana*. If desired, African stock could be imported into Europe, which implies a reintroduction of the same species in its former grounds. However, we should be aware of the fact that the correlation between the genetic variance in traits of ecological significance and molecular diversity is weak, with molecular measure explaining only about 4% of the variation in quantitative traits (Reed and Frankham 2001; Gilligan et al. 2005). Therefore, the potential for evolutionary response in quantitative traits cannot be predicted by use of molecular measure, and the lack of divergence at the molecular level cannot exclude genetic divergence in quantitative traits. Hence, we cannot exclude the possibility of a genetic adaptation of *T. s. lepurana* due to the environment where extant populations are living, which should be taken into account when planning a reintroduction action. Biological considerations including knowledge of genetics, demographics, behaviour, disease, and habitat requirements are critical for determining if the reintroduction should be attempted, if it could be successful, and how it could be implemented in an efficient and effective manner.

Among the most important points in assessing a release site are determining the amount and type of habitat required, and the cause of decline of the species to be reintroduced. It is also extremely important to identify and remove completely the factors of extinction in southern Spain and assess with high confidence that no indigenous Andalusian hemipode populations are still surviving in southern Spain and in northern Africa, before planning reintroduction from southern Africa.

The last recorded Andalusian hemipodes were living in hunting states currently within the limits of Doñana National Park. No more hunting is allowed there and habitat characteristics have not changed significantly. Even though we do not know whether human persecution was a significant factor responsible for the demise of the buttonquail in Doñana, we may assume at least that the protected area is large enough (>75,000 ha) and should be able to hold a viable population of buttonquails.

Given that kurrichane buttonquails have been bred repeatedly in captivity (Rutgers and Norris 1970; Spenkelink-Van Schaik 1984), we suggest a reintroduction plan with the following steps: (a) Capture of wild birds in Africa. In several southern countries, such as Namibia and South Africa, *T. sylvatica* is a common game species that can be legally trapped. Some individuals may be captured to be kept in captivity for propagation purposes. This should be made in the same general area of capture in collaboration with local authorities to develop breeding technology and to multiply the captive population. (b) Translocation of captive-bred stock to a game farm or zoo in southern Spain near Doñana for acclimatization and further propagation. (c) Relocation of captive-bred buttonquails to large aviaries built within Doñana. (d) Release of buttonquails hatched in the Doñana aviaries into the wild.

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