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ORIGINAL ARTICLE

Comparative phylogeography of two African carnivorans presumably introduced into Europe: disentangling natural versus human-mediated dispersal across the Strait of Gibraltar

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ABSTRACT

Aim Natural processes of colonization and human-mediated introductions have shaped current patterns of biodiversity in the Mediterranean Basin. We use a comparative phylogeographic approach to investigate the genetic structure of *Herpestes ichneumon* and *Genetta genetta* (Carnivora) across the Strait of Gibraltar, and test for their supposedly contemporaneous introduction into Iberia.

Location Mediterranean Basin and Africa.

Methods We sequenced two mitochondrial fragments (cytochrome *b* and control region) of 91 (*H. ichneumon*) and 185 (*G. genetta*) individuals, including the sole archaeological record of *G. genetta* in Iberia, dating from the Muslim occupation. We used phylogenetic and tokogenetic methods, summary statistics, neutrality tests, geographic–genetic pairwise comparisons and coalescent estimates to explore the history of the two species in the Mediterranean Basin.

Results In North Africa, an autochthonous (Clade I) and a western African mtDNA clade, coalescing in the Middle to Late Pleistocene, co-occurred in both species. Only Clade I was present in Europe. In *H. ichneumon*, the European pool showed deep coalescence (median = 335 kyr) and high genetic differentiation and diversity compared with its North African counterpart, suggesting long-term stability of female effective population size. In sharp contrast, *G. genetta* in Europe exhibited lower genetic diversity, weak differentiation with North Africa and recent demographic expansion; however, Andalusia and Catalonia (Spain) showed distinctly higher genetic diversity, and the archaeological specimen had the predominant European haplotype.

Main conclusions The co-occurrence of autochthonous and sub-Saharan lineages in North Africa (1) supports a new, emerging biogeographic scenario in North Africa, and (2) suggests a connection through the Sahara, possibly from the Middle Pleistocene onwards. Our results refute the idea that *H. ichneumon* was introduced into Europe contemporaneously with *G. genetta*. Instead, they support a scenario of sweepstake dispersal during Late Pleistocene sea-level fluctuations, followed by long-term *in situ* evolution throughout the last glaciation cycles. *Genetta genetta* appears to have undergone a recent spread from at least two independent introduction 'hotspots' in Catalonia and

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Andalusia, possibly following antique trade routes and/or Muslim invasions. Despite their contrasting histories, the European gene pools of both species represent unusual cases leading to the preservation of autochthonous, North African lineages.

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Keywords

Ancient DNA, coalescence, comparative phylogeography, *Genetta genetta*, *Herpestes ichneumon*, introduction, Mediterranean Basin, mitochondrial DNA, North Africa, transmarine dispersal.

INTRODUCTION

In the Mediterranean Basin superimposed forces, including climatic fluctuations, natural processes of colonization and human-mediated introductions, have deeply impacted current patterns of biodiversity (Blondel & Vigne, 1993). During the Late Miocene Messinian salinity crisis, the *c.* 200-m lowering of sea level created a continuous land bridge across the western Mediterranean (Rouchy *et al.*, 2007), which allowed intense dispersal between North Africa and south-western Europe, notably in mammals (van der Made *et al.*, 2006). The opening of the Strait of Gibraltar (SG) in the Early Pliocene (Loget & Van Den Driessche, 2006), now 14 km wide, has long been considered a biogeographic barrier to the natural dispersal of non-flying vertebrates (Dobson & Wright, 2000; O'Regan *et al.*, 2006). However, recent palaeontological data show that North Africa lost its predominantly Afro-tropical affinities during the Late Pleistocene through the combined effects of desiccation and invasion by European immigrants (Geraads, 2010). This supports a scenario of sweepstake migrations across the SG during sea-level fluctuations associated with the last glaciations (Flemming *et al.*, 2003), when large vegetated islands resulted from a sea-level depression of 140 m relative to the present (Masseti, 2009).

Although it is difficult to distinguish between natural crossings during the Late Pleistocene and prehistorical, human-mediated introductions (see Zeder, 2008), phylogeographic analyses, along with routine ancient DNA techniques, have recently provided evidence for a complex, temporally superimposed, dispersal framework across the SG, which included (1) episodic permeability to the natural dispersal of non-flying vertebrates from North Africa, from the Upper Pliocene to approximately the Last Glacial Maximum (e.g. Harris *et al.*, 2002; Pinho *et al.*, 2007), and (2) several early translocations by humans from both sides of the western Mediterranean (e.g. Cosson *et al.*, 2005; Beja-Pereira *et al.*, 2006; Recuero *et al.*, 2007).

Facing the potential multiplicity of geographic sources and time-scales of species dispersal, we investigate here the comparative phylogeography of two small carnivorans (Mammalia), the Egyptian mongoose, *Herpestes ichneumon* (Linnaeus, 1758), and the common genet, *Genetta genetta* (Linnaeus, 1758), both presumably introduced from North Africa to Europe during a contemporaneous historical period (Morales, 1994; Dobson, 1998; Amigues, 1999; Riquelme-Cantal *et al.*, 2008; but see Campanella & Wilkens, 2004). Both species are opportunistic, ubiquitous carnivores that share similar distributions (Fig. 1) and ecological requirements within their native ranges (Delibes & Gaubert, in press; Palomares, in press). However, the mongoose is restricted to the south-western quarter of Iberia (Delibes, 1982; Barros, 2009), whereas the genet shows a more 'successful' establishment in Europe, from Iberia to south-eastern France (Gaubert *et al.*, 2008). The two species are ideal cases for studying dispersal routes in the western Mediterranean because (1) they never shared a circum-Mediterranean distribution that would have allowed continental diffusion through the eastern Mediterranean (Fig. 1), (2) the fossil record attests their presence in the Pleistocene–Holocene of North Africa, but not in Europe (Kurten, 2007; Werdelin & Peigné, 2010), (3) archaeozoological records in continental Europe associate them with the last Muslim dynasty ruling Iberia, the Almohads (Morales, 1994; Riquelme-Cantal *et al.*, 2008), and (4) they exhibit different European, invaded ranges, the common genet also being present in the Balearic Islands (Delibes & Gaubert, in press).

We generated a new mitochondrial DNA (mtDNA) data set for the Egyptian mongoose, and completed a previously published data set on the common genet (Gaubert *et al.*, 2009). In both cases, we used ancient DNA techniques to include representatives from crucial, under-represented areas, such as the unique European archaeozoological record of the common genet from Portugal (Morales, 1994). We used batteries of exploratory methods, including summary statistics, neutrality tests and coalescent estimates, to test the following predictions based on contemporaneous introduction into Europe of the Egyptian mongoose and the common genet: (1) the two species share common phylogeographic and demographic patterns in North Africa, suggesting that similar historical processes shaped their matrilineal genealogies, (2) the two species dispersed from the same geographic source into Europe, and (3) the Egyptian mongoose exhibits a genetic structure and demographic history similar to those

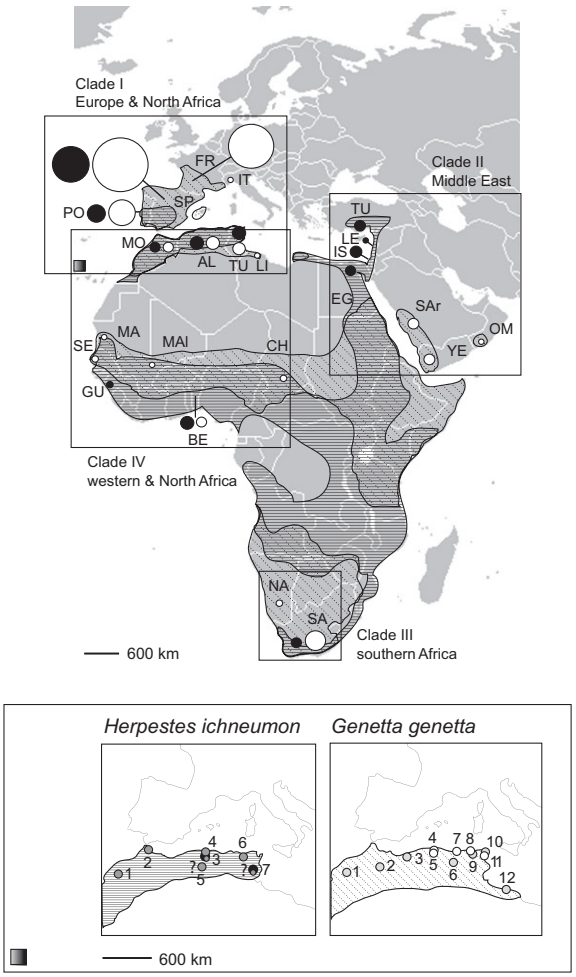

Figure 1 Distribution ranges of the Egyptian mongoose, *Herpestes ichneumon* (horizontal lines), and the common genet, *Genetta genetta* (dashed, diagonal lines), with each country's proportion of the total sample set given as black and white circles, respectively. The four boxes delimit the co-distributed, mtDNA geographic clades into which the two species were partitioned (Clade I in *H. ichneumon* also included one specimen from Benin). Country acronyms are defined in Appendix S1. The inset (bottom) shows the North African distribution of Clade I and IV haplotypes in both species. For *H. ichneumon* (grey and black circles for Clade I and IV representatives, respectively): 1, Marrakech; 2, Ceuta; 3, Forêt de Zéralda; 4, Fondouk; 5, unknown Algerian localities; 6, Ain Draham; 7, captive specimens from Tunisia. For *G. genetta* (white and light grey circles for Clade I and IV representatives, respectively): 1, Marrakech; 2, Missoura; 3, Tlemcen; 4, Algiers; 5, Forêt de Zéralda; 6, Batna; 7, Jijel; 8, El-Kala NP; 9, Kroumirie; 10, Tunis; 11, Zagouan; 12, Tripoli.

of the common genet, compatible with historical introduction in Europe (e.g. low genetic differentiation between European and source populations, mtDNA signature of a demographic event reflecting introduction, and decrease of genetic diversity with geographic distance from putative sites of introduction).

MATERIALS AND METHODS

Geographic coverage and nature of samples

Tissue and hair samples from trapped or road-killed animals were gathered opportunistically between 1999 and 2009 through a network of collaborators (see Appendix S1 in Supporting Information). We examined nucleotide variability in 91 and 185 individuals of *H. ichneumon* and *G. genetta*, respectively. Because our main interest lies in comparative phylogeography across the SG, we focused our sampling effort on Europe and North Africa (*c.* 74 and 82% of the sample sets, respectively). We achieved a representative geographic coverage of the species' native ranges in western and southern Africa and the Middle East (Fig. 1). We strengthened our geographic sampling in critical areas such as North Africa and in poorly represented regions in Europe through the use of museum specimens. In the case of *H. ichneumon*, we extracted DNA from four individuals from central Spain (Castilla-La Mancha and Castilla-Leon), four from Maghreb (Algeria and Tunisia) and one from Lebanon. Regarding *G. genetta*, we added 52 new individuals to the sample set of Gaubert *et al.* (2009), including three museum specimens representing Haute-Normandie (France), Tunisia, and the only known individual collected from Libya. We also included the sole archaeological record of *G. genetta* found in Europe, from the Almohad levels of Mértola (first quarter of the 13th century; Morales, 1994) (Appendix S1).

Ancient-DNA laboratory procedures

We extracted DNA from a total of 26 skeletal, dental, pad and connective tissue samples, which included museum specimens (Appendix S1). Extraction was performed in isolated, ancient-DNA-dedicated boxes equipped with an autonomous ventilation system and UV-irradiation, in two laboratories: (1) Laboratorio de Sistemática Molecular, at the Museo Nacional de Ciencias Naturales of Madrid (MNCN collection samples), and (2) Service de Systématique Moléculaire, at the Muséum National d'Histoire Naturelle of Paris (MNHN collection samples). No sample of either species had been extracted using the ancient-DNA boxes prior to this study. At MNHN, we first processed the archaeological material of a common genet, a superbly preserved right hemipelvis. The bone had been in an undisturbed deposit (a sealed cesspit at the entrance of a house), among abundant micro-vertebrate debris, mostly of the black rat (*Rattus rattus*) (Morales & Rodriguez, 1997). All extractions were performed twice independently in the two laboratories, and polymerase chain reaction (PCR) products were checked for consistencies in nucleotide polymorphism. DNA was extracted from bones and teeth following a modified protocol of Rohland *et al.* (2004). The material was gently washed with Tris-EDTA buffer (pH = 9) solution and was then incubated under gentle rotation in the dark in 1.5 to 60 mL of GuSCN-based buffer (depending on sample size) at 40 °C for 5 days, along with negative extraction controls.

Purification consisted of two successive washes of chloroform-isoamyl alcohol (CIA 96:4). DNA was precipitated for 2 h at −20 °C, adding 2/3 volume of isopropanol and 0.1 volume of 3M sodium acetate. The final elution volume was 50 μL H_2O. We followed Gaubert & Zenatello (2009) for the extraction of the remaining samples, representing tanned, smoked or badly preserved tissues.

PCR amplification and sequencing

Two mtDNA fragments from the cytochrome *b* (cyt *b*) and control region (CR) were PCR-amplified. Given that cyt *b* tends to evolve at a slower rate than the CR in mammals, combining information from these two markers can avoid misleading effects on phylogenetic and genealogical reconstruction of possible homoplasy in the non-coding, fast-evolving CR (Phillips *et al.*, 2009). In the case of ancient-DNA protocols, PCRs were performed twice for each sample – resulting in four PCR replicates for each fragment – with negative amplification controls each time. The specific primers used to amplify mtDNA in *H. ichneumon* are given in Gaubert & Zenatello (2009). Four new pairs of primers were designed for *G. genetta* using Primer3 (Rozen & Skaletsky, 2000), fixing different 'Targets' values to obtain overlapping sets of PCR products. Because the presence of long tandem repeats in the first hypervariable region of CR in genets (Gaubert & Begg, 2007) may rend amplifications of short fragments difficult, we designed a new pair of primers that amplifies within the 5′ flanking region of CR, excluding the repeated motifs. PCR amplification protocols for ancient-DNA and fresh sample extracts are detailed in Gaubert & Zenatello (2009) and Gaubert *et al.* (2009), respectively. Complementary cyt *b* sequences were also produced for all of the CR-sequenced Clade I representatives (Europe) from Gaubert *et al.* (2009). The list of primer pairs, corresponding annealing temperatures and product sizes are given in Table 1. PCR products were visualized on a 1.5% agarose gel, purified and directly sequenced in both directions on 3730xl DNA Analyzer 96-capillary sequencers (Applied Biosystems, Foster City, CA, USA) at GenoScreen (Lille, France), Genoscope (Evry, France) and Secugen (Madrid, Spain).

The sequences produced from this study were deposited in GenBank under the accession numbers GU183490–GU183545 and GU189389 (Appendices S2–S4).

Phylogenetic analysis, coalescent events and genetic diversity among the main geographic lineages

We generated cyt *b* and CR sequences for outgroups chosen as closely related, non-monophyletic pairs with *H. ichneumon* (*Herpestes javanicus* and *Galerella sanguinea*) and *G. genetta* (*Genetta johnstoni* and *Genetta maculata*) (Gaubert & Begg, 2007; Patou *et al.*, 2009). We followed Gaubert & Begg (2007) by removing the third repeat motif of 81 bp (TR3′) responsible for cases of heteroplasmy in *G. genetta*, before proceeding to the alignment of CR sequences. Final alignments were reconstructed by eye using BioEdit 7.0.9 (Hall, 1999), representing 93 individuals and 926 bp (cyt *b*: 402 bp; CR: 524 bp) for *H. ichneumon*, and 187 individuals and 913 bp (402 bp; 511 bp) for *G. genetta*.

Models of molecular evolution for statistical phylogenetic analyses were selected from alignments pruned of indels and incomplete nucleotide sequences using ModelGenerator 0.85 under the Bayesian information criterion (Keane *et al.*, 2006). We performed model selection with four gamma categories for the separated data sets (cyt *b*/CR), with and without outgroups (see below). Best-fitting models and associated parameters are detailed in Appendix S5.

We ran maximum parsimony (MP) analyses with paup* 4.0b10 (Swofford, 2003) using tree bisection–reconnection (TBR) branch-swapping and fixing 'maxtrees' at 10,000. Indels were coded as binary characters using GapCoder (Young & Healy, 2003). Maximum likelihood (ML) analyses were run with TreeFinder (October 2008 version; Jobb *et al.*, 2004), injecting models for cyt *b* (HKY + I) and CR [HKY + Γ (*H. ichneumon*)/TrN + Γ (*G. genetta*)] as selected from ModelGenerator. Node support was assessed by 1000 bootstrap pseudo-replicates (Felsenstein, 1985) in MP and ML analyses. We used beast 1.4.8 (Drummond & Rambaut, 2007) to run Bayesian Markov chain Monte Carlo (MCMC) phylogenetic estimates. Model parameters followed ModelGenerator outputs, fixing uniform priors. Phylogenetic relationships were reconstructed under the Yule speciation process (Steel & McKenzie, 2001), with enforced monophyly of the ingroup. The mean substitution rate was unfixed, and branch rates were estimated from an uncorrelated lognormal distribution to accommodate possible rate variation among lineages. In order to separate parameter estimates for cyt *b* and CR, we partitioned the data sets by editing the xml source files, according to various sources of information (Drummond *et al.*, 2007; http://beast.bio.ed.ac.uk/Tutorials; http://groups.google.com/group/beast-users; http://tlpcouvreur.googlepages.com/beastpartitioning). Operators were tuned manually after screening of effective sample size values from preliminary runs, to maximize their efficiency and obtain convergence in poorly estimated parameters (Drummond *et al.*, 2007). Chain lengths were 50 to 100,000,000, sampled every 5 to 10,000 generations. Analyses were run two to four times independently using the web-accessible platform of the Computational Biology Service Unit (CBSU, Cornell University, NY; http://cbsuapps.tc.cornell.edu/beast.aspx). Convergence and stability of estimated parameters were checked using Tracer 1.4.1 (Rambaut & Drummond, 2008). Log and tree files were concatenated under LogCombiner 1.4.8 (Drummond *et al.*, 2007) with a final burn-in of 1000. Trees were summarized as maximum clade credibility trees using TreeAnnotator 1.4.8 (Drummond *et al.*, 2007), and were visualized and edited using FigTree 1.2.2 (Rambaut, 2009).

We estimated times to most recent common ancestors (TMRCAs) for the main geographic lineages through beast, restricting our data sets to the ingroup and fixing a coalescent model of expansion growth (see Pybus & Rambaut, 2002).

Table 1 List of the primer pairs used to amplify fragments of the cytochrome *b* and control region of the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta*, together with their corresponding annealing temperatures and product sizes. Primers specifically designed to amplify short mitochondrial fragments are given under 'aDNA' (ancient-DNA protocol).

	Primer pairs	Annealing temperature (°C)	Product size (excluding primers) (bp)	Source
Herpestes ichneumon				
Cytochrome *b*	GVL14724 5′ GAT ATG AAA AAC CAT CGT TG 3′	50	402	Modified from Irwin *et al.* (1991)
	H15149 5′ CTC AGA ATG ATA TTT GTC CTC A 3′			Modified from Kocher *et al.* (1989)
Cytochrome *b* (aDNA)	HERP-cytb-41L 5′ TAA TGA CCA ACA TCC GCA AA 3′	52	124	Gaubert & Zenatello (2009)
	HERP-cytb-204H 5′ GTG CAT GGC TAG GAA TAG GC 3′			Gaubert & Zenatello (2009)
	HERP-cytb-185L 5′ GCC TAT TCC TAG CCA TGC AC 3′	52	206	Gaubert & Zenatello (2009)
	HERP-cytb-433H 5′ AAC CTA TGA AGG CAG TTG CTA TT 3′		Total: 350	Gaubert & Zenatello (2009)
Control region	HERP-DL93L 5′ CAA CTC CAC CCC ACA ACT CT 3′	51	443	Gaubert & Zenatello (2009)
	HERP-DL656H 5′ TGT GTG ATC ATG GGC TGA TT 3′			This study
Control region (aDNA)	HERP-DL93L	52	118	Gaubert & Zenatello (2009)
	HERP-DL251H 5′ TTC ATG GGG ACA AGC AGT TA 3′			Gaubert & Zenatello (2009)
	HERP-DL213L 5′ AAC TGC TTG TCC CCA TGA AT 3′	58	127	Gaubert & Zenatello (2009)
	HERP-DL379H 5′ GCA AGG GTT GAT GGT TTC TC 3′			Gaubert & Zenatello (2009)
	HERP-DL324L 5′ CCT CAA CTG TCC GAG AGA GC 3′	61	112	Gaubert & Zenatello (2009)
	HERP-DL475H 5′ ATG GCC CTG AGG TAA GAA CC 3′		Total: 362	Gaubert & Zenatello (2009)
Genetta genetta				
Cytochrome *b*	GVL14724	id.	id.	id.
	H15149			id.
Cytochrome *b* (aDNA)	GG-cytb1L 5′ TGA CCA ACA TCC GAA AAT CC 3′	54	143	This study
	GG-cytb1H 5′ GAA GGC AGT TAT TGT ATC TGA TGT G 3′			This study
	GG-cytb2L 5′ CGG CTC TTT ATT AGG AGT TTG C 3′	58	140	This study
	GG-cytb2H 5′ AAA TAG ACA GAT GAA GAA CAT GGA G 3′			This study
	GG-cytb3L 5′ TTC TCA TCA GTA ACT CAC ATC TGC 3′	54	154	This study
	GG-cytb3H 5′ TGA AGG CTG TAG CCA TAA CTG 3′			This study
	GG-cytb4L 5′ GCC TCC ATG TTC TTC ATC TGT 3′	50	134	This study
	GG-cytb4H 5′ CGG TTG CTC CTC AGA ATG AT 3′		Total: 381	This study
Control region	CR1F 5′ CCA CTA TCA GCA CCC AAA GC 3′	61	507 to 590	Palomares *et al.* (2002)
	CR2R 5′ CCC GGA GCG AGA AGA GG 3′			Palomares *et al.* (2002)
Control region (aDNA)	GG-CR1L 5′ ACC AAA AAT CAA CCC AAC AA 3′	58	127	This study
	GG-CR1H 5′ TAA TAT TCA TGG GGC GGT TG 3′			This study
	HVGg-H 5′ CAC GAT ATA CAT AGT ATG YCT 3′	58	204	Gaubert *et al.* (2009)
	HVGg-L 5′ GAA ATT CTT TTT AAA CTA TTC CTT 3′			Gaubert *et al.* (2009)

TMRCAs and credibility intervals (95% highest posterior density – 95% HPD) were calculated using an uncorrelated lognormal relaxed clock function (Drummond *et al.*, 2006), fixing model parameters estimated from ModelGenerator [cyt *b*: HKY + I; CR: HKY + I (*H. ichneumon*)/TrN + Γ + I (*G. genetta*)]. We used fossil data to calibrate the root height of the tree. For *H. ichneumon*, we fixed a uniform prior (lower bound of 3.5 Ma to upper bound of 3.8 Ma) reflecting the time frame of the species' first record in Laetoli, Tanzania (Werdelin & Lewis, 2005). In the case of *G. genetta*, some uncertainty is caused by a possible first record from Sterkfontein Jacovec Cave, South Africa (4.2–3.5 Ma; Werdelin & Peigné, 2010), whereas remains confidently identified at the species level have been described from Koobi Fora, Kenya (2.5 Ma; Werdelin & Lewis, 2005). To take this into account, we used a lognormal prior (Drummond *et al.*, 2007), with the lower, hard bound fixed at 2.5 Ma and the lognormal standard deviation value fixed at 0.8725, so that the upper, soft bound reaches its 95% credibility interval at 4.2 Ma (initial value = 3.5 Ma).

We used DnaSP 5.00.07 (Librado & Rozas, 2009) to calculate the number of polymorphic sites (*S*), nucleotide diversity (π), number of haplotypes (*h*) and haplotype diversity (*Hd*) among the main geographic lineages. Given that DnaSP considers gaps or missing data as unique features, 'new' haplotypes based on missing data distribution along sequences may bias estimates of genetic diversity. We thus removed nucleotide sequences with a fair level of missing data, when they were 100% identical to at least one complete haplotype. In order to maintain sequences with unique

polymorphisms, we reconstructed 'most parsimonious' sequences by replacing missing data with the aligned portion of their phylogenetically closest relative. This resulted in a final alignment of 64–174 individuals and 845–925 bp, including three most-parsimonious sequences of *H. ichneumon* from Egypt (T865) and Benin (T701, T702), and three of *G. genetta* from Benin (T298, T304) and Chad (T328).

Genetic structure, historical demography and time of crossing of the SG: tests of introduction signatures in Europe

To compare the history of dispersal between North Africa and Europe in the two species, we applied population-level methods to Clade I, to which all the European populations belong. We built a median-joining network from the 40 and 137 cyt *b* + CR Clade I sequences (*H. ichneumon* and *G. genetta*, respectively) using Network 4.5.1.0 (http://www.fluxus-engineering.com), with the parameter ε fixed at 0 to minimize alternative median networks. Fixing ε at 70 (*H. ichneumon*) and 150 (*G. genetta*), following the user's guide recommendations (Polzin & Daneshmand, 2008), when maximum pairwise differences are respectively 7 and 15 (character weight = 10; transversion/transition weighting = 1:1), did not produce discordant branching hypotheses.

We estimated the level of genetic differentiation between North Africa and Europe (and the Balearic Islands in the case of *G. genetta*) by calculating pairwise genetic distances (Φ_{ST}) (Reynolds *et al.*, 1983) in Arlequin 3.1 (Excoffier *et al.*, 2005). The significance of Φ_{ST} values was assessed through 10,000 permutations for each pair. We performed mismatch analysis (distribution of pairwise genetic differences) for European gene pools and assessed the fit of the observed distributions to the Rogers' sudden expansion model (Rogers & Harpending, 1992) by calculating (1) the sum of squared deviations (SSD) between observed and expected distributions, and (2) the Harpending raggedness index (*r*), as implemented in Arlequin, using 1000 bootstrap replicates (Schneider & Excoffier, 1999).

We screened for historical, demographic signatures in European gene pools by testing for deviation from neutrality. Neutrality tests have proved to be useful in detecting demographic events, especially when assumptions of recombination or selection can be discarded (Ramos-Onsins & Rozas, 2002; Depaulis *et al.*, 2003). We believe that there is a strong probability of these assumptions being met in our data sets, because most of the nucleotide variation is contained in the non-coding CR fragment (see Results), and recombination events remain rare in the mitochondrial genome (White *et al.*, 2008). We used statistics (1) based on mutation frequencies (Tajima's *D*, Fu and Li's D^* and F^*, Ramos-Onsins and Rozas' R_2), and (2) derived from the distribution of haplotypes (Fu's F_S, Nei's *Hd*) or linkage disequilibrium (Wall's *B* and *Q*, Kelly's Z_{nS}, Rozas' Z_A and *ZZ*) as implemented in DnaSP (see Ramírez-Soriano *et al.*, 2008, for a detailed review). We ran 1000 replicates, assuming a coalescent process with a neutral, infinite-sites model and large constant population sizes (Hudson, 1990), to calculate the *P*-value of each observed statistics. The detection of demographic expansion, contraction and bottlenecks, together with their level of intensity and relative temporality, followed the decision tables provided by Ramírez-Soriano *et al.* (2008, tables 3–5: polymorphic sites *c.* 10; *c.* 20 < number of individuals <100).

In order to detect the signature of putative introduction events in Europe, we investigated the geographic structure of genetic variation among European 'subpopulations' (region-level partitions; see Appendix S1). We assessed the correlation between geographic and genetic distances through the Mantel test (Smouse *et al.*, 1986), as implemented in Arlequin. In the case of historically introduced populations, we expect an absence of isolation by distance because of (1) null *in situ* genetic drift, and (2) a random distribution of genetic diversity via human-mediated propagation. Geographic distances were calculated by taking into account continental contours, using the 'Rule' tool and the 'Traject' option in Google Earth©. We tested the correlation between pairs of matrices expressed either as linear geographic distances versus pairwise Φ_{ST} or as ln-transformed geographic distances versus linearized Φ_{ST} (Slatkin, 1995), as recommended by Rousset (1997). We ran 1000 permutations to assess the significance level of the squares of the observed correlation coefficients (Smouse *et al.*, 1986).

The geographic structure of genetic variation in continental Europe was further assessed through spatial analysis of molecular variance (SAMOVA) using samova 1.0 (Dupanloup *et al.*, 2002). Through a simulated annealing procedure, SAMOVA seeks to establish a user-defined number of *k* groups of geographically proximate populations that maximizes the proportion of total genetic variance arising from differences among groups of populations (Φ_{CT}). Although this method was first tested in a case study including introduced populations (Dupanloup *et al.*, 2002), it is only recently that SAMOVA has been used to investigate translocation patterns (Tomimatsu *et al.*, 2009). We expect that distinct introduction 'hotspots' (i.e. focal regions where populations have been introduced) will be identified as well-differentiated groups not separated by apparent geographic barriers. We let *k* vary from 2 to 20, and ran 100 simulated annealing processes to modify group structure and test the significance of Φ_{CT} values (Dupanloup *et al.*, 2002).

We also tested for signatures of introduction by observing the distribution trend of a series of genetic indexes relative to geographic distance from putative introduction hotspots. On the basis of the distribution of haplotype diversity and network pattern (see Results), we specifically tested the following hypotheses: (1) Andalusia constituted the introduction hotspot of *H. ichneumon* in Europe, and (2) both Andalusia and Catalonia represent distinct introduction hotspots for *G. genetta* in continental Europe. We made the assumption that, under a scenario of a single, recent introduction in Europe, pairwise genetic distances (Φ_{ST}) among introduction hotspots and the other geographic subpopulations should not exhibit any specific correlation with geographic distances, although one may

observe a positive trend owing to the loss of intra-population variability in diffusing populations. On the other hand, the propagation of a subset of the original genetic pool from an introduction hotspot accompanying the geographic progression of humans is likely to result in a loss of haplotype diversity (e.g. Anderung *et al.*, 2005), here measured as *h*, and in a decrease in the number of shared haplotypes (*sh*), correlated with geographic distance. We used Spearman's r_s (*H. ichneumon*) and Pearson's *r* (*G. genetta*) to assess the significance (1000 permutations) of correlation among geographic distances and those genetic indexes.

RESULTS

Phylogenetic analyses, TMRCAs and genetic diversity among the major geographic lineages

Genetic variability within *H. ichneumon* was much lower than in *G. genetta*, with the number of polymorphic sites varying from 10 (cyt *b*) to 37 (CR) vs. 32 to 73, and phylogenetically informative sites ranging from 10 (cyt *b*) to 33 (CR) vs. 29 to 65 (including one indel), respectively. Relative to Gaubert *et al.* (2009), nine new haplotypes were recorded in *G. genetta*, from France (Pays-de-la-Loire: CRH3), Spain (Catalonia: CRH12, CRH13, CRH14; Galicia: CRH8), Tunisia and Benin (CRH28) and South Africa (cytbH12/CRH42, CRH47) (see Appendices S2–S4).

The three methods of phylogenetic reconstruction resulted in congruent topologies in each species, the mtDNA tree of *G. genetta* being very similar to that found by Gaubert *et al.* (2009; see below). Strikingly, like *G. genetta*, *H. ichneumon* exhibited four mtDNA geographic lineages as follows: Clade I (Europe and North Africa), Clade II (Middle East), Clade III (southern Africa) and Clade IV (western and North Africa) (Figs 1 & 2; phylogenetic trees given in Fig. 2 are fully detailed in Appendixes S6 and S7). In *H. ichneumon*, phylogenetic relationships among these major geographic clades were poorly supported, except for the sister relationship between Clades III and IV (Fig. 2). Nevertheless, all the main mtDNA lineages were strongly supported (Bayesian posterior probabilities = 1.00) under a coalescent model of expansion growth discarding outgroups (same observation for *G. genetta*; Fig. 3).

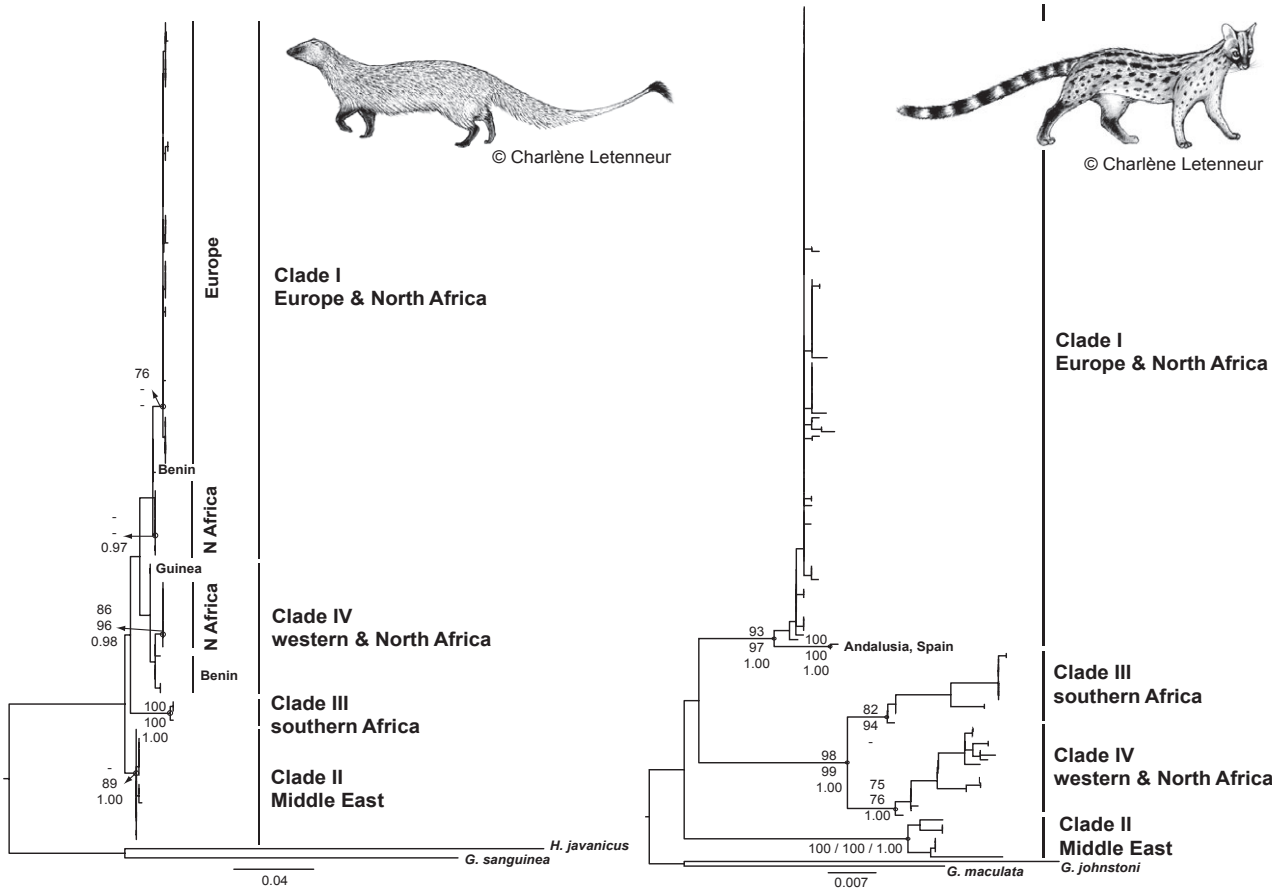

Figure 2 Summarized maximum likelihood phylogenetic trees of the Egyptian mongoose, *Herpestes ichneumon* (left), and the common genet, *Genetta genetta* (right), based on the partitioned analysis of cytochrome *b* and control region data sets. Values at major nodes indicate, from top to bottom, maximum parsimony and maximum likelihood bootstrap support (>75%), and Bayesian posterior probability (>0.95). Scale bars indicate sequence divergence. The first two taxa at the base of the tree represent the outgroups. Clade numbering and delimitation refer to Fig. 1. The detailed phylogenies are given in Appendices S6 and S7.

In *H. ichneumon*, Clade I was extended to western Africa, with the inclusion of an individual from Benin. The distributions of Clades I and IV partially overlapped in North Africa in both species (Fig. 1). TMRCA estimates suggested a Middle to Late Pleistocene origin of the main geographic clades for both species (Fig. 3). Clade I had the oldest coalescent in *H. ichneumon* [735 kyr (95% HPD = 235–1560)], whereas it was the youngest lineage in *G. genetta* [358 kyr (73–1004)]. Within *H. ichneumon* Clade I, the European and African gene pools were clearly differentiated and had similar TMRCAs, between 335 kyr (108–726) and 272 kyr (39–750), respectively. In *G. genetta* Clade I, the North African and European haplogroups were intermixed without apparent structure, with the exception of a distinct lineage restricted to two Andalusian (Spain) individuals, coalescing at 15 kyr (0–83).

In *H. ichneumon*, Clade I showed genetic diversity estimates that were intermediate or high relative to the three other geographic clades (*Hd* and π), whereas in *G. genetta* those were markedly lower [*Hd* (cyt *b*), π], despite an important number of CR haplotypes (Table 2). Within *H. ichneumon* Clade I, populations from Europe had a similar level of genetic diversity to those from Africa [*Hd* and π (CR)]. On the other hand, European populations of *G. genetta* exhibited a markedly lower genetic diversity compared with that from North Africa [*Hd* and π (CR)].

Genetic structure and potential signatures of introduction events within Clade I

In the network analysis of *H. ichneumon*, North African (3) and European (11) cyt *b* + CR haplotypes were clearly separated by four mutation steps (Fig. 4), whereas the number of mutations within the two haplogroups was low (1–2). Andalusia had the greatest genetic diversity ($h = 7$). The commonest haplotype in Europe was H2 (*c.* 47%); however, it was present in only three regions out of eight (Andalusia, Alentejo and Centro Portugal). Four regions featured unique haplotypes: Extremadura (H7), Castilla-Leon (H10), Alentejo (H9) and Centro Portugal (H10, H11). In *G. genetta*, three haplotypes were endemic to North Africa (Algeria and Tunisia), while a fourth (H10) was shared with two individuals from Catalonia. The predominant European haplotype (H1; *c.* 61%) was distributed in 24 out of 29 regions. The number of mutations among haplotypes varied from 1 to 4, with the exception of an Andalusian haplogroup (H16, H17) 10 mutations away from H10. Structuring among the 20 haplotypes found in Europe was not clear-cut, although we could observe (1) a star-like configuration originating from H1 and involving the majority of the European haplotypes (including Mallorca and Cabrera), and (2) close affinities among H10 (Algeria and Catalonia), other North African haplotypes (H21 to H23), and

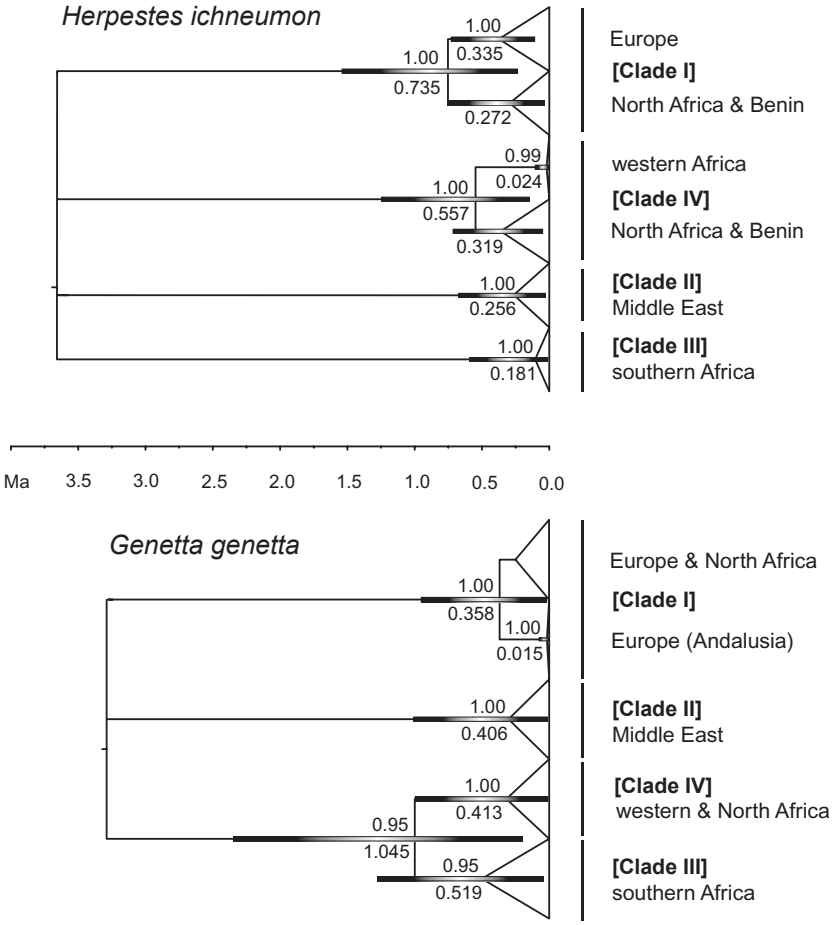

Figure 3 Times to most recent common ancestors among major geographic lineages in the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta*, estimated in BEAST under a coalescent model of expansion growth. Bayesian posterior probabilities and median height values ($yr.10^6$) are given above and below nodes, respectively. Horizontal bars show credibility intervals of node heights (95% highest posterior density).

Table 2 Genetic diversity in the four main mtDNA geographic lineages found in the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta*. Clade numbering follows Fig. 1.

	h		*Hd* (CI)		*S*		$\pi \times 10^{-3}$	
	cyt *b*	CR	cyt *b*	CR	cyt *b*	CR	cyt *b*	CR
Herpestes ichneumon								
Clade I	5	9	0.463 (0.178)	0.771 (0.102)	4	8	1.29	3.41
Clade II	2	3	0.467 (0.259)	0.600 (0.257)	1	2	1.16	1.50
Clade III	1	2	–	1.000 (0.980)	0	2	–	5.22
Clade IV	2	5	0.182 (0.282)	0.667 (0.323)	1	8	0.45	5.87
Clade I – Africa	1	3	–	0.833 (0.435)	0	2	–	2.63
Clade I – Europe	4	6	0.344 (0.188)	0.717 (0.118)	3	5	0.91	2.16
Genetta genetta								
Clade I	2	23	0.029 (0.039)	0.650 (0.088)	1	31	0.07	2.64
Clade II	3	4	0.667 (0.206)	0.750 (0.220)	10	9	8.84	6.16
Clade III	4	5	0.525 (0.269)	0.675 (0.227)	8	12	5.99	7.92
Clade IV	5	10	0.576 (0.320)	0.970 (0.086)	5	17	3.09	10.08
Clade I – North Africa	1	4	–	0.900 (0.316)	0	6	–	4.59
Clade I – Balearic Islands	1	3	–	0.538 (0.225)	0	4	–	2.23
Clade I – continental Europe	2	18	0.030 (0.041)	0.626 (0.094)	1	26	0.07	2.41

h, number of haplotypes; *Hd*, haplotype diversity; *S*, number of polymorphic sites; π, nucleotide diversity.

haplotypes from Ibiza (H19) and Portugal (H20) (Fig. 4). The archaeological sample from Alentejo, southern Portugal, had the H1 haplotype.

In *H. ichneumon*, pairwise genetic distances (Φ_{ST}) between the North African and European gene pools were high and significant (0.747) (Table 3). In *G. genetta*, the level of genetic differentiation was lower but still significant between North Africa and Europe (0.430) or the Balearic Islands (0.297). The mismatch distribution in European *H. ichneumon* differed significantly from a sudden-expansion model ($P < 0.05$), whereas it conformed to the latter in the continental population of *G. genetta* (Table 3; Fig. 5). The sudden-expansion model was partly rejected (*r*) in island populations. In Europe, *H. ichneumon* showed deviation from neutrality through a significantly negative F_S value, fitting a scenario of a strong, ancient bottleneck or sudden expansion, and a high, significant *Hd* value (0.841 vs. 0.833 in Africa). In *G. genetta*, the continental population deviated from neutrality as evidenced by significant, high negative values of *D*, D^*, F^*, R_2 and F_S, fitting a scenario of a strong, recent sudden expansion (Table 3; Ramírez-Soriano *et al.*, 2008). On the other hand, no deviation was detected within island populations. *Hd* values in both continental Europe and islands were lower than for North Africa (0.626 and 0.538 vs. 0.900).

We did not detect any significant correlation between geographic and genetic distances in continental Europe using the Mantel test (*H. ichneumon*: $r = -0.428/-0.424$; $P = 0.984/0.996$; *G. genetta*: $r = -0.012/-0.061$; $P = 0.553/0.778$). The SAMOVA suggested a partition into $k = 4$ groups (highest significant $\Phi_{CT} = 0.252$; $P = 0.039$) for *H. ichneumon* in Europe, consisting of Castilla-La Mancha, Extremadura, Castilla-Leon, and the rest of the Iberian subpopulations (Fig. 4). The markedly greater Φ_{ST} values of these three first regions were caused by the representation of a single haplotype. In *G. genetta*, SAMOVA yielded less straightforward results, because Φ_{CT} values reached a plateau between $k = 4$ and 6, and then increased continuously until the upper limit of $k = 20$. Given that *k*-values between 7 and 20 did not have significant estimates of variance among populations within groups (Φ_{SC}), we opted for $k = 6$ as the number of groups maximizing Φ_{CT} (0.455) and for which Φ_{SC} was still significant (-0.166; $P < 0.001$). Eventually we recognized the following partitions: Pays-de-la-Loire–Centre, Poitou-Charentes, Castilla-Leon, Andalusia, Catalonia–PACA, and the rest of the European, continental subpopulations (Fig. 4).

The distribution of pairwise genetic distances among Andalusian (*H. ichneumon* and *G. genetta*) or Catalonian (*G. genetta*) and the rest of the European subpopulations was not significantly correlated with geographic distance (Appendix S8). The negative correlation ($r_s = -0.290$) observed in *H. ichneumon* was caused by the higher level of intrapopulation variance within the Portuguese groups compared with their Spanish counterparts. Moreover, *h*-values were positively correlated with geographic distance (no specific trend for *sh*) in the latter species, whereas both *h* and *sh* were negatively correlated in *G. genetta*, with either Andalusia or Catalonia as 'anchor points', although neither *h* nor *sh* was significant.

DISCUSSION

Evolutionary history of North Africa and origin of the European populations of the Egyptian mongoose and common genet

The Egyptian mongoose and the common genet show remarkable concordance in their phylogeographic patterns,

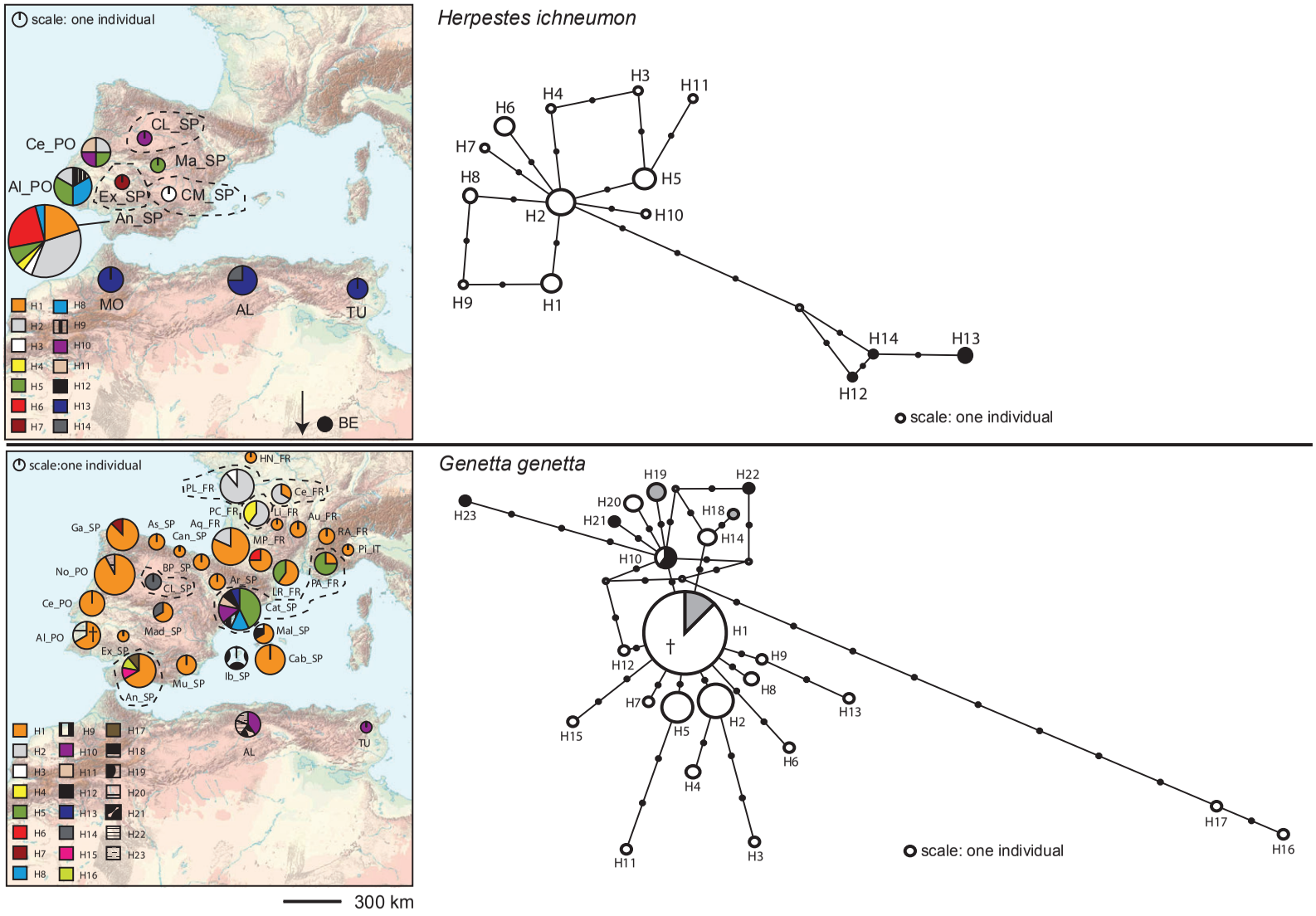

Figure 4 Distribution frequencies of combined (cytochrome *b* + control region) Clade I haplotypes in the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta* (left panels), and related median-joining networks (right). Regional acronyms and numbering of haplotypes refer to Appendices S1 and S2–S4, respectively. In the distribution frequency panels, dashed lines delimit geographic groupings that maximize the proportion of total genetic variance (SAMOVA). In the network, black, grey and white circles correspond to African, Balearic Islands and continental European representatives, respectively. Black and grey points along the branches represent single mutations and hypothetical haplotypes, respectively. † shows the haplotype attribution of the archaeological sample from Mértola, southern Portugal (first quarter of the 13th century).

Table 3 Estimates of genetic differentiation (Φ_{ST}), fit of the observed mismatch distributions to the Rogers' sudden expansion model (SSD, *r*) and deviation from neutrality (Tajima's *D*, Fu and Li's D^* and F^*, Fu's F_S, Ramos-Onsins and Rozas' R_2, Nei's *Hd*, Wall's *B* and Q, Kelly's Z_{nS}, Rozas' Z_A and *ZZ*) interpreted after Ramírez-Soriano *et al.* (2008), characterizing European populations of the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta*. Significance levels of observed statistics are expressed as follows: ***($P < 0.001$), **($0.001 < P < 0.01$), *($0.01 < P < 0.05$). R and NR mean Rogers' sudden expansion model 'rejected' and 'not rejected', respectively. SSD, sum of squared deviations.

	Source	No. sequences (Europe – North Africa)	Alignment	Φ_{ST}	SSD	*r*	*D*	D^*	F^*	R^2	F_S	*Hd* (North Africa)
Herpestes ichneumon	This study	36–4	*cyt b* + CR: 845 bp	0.747***	0.027R	0.162R	−0.918	−0.674	−0.878	0.080	−6.048**	0.841* (0.833)
Genetta genetta – continental Europe	This study and Gaubert *et al.* (2009)	118–5	*cyt b* + CR: 925 bp	0.430***	0.001NR	0.059NR	−2.194***	−1.087	−1.824	0.024**	−11.860***	0.626 (0.900)
Genetta genetta – Balearic Islands	This study and Gaubert *et al.* (2009)	14–5	*cyt b* + CR: 925 bp	0.297**	0.188NR	0.706R	−0.704	−0.555	−0.676	0.156	−0.445	0.538 (0.900)

	B	Q	Z_{nS}	*ZA*	Inference from Ramírez-Soriano *et al.*'s (2008) decision tables
Herpestes ichneumon	0.000	0.000	0.014	0.027	Strong, ancient bottleneck/Strong, ancient sudden expansion
Genetta genetta – continental Europe	0.077	0.111	0.142	0.090	Strong, recent sudden expansion
Genetta genetta – Balearic Islands	0.000	0.000	0.251	0.019	No departure from neutrality

with four co-distributed mtDNA clades, grouped into Europe–North Africa, the Middle East, southern Africa, and western–North Africa (Fig. 1). Those lineages coalesced in a contemporaneous time frame, from the Middle to Late Pleistocene, a time of intense, cyclical alternation of wet and dry periods (deMenocal, 2004) that has impacted the genetic structure of populations, notably in Africa (e.g. Hewitt, 2004; Brown *et al.*, 2007). The phylogeographic pattern we observed is echoed by the 'West – South – East' genetic partition of the white-tailed mongoose, *Ichneumia albicauda* (Cuvier, 1829) (Dehghani *et al.*, 2008), suggesting that common palaeoclimatic factors may have shaped the maternal evolutionary history of savanicole, small African carnivorans in sub-Saharan Africa.

In North Africa, the Egyptian mongoose and the common genet also exhibit concordant patterns, through the co-occurrence of two distinct lineages, representing (1) a probably autochthonous, North African clade (Clade I), and (2) a migrant pool likely of western African origin (Clade IV). Given the relatively low number of samples representing western Africa in our study, the exact geographic delimitation of Clade IV – especially for the Egyptian mongoose – remains uncertain. In *H. ichneumon*, the level of genetic diversity and deep TMRCA observed in Clade I provide evidence of the long-term stability of effective population size. This is in contrast to what we observed in *G. genetta*, where Clade I probably underwent a Plio-Pleistocene bottleneck resulting in the loss of genetic diversity and a more recent TMRCA (Gaubert *et al.*, 2009). We thus hypothesize that the existence of recurrent arid phases in North Africa during that period (deMenocal, 2004), resulting in the extreme contraction of forest cover (Dobson, 1998; Jolly *et al.*, 1998), did not significantly affect the historical demography of the Egyptian mongoose. This scenario is supported by (1) the species' tolerance of semi-desert conditions in the presence of water points (Osborn & Helmy, 1980; Kasparek, 1993; Cuzin, 2003), and (2) the southward-extended range of Clade I, which indicates the crossing of the Sahara by North African migrants.

TMRCAs of Clade IV in both species suggest a northward passage of western African individuals through the Saharan belt, possibly as early as the Middle Pleistocene, in concordance with the peak of faunal similarity between North and sub-Saharan Africa at that period (Geraads, 2010). Dispersal across the desert was indeed possible during Quaternary cyclical contractions of the hyper-arid area of the Sahara (Le Houérou, 1997), which culminated in the so-called 'African Humid Period', leading to a verdant Sahara *c.* 14.8–5.5 ka (deMenocal *et al.*, 2000). Trucchi & Sbordini (2009) recently found a similar genetic signature in the crested porcupine (*Hystrix cristata*), observing the co-occurrence of an autochthonous and a sub-Saharan lineage in North Africa.

Our results do not fit a previously observed pattern of 'western'/'eastern' genetic differentiation in Maghreb (e.g. Cosson *et al.*, 2005; Modolo *et al.*, 2005). Instead, we show that North Africa and the Middle East were distinct evolutionary entities, a picture that has also emerged through other

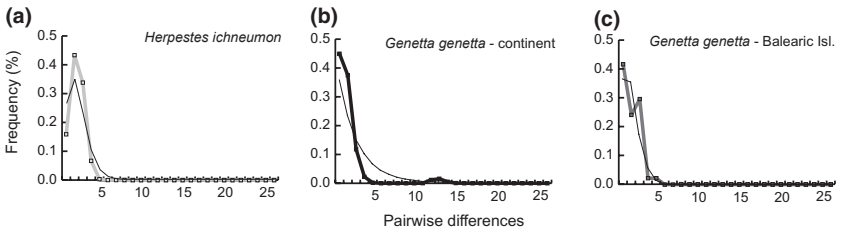

Figure 5 Mismatch distributions of cytochrome *b* + control region sequences in European populations of (a) the Egyptian mongoose, *Herpestes ichneumon*, and the common genet, *Genetta genetta* from (b) the continent and (c) the Balearic Islands. Thick grey or black curves represent the observed distributions relative to a sudden expansion (thin black curve) following the Rogers model (Rogers & Harpending, 1992).

recent investigations (e.g. Calvignac *et al.*, 2009). There have been few phylogeographic studies on species distributed on both sides of the Sahara. Nevertheless, (1) the co-occurrence of autochthonous and allochthonous lineages, and (2) the refuge-like structuring within autochthonous lineages, implying periods of *in situ* evolution, support an emerging biogeographic scenario that adds to the complexity of the North African evolutionary 'platform' (Dobson & Wright, 2000).

Our results show that the European gene pools of both the Egyptian mongoose and the common genet originated from the autochthonous North African mtDNA lineage (Clade I). The exhaustive representation of European individuals in our study, along with the level of haplotype diversity observed, leave little room for undetected haplotypes representing Clade IV (see Gaubert *et al.*, 2009). The use of museum samples and ancient-DNA techniques contributed to further refining the range of co-occurring North African lineages. In *H. ichneumon*, Clade I is largely distributed in North Africa, whereas in *G. genetta* it is restricted to coastal north-eastern Algeria and northern Tunisia. These two lineages are at least partly sympatric in both species, suggesting an absence of ecological differentiation. Further sampling efforts will be necessary to establish precise details of the evolutionary relationships and ecological interactions between lineages co-existing in North Africa.

Evidence for the natural dispersal of the Egyptian mongoose into Europe

Our results radically contradict the established idea that the Egyptian mongoose was introduced into Europe, contemporaneously with the common genet, during the invasion of Iberia by the Muslim armies (Delibes, 1982; Dobson, 1998; Riquelme-Cantal *et al.*, 2008). The coalescence of Clade I (median = 735 kyr) can be considered as a fair approximation of the divergence time between the North African and European clades, given the deep phylogenetic divergence between the two, suggesting no or very limited past gene flow (see Rosenberg & Feldman, 2001). Our analyses point to a natural crossing of the Mediterranean Sea, long before the earliest Palaeolithic contacts across the SG (Straus, 2001). The strong genetic differentiation between European and North African Clade I haplogroups, the significant level of genetic diversity found in Europe, and the important morphological differences between European and North African mongooses reported by Cabrera (1914) all point to a scenario of long-term, separated *in situ* evolution of European populations, which is supported by the relatively deep coalescence of both the European and North African pools (median = 335 and 272 kyr, respectively).

Our molecular results support, for the first time in a non-micro-mammal, the hypothesis that natural dispersal across the SG was possible for non-flying vertebrates during the Middle to Late Pleistocene cyclical lowering of sea levels (see Cosson *et al.*, 2005; Carranza *et al.*, 2006). The swimming abilities of the Egyptian mongoose (Osborn & Helmy, 1980; Delibes, 1982) make plausible a sweepstake migration using a partially emerged shoal across the SG, such as the archipelago of Cape Spartel that is now 56 to 200 m below sea level (Collina-Girard, 2001). The natural dispersal of the species into Europe implies that individuals migrated from the tip of Morocco, consistent with the detection of a Clade I haplotype in Ceuta.

Although the North African fossil record concurs with our molecular estimates in attesting the presence of *H. ichneumon* since the Middle Pleistocene (Werdelin & Peigné, 2010), the absence of palaeontological remains in Europe contradicts the Late Pleistocene coalescence of the European pool. The radiocarbon dating of an Egyptian mongoose skull from the Cueva de Nerja (Malaga) to the Almoravid–Almohad period led Riquelme-Cantal *et al.* (2008) to link the introduction of the species to the Muslim invaders. There is, however, no evidence for the domestication or taming of *H. ichneumon* by North African people (contrary to what occurred in Egypt; Osborn & Osbornova, 1998), although the discovery of a tibia from a Punic tank from the *c.* 5th–4th centuries BC in Sardinia is evidence for the historical translocation of the species (Campanella & Wilkens, 2004). Instead, the long-term stability of female effective population size in Europe is suggested by (1) the restricted range of the commonest haplotype found in Europe (H2), (2) the presence of unique haplotypes in about half of Europe, (3) the negative correlation between pairwise genetic and geographic distances in Andalusia and relatively high intra-population variance in Portuguese populations, (4)

an increasing number of haplotypes and shared haplotypes with geographic distance from Andalusia, and (5) the non-specific contribution of Andalusia in terms of among-population variance (SAMOVA). The hypothesis of a stable population throughout the last glaciations is further supported by the remarkable correspondence between the limits of the proposed ice age refugium in south-western Iberia (see Hewitt, 1996) and the distribution of suitable ecological conditions for the species (specifically, low rainfall and warm temperatures; Borralho *et al.*, 1996).

We envisage the higher genetic diversity observed in Andalusia as an imprint of the arrival of a fairly large number of migrants into southern Iberia. The northerly spread was perhaps through long-distance dispersal, given the geographic structuring of the haplotype distribution (Nichols & Hewitt, 1994) and the absence of evidence for isolation by distance (Wright, 1943). Mismatch analysis did not detect a signature of demographic expansion, despite field data indicating recent range fluctuations (Delibes, 1982; Palacios *et al.*, 1992; Borralho *et al.*, 1996; Gragera, 1996). On the other hand, the deviation from demographic neutrality fits with a scenario of a strong and ancient bottleneck or sudden expansion in Europe, following dispersal into south-western Iberia.

Multiple introductions of the common genet: a European success story starting before Muslim invasions?

Our results confirm the introduction of Clade I representatives of the common genet into Europe from North Africa (Gaubert *et al.*, 2009). The lower genetic diversity and weak differentiation of the European and Balearic gene pools relative to North Africa, together with the lack of reciprocal monophyly of the European and North African haplogroups, argue for a recent introduction. This is supported by the homogeneity between European and North African phenotypes (Delibes & Gaubert, in press).

Newly sequenced European samples allowed us to reinforce the picture that (1) Catalonia, with three new unique haplotypes, was a probable introduction hotspot, distinct from Andalusia, and (2) western France, with an additional unique haplotype, constituted a region dominated by private alleles (a picture further confirmed by preliminary microsatellite data; P. Gaubert *et al.*, unpublished data). The lack of isolation by distance and absence of specific geographic structuring suggest that there was no long-term *in situ* evolution in continental European populations. Rather, the greater genetic diversity observed in Andalusia and Catalonia, the negative correlation between the number of shared haplotypes and geographic distances from those two regions, and the significant contribution of those latter in terms of inter-population variance (SAMOVA) argue for at least two introduction hotspots on the continent with different sources. The remaining regions identified by SAMOVA (western France and Castilla-Leon) may reflect additional translocations, possibly motivated by the Middle Age fashion for the species at European courts (Delort, 1978; Gaubert & Mézan-Muxart, 2010), although random allelic fixation cannot be ruled out.

It is most probable that the species was translocated intentionally, since middle-sized carnivorans would hardly pass unseen in holds of trading boats. The fact that only Clade I haplotypes were introduced into Europe suggests a cultural constraint conditioning the translocation of *G. genetta* (Gaubert *et al.*, 2009). Although Morales (1994) described from Mértola the tibia of a hare exhibiting both cut marks made by humans and a chew mark left by a genet's upper carnassial (P4), there is still no direct evidence of the use of the species as a pre-cat, commensal or domesticated predator of rodents. However, we suggest that the distribution of territories and trade networks of the Phoenicians, and later Punics, with Greek colonies, could relate to its pattern of introduction from Maghreb to Ibiza, Andalusia and Catalonia (Appendix S9; see Semmler, 2002; Bierling & Gitin, 2002). Nevertheless, Muslim conquerors introduced a series of domestic and wild species into south-western Europe (e.g. Morales *et al.*, 1995; Bermejo & Sánchez, 1998), and the most common European haplotype H1 of the genet was found in the archaeological Almohad levels of Mértola. This could fit with suggestions that Muslim invaders 'made popular' the common genet in south-western Europe (Amigues, 1999), notably as political gifts to Christian monarchs (D. Morales, Universidad Autónoma de Madrid, Spain, pers. comm.). The significant, recent sudden expansion in the European, continental populations of the species (see also Gaubert *et al.*, 2009) demonstrated through mismatch distribution and neutrality tests are not in conflict with a scenario of recent, independent introductions (Excoffier, 2004) possibly associated with an artificial spread of the species, represented by the dominant European haplotype (H1). The question remains open as to the *in situ* evolution within Europe of the divergent, Andalusian haplogroup H16-H17, after a natural crossing of the SG during the Last Glacial Maximum, or its later, human-mediated translocation from an as yet undetected Clade I haplogroup in Morocco.

CONCLUSIONS

We established dramatically different scenarios of dispersal across the Strait of Gibraltar for the Egyptian mongoose and the common genet. The presence of the former in south-western Europe appears to be the result of Pleistocene sweepstake dispersal, whereas the latter was probably introduced several times during the historical period. From a biogeographic perspective, our investigations show the value of the comparative phylogeographic approach for unravelling part of the complex, superimposed history of colonizations within the Mediterranean Basin.

The Mediterranean Basin is one of the biodiversity hotspots that suffers most from the pressure of introduced species (e.g. Gritti *et al.*, 2006). Paradoxically, historical introductions, being sometimes considered a component of bio-cultural heritage (e.g. Masseti, 2009), have contributed to the inflation of the number of species counted as native to the region

(Gippoliti & Amori, 2006). As a contribution to the refinement of this picture, we propose that the Egyptian mongoose should be considered a natural colonizer of south-western Europe, whereas the common genet should not. Regardless of their dispersal scenarios, the European gene pools of both *H. ichneumon* and *G. genetta* are pure representatives of two autochthonous, North African mitochondrial lineages (Clades I) that have probably been impacted by genetic pollution and habitat loss in their North African, native ranges.

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SUPPORTING INFORMATION

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

Appendix S1 Geographic sampling within *Herpestes ichneumon* and *Genetta genetta*, together with their respective outgroups.

Appendix S2 Geographic distributions of cytochrome *b* and control region haplotypes.

Appendix S3 Cytochrome *b* and control region haplotypes with corresponding GenBank accession numbers.

Appendix S4 Numbering of cytochrome *b* + control region haplotype combinations.

Appendix S5 Best-fitting models and parameters estimated from ModelGenerator.

Appendix S6 Detailed phylogenetic tree of *Herpestes ichneumon.*

Appendix S7 Detailed phylogenetic tree of *Genetta genetta.*

Appendix S8 Trends among geographic distances and genetic indices, from Andalusia (*Herpestes ichneumon* and *Genetta genetta*) and Catalonia (*G. genetta*).

Appendix S9 Overlap among the Clade I distribution in *Genetta genetta* and the main Mediterranean civilizations.

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