Geographical origin and endemism of Corsican Kuhl's pipistrelles assessed from mitochondrial DNA

A. Evin[1,2,3], V. Nicolas[1,2], G. Beuneux[4], R. Toffoli[5], C. Cruaud[6], A. Couloux[6] & J.-M. Pons[1,2]

1 Muséum National d'Histoire Naturelle, Département Systématique et Evolution, Origine Structure et Evolution de la Biodiversité, UMR 7205, Paris, France
2 Service de Systématique moléculaire, UMS 2700 CNRS, Paris, France
3 Department of Archaeology, University of Aberdeen, Aberdeen, Scotland
4 Groupe Chiroptères Corse, Corte, France
5 Via Tetto Mantello, Borgo San Dalmazzo CN, Italy
6 Génoscope, Centre National de Sequençage, Evry Cedex, France

Abstract

Previous genetic analyses have demonstrated that two divergent lineages of *Pipistrellus kuhlii* are spread over Europe and North Africa, and it has been proposed that *Pipistrellus maderensis*, a taxon endemic to the Canary Archipelago and Madeira, was its sister species. In this study, we used mitochondrial DNA sequences to investigate the level of endemism achieved by Corsican lineages with regard to their continental counterparts and to propose hypotheses about the geographical origin of Corsican bats. Our results suggest that Corsican Kuhl's pipistrelles are not endemic. Such a lack of genetic endemism in Corsica can result from current gene flow with French and Italian populations and/or recent colonization of this island. Additionally, our results demonstrate that Corsica was colonized independently from Europe by two divergent lineages (genetic distance = 5.8%) widespread in the western Palaearctic and clearly suggest that North Africa probably does not play any significant role in the colonization of Corsica by the Kuhl's Pipistrelle. Additional morphometric, acoustic and ecological studies are needed to soundly ascertain the respective taxonomic status of these two divergent lineages and the level of distinctiveness achieved by Corsican bats.

Introduction

Phylogeographic approaches have been used intensively in the last two decades (Ruedi & McCracken, 2009) to understand a large array of evolutionary issues ranging from historical biogeography to speciation processes and the current geographic distributions of species and lineages. Regarding European bats, previous phylogeographic analyses revealed the existence of high levels of cryptic diversity and led to the description of several new taxa (Castella *et al.*, 2000; Mayer & von Helversen, 2001; Ibáñez *et al.*, 2006; Hulva *et al.*, 2007; Mayer, Dietz & Kiefer, 2007; García-Mudarra, Ibáñez & Juste, 2009). For instance, four species of *Pipistrellus* were classically recognized in the western Palaearctic region: *Pipistrellus pipistrellus*, *Pipistrellus nathusii*, *Pipistrellus maderensis* and *Pipistrellus kuhlii*. From acoustic data, and molecular genetic validation, *Pipistrellus pygmaeus*, a sister-species of *P. pipistrellus*, was discovered recently (Barratt *et al.*, 1997). After that, *Pipistrellus hanaki* (Benda, Hulva & Gaisler, 2004) was described from Cyrenaica and *P. pygmaeus cyprius* from Cyprus (Benda *et al.*,

2007). In addition, four distinctive *Pipistrellus* lineages, distributed, respectively, in Crete, Morocco, Corsica and Sicily, were recently highlighted (Hulva *et al.*, 2007). The population from Crete was subsequently named *P. hanaki creticus* (Benda *et al.*, 2009).

All these studies suggested that the cryptic diversity among Palaearctic *Pipistrellus* taxa, and especially, across the Mediterranean region, may still be undiscovered. Regarding *P. kuhlii*, two divergent European lineages were highlighted using mitochondrial markers (Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009). *Pipistrellus maderensis*, the sister-species of *P. kuhlii*, was firstly described from Madeira (Dobson, 1878) on the basis of slight morphological differences and later found in the Canary Islands (Bannerman, 1922). Based on mitochondrial markers, Pestano *et al.* (2003) suggested that these two species were sympatric throughout the Canary archipelago and demonstrated that *P. kuhlii* as currently defined was paraphyletic with respect to *P. maderensis*. However, as their study included only three individuals from continental Europe and no individuals from North Africa, Pestano *et al.* (2003)

underlined the need for additional studies before specifying the taxonomic status of these two taxa.

Geographic barriers, like mountains or seas, may prevent the dispersion of many terrestrial vertebrate species. Because of their ability to fly, bats are potentially good dispersers. However, their ability to colonize islands seems to vary according to the species and geographical context (Ruedi & McCracken, 2009). Whereas large sea barriers do not prevent the colonization of remote islands (Palmeirim, 1979; Carstens *et al.*, 2004; Juste *et al.*, 2004), narrow barriers of open water can limit or suppress movements depending on species. For example, the Straits of Gibraltar prevent the dispersal of at least 30% of the bats species between southern Spain and Morocco but not the dispersal of other bat species with comparable flight abilities (García-Mudarra *et al.*, 2009). In the same way, the Strait of Bonifaccio (12 km wide), between the islands of Corsica and Sardinia, strongly reduces gene flow for *Myotis punicus* with no female exchange and limited male gene flow between the islands (Biollaz *et al.*, 2010).

Corsica is a continental Mediterranean island 160 km from the French coast and nearly 50 km from the Island of Elba, which is 10 km from the coast of Italy. As in other Mediterranean islands, all extant Corsican terrestrial mammals were introduced by humans (Vigne, 1987, 1988). Consequently, bats are the only mammals that naturally colonized the island. Despite the longstanding interest of naturalists in the biogeography of the Corsican fauna and flora (Allorge *et al.*, 1926), little is known about the evolutionary history of Corsican bats and their affinities with surrounding continental populations. It has been shown that two species, namely *M. punicus* (Castella *et al.*, 2000) and *P. pipistrellus* (Hulva *et al.*, 2007), have a North African origin and do not originate from the closest European continent. For these two species, the insular populations are genetically (Castella *et al.*, 2000; Hulva *et al.*, 2007) and morphometrically (Evin *et al.*, 2008) distinct from their closely related continental counterparts, suggesting a rather high level of endemism. Overall, the level of endemism that may characterize the Corsican bat assemblage remains to be established not only for obvious scientific reasons but also for conservation purposes.

To improve our understanding of the phylogeography of *P. kuhlii* in western Europe, our phylogeographic analyses were based on a large geographical sampling including new mitochondrial DNA sequences of the cytochrome *b* gene from France, Italy and Corsica in addition to genetic data published previously (Pestano *et al.*, 2003; Stadelmann *et al.*, 2004; Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009). We specifically aimed to answer the following questions: (1) are Corsican Kuhl's pipistrelles endemic to Corsica? (2) do Corsican Kuhl's pipistrelles originate from Europe or North Africa?

Material and methods

This study included 98 specimens of *P. kuhlii–P. maderensis*, listed in Appendix S1, among which 26 new specimens were from continental France (*n* = 8), Corsica (*n* = 9) and North Italy (*n* = 9). Previously analysed specimens came from Spain (*n* = 19, Pestano *et al.*, 2003; Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009), Morocco (*n* = 18, García-Mudarra *et al.*, 2009), Switzerland (*n* = 3, Ibáñez *et al.*, 2006), Italy (*n* = 2, García-Mudarra *et al.*, 2009) and Greece (*n* = 1, Stadelmann *et al.*, 2004). The specimens from the Canary archipelago were split into *P. kuhlii* (*n* = 10) and *P. maderensis* (*n* = 20) according to Pestano *et al.*, 2003. Three species were used as outgroups: *P. pipistrellus* (GenBank DQ120854), *P. pygmaeus* (GenBank DQ120856) and *N. noctula* (GenBank AJ841967).

A 2 mm diameter wing biopsy punch was taken for tissue sampling (Worthington-Wilmer & Barratt, 1996) under appropriate license. Biopsies were stored in 70% ethanol and DNA was extracted using a CTAB procedure (Winnepenninckx, Backeljau & De Watcher, 1993). A 735 bp segment of the cytochrome *b* gene was amplified with the primers Molcit-F (Ibáñez *et al.*, 2006) and MVZ-16 (Kocher *et al.*, 1989; Smith & Patton, 1993). The PCR consisted of 3 min of denaturation at 94 °C, followed by 35 cycles of 30 s at 94 °C, 40 s at 47 °C and 90 s at 72 °C, followed by a final extension at 72 °C for 10 min. PCR products were purified using ExoSap (Genoscope, Evry, France) and sequenced in both directions and run on an automated DNA sequencer (Applied Biosystems 3100, Courtaboeuf, France). Sequences were assembled and edited using Sequencer 4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA) and aligned using BioEdit (Ibis Therapeutics, Carlsbad, CA, USA) (Hall, 1999). We controlled for possible pseudogene amplification using steps proposed by Song *et al.* (2008): no double bands, no ambiguities in the chromatograms, no stop codon or insertion/deletion in the reading frame. Some of our haplotypes were similar to the sequences published previously. As the sequences acquired in GenBank were 515 bp in length, our new sequences were cut short to match this length.

Relationships among individuals and haplotypes

Evolutionary relationships among individuals were estimated using neighbour joining (NJ) and the Bayesian Markov chain Monte Carlo method. The NJ analysis was performed using MEGA v4.0 (Tamura *et al.*, 2007). To select the model of nucleotide substitution that better fitted our data, we used MrModeltest 2.3 (Nylander, 2004) and the Bayesian analysis was then performed using MrBayes v3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Three heated chains and a single cold chain were used in the Bayesian analysis, and runs were initiated with random trees. Two independent runs were conducted with 1 million generations per run. Trees and parameters were saved every 100 generations. Stationarity was assessed by examining the average standard deviation of split frequencies and the potential scale reduction factor (Ronquist, Huelsenbeck & Van der Mark, 2005). For each run, the first 25% of sampled trees were discarded as burn-in. Because only a few mutations were present within each clade, the

relationships among haplotypes were also represented by networks using the statistical parsimony algorithm implemented in TCS v1.21 (Clement, Posada & Crandall, 2000) and networks were reconstructed with a 95% confidence interval for between-haplotype divergence. Clades recovered in the phylogenetic analysis were too divergent to be unambiguously connected. Therefore, TCS performed one network separately for each clade.

Genetic diversity and genetic structure

To describe and compare the clades recovered in phylogenetic analyses, we estimated genetic diversity and genetic structure using different approaches. We computed genetic distances among clades using K2P distances and *p*-distances with MEGA v4.0 (Tamura *et al.*, 2007) to provide distances comparable to those depicted classically in the literature. Within each clade, nucleotide diversity (Pi) and haplotype diversity (Hd) (Nei, 1987) were calculated using ARLEQUIN v3.0 (Excoffier, Laval & Schneider, 2005). Where possible, we investigated the geographical structure of the genetic variation within each clade using the analysis of molecular variance (AMOVA) (ARLEQUIN v3.0, Excoffier *et al.*, 2005). The approximate times of divergence among lineages were estimated using a Bayesian-coalescence approach performed with BEAST v1.4.8 (Drummond & Rambaut, 2007). The time to the most recent common ancestor was estimated for the main clades recovered in our phylogenetic analysis. Two independent runs of 10^8 generations, each with a burnin of 10^6 steps, were performed. These two runs were then combined in TRACER v1.4 (Rambaut & Drummond, 2007), which also provides options for examining effective sample size (ESS) values and frequency plots in order to check whether the mixing of the chain was adequate. MrModelTest 2.3 was used to identify the model of sequence evolution used in BEAST v1.4.6 and a Bayesian skyline coalescent model of population size was specified. Divergence times and their credibility intervals were estimated using a relaxed clock model with branch rates drawn from an uncorrelated lognormal distribution. To calibrate the divergence time between lineages, we used two calibration dates: the dichotomy between *Myotis daubentonii* and *Myotis bechsteinii* dated at 5 million years (MY) (Topál, 1983) and the dichotomy between *Myotis nattereri* and *Myotis schaubi* dated at 6 MY (Horácek & Hanák, 1983) (the sequences are listed in Appendix S1). We used a standard deviation of 1 MY.

Results

Phylogenetic analyses and divergence times

The entire dataset comprised 515 bp cytochrome *b* sequences for 98 bats (ingroup). Within the ingroup, there were 59 variable sites and 25 haplotypes. According to the Akaike information criterion, the model that best fitted our data was the generalised time-reversible nucleotide substitution model with a fixed proportion of invariant sites (GTR + I). Three main clades were recovered in the Baye-sian phylogenetic tree (Fig. 1). The first clade to diverge in our phylogenetic tree (clade I) comprised only western European bats, except one individual from Corsica. The clade II, which had the largest geographic range (eastern Europe, Morocco, Corsica, Canaries) together with the clade III, restricted to the Canary Islands, formed a highly supported monophyletic group. All the Corsican sequences were clustered within clades I and II, whereas all Canarian sequences were grouped within clades II and III. Branching orders among haplotypes within each clade were more or less strongly supported depending on the localities (Fig. 1).

The inter-clade K2P genetic distances were high, ranging from 4.3% between clades II and III to 6.4% between clades I and III, whereas its value was 5.8% between clades I and II (*p*-distances are, respectively, 4.1, 6.1 and 5.5%). Divergence among the three clades (node A) dated back to the end of Pliocene or to the beginning of the Pleistocene [~2 millions-years ago (MYA)] (Table 1), whereas clades II and III (node B) diverged roughly ~1.2 MYA. Divergences within clade II (node D) took place at ~0.4 MYA, within clade III (node E) at ~0.25 MYA, whereas the clade I (node C) differentiated more recently at ~0.17 MYA. Within clade II, the Moroccan clade (node G) diverged roughly at ~0.17 MYA, whereas the Canarian clade (node F) diverged at ~0.09 MYA. As the confident intervals obtained are, however, very large and knowing that calibration points were determined from another genus only distantly related to *Pipistrellus*, our date estimates should be considered with caution. They nevertheless clearly suggest that all divergence events that led to extant lineages took place recently throughout the Pleistocene.

Parsimony networks

Clade I

The star-like network corresponding to clade I comprised 26 sequences and six haplotypes (Fig. 2, Table 2). One ancestral haplotype shared by almost all individuals (*n* = 21) was distributed throughout our study area (Spain, France, Switzerland, Italy and Corsica). Five derived Spanish haplotypes, each one found only in one specimen, diverged from the most frequent haplotype by only one mutation step (Fig. 2). The nucleotide diversity was 10-fold lower and the haplotype diversity was twofold lower than the values obtained for the other two clades (Table 2).

Clade II

The network corresponding to clade II comprised 52 specimens and 11 haplotypes (Table 2, Fig. 2). The central haplotype had a wide geographical range, being found eastwards up to Greece and southwards up to Morocco. The haplotypes derived from this ancestral sequence also had a widespread distribution including the Canary archipelago, Corsica, Italy, Spain, Morocco and France. Two Corsican haplotypes were found: the most frequent was also found in Italy while the second one was shared with France.
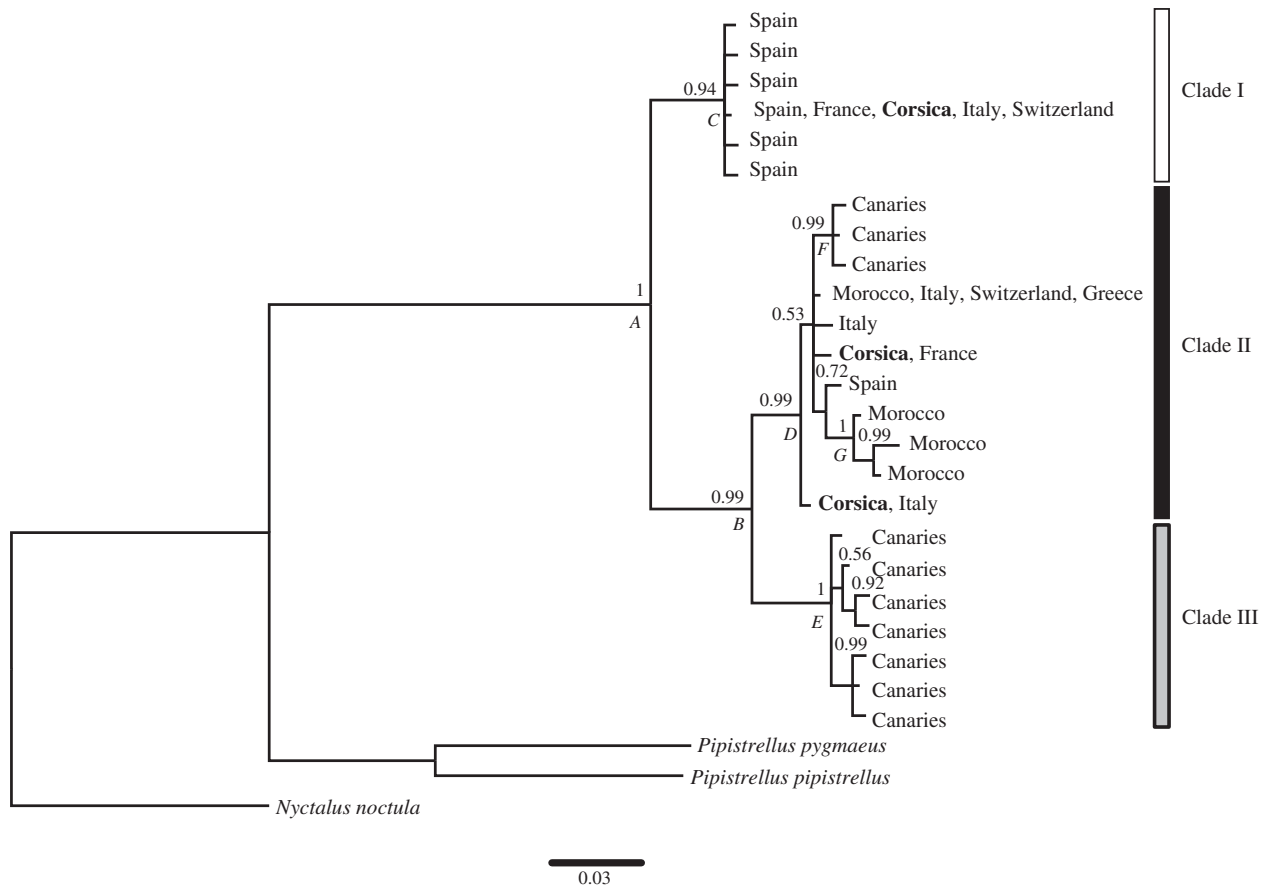
Figure 1 Bayesian phylogenetic relationships among haplotypes under the GTR + I model of DNA substitution. The number above nodes indicates the posterior probabilities. In bold, specimens from Corsica. Letters correspond to node labels.

Table 1 BEAST estimates of time to most recent common ancestor (million years ago) for the main clades identified in the phylogenetic analysis (Fig. 1)

Node	A	B	C	D	E	F	G
95% HPD lower	0.927	0.543	0.055	0.161	0.094	0.016	0.044
Mean	2.077	1.184	0.166	0.418	0.247	0.094	0.167
95% HPD upper	3.568	2.17	0.352	0.804	0.5	0.198	0.346

The mean and range (95% confidence intervals derived from the Bayesian probability distribution) over two runs are given.

One haplotype was found only in Spain and another one was restricted to Italy. The three Moroccan haplotypes were related and separated by two to three mutations. These Moroccan haplotypes were related to the Spanish haplotype and to the most widespread haplotype by four to nine mutations. The three Canarian haplotypes were closely related and differed from each other by only one mutation.

Clade III

The network corresponding to clade III comprised 20 sequences and eight haplotypes only found in the Canary Archipelago (Table 2, Fig. 2). The genetic variability of this insular clade was similar to that observed in clade II despite a much more restricted geographical range (Table 2).

Geographic distribution of clades

To investigate the potential role of geographic barriers in shaping the genetic variability of *P. kuhlii*, we compared the genetic structure of French versus Spanish populations within the clade I as well as the genetic structure of Corsican versus Italian populations and of Moroccan versus Italian populations within the clade II. Results of the AMOVA computed between the Corsican and the Italian populations indicated significant geographic partitioning of the genetic
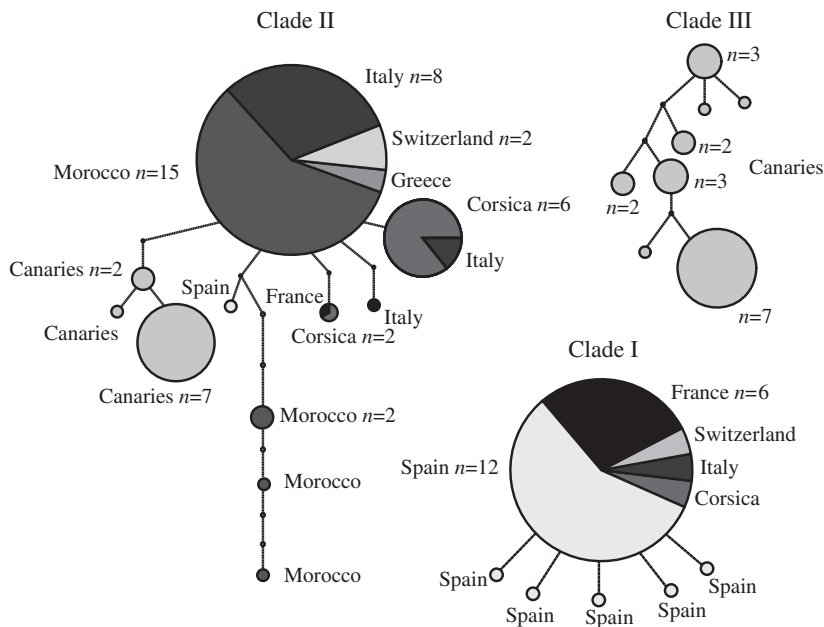
Figure 2 Statistical parsimony networks (TCS). Each circle represents one haplotype and its size is proportional to its frequency. Clade I (*Pipistrellus kuhlii*), clade II (*P. kuhlii*), clade III (*Pipistrellus maderensis*).

Table 2 Genetic diversity estimates for each of the three clades identified in the phylogenetic analyses

	Clade 1	Clade 2	Clade 3
N	26	52	20
Nh	6	11	8
Np	5	19	10
Pi	0.0007	0.005	0.006
Hd	0.35	0.72	0.85

N, number of specimens; Nh, number of haplotypes, Np, number of polymorphic sites; Pi, nucleotide diversity; Hd, haplotype diversity.

variability, with 34.13% ($P = 0.003$) of the variance explained by differences between populations. By contrast, we failed to find any geographical structure of the genetic variability either between Italian and Moroccan populations ($P > 0.1$) within clade II or between French and Spanish populations ($P > 0.1$) within clade I.

Whereas clades I and II were found throughout Europe, their respective frequency sharply varied across the study area and thus defined distinct phylogeographic units (Fig. 3). As clade I was much more frequent than clade II in western populations, France, Spain and Switzerland can be gathered in the same phylogeographic unit 1. The reverse trend was observed in the eastern part of our European study area, Corsica and in North Africa where clade II was the most common (phylogeographic unit 2, Fig. 3). The third phylogeographic unit is restricted to the Canary archipelago, where clade II is found together with a well-differentiated endemic clade.

Discussion

Our results corroborate the presence of three divergent lineages of *P. kuhlii* complex in the western Palaearctic

detected by Pestano *et al.* (2003), of which two are widespread across the western Europe (Ibáñez *et al.*, 2006; Mayer *et al.*, 2007; García-Mudarra *et al.*, 2009). The complex can be described as: a western clade (clade I), an eastern and southern clade (clade II), and a Canarian clade (clade III) that corresponds to *P. maderensis* was also suggested in several other studies.

Our results, based only on a small portion of mitochondrial DNA, suggest that the Canary Islands might have been colonized at least twice by Kuhl's pipistrelle-like bats: a first time, by an older lineage that probably differentiated (Fig. 1) during the middle Pleistocene to give the *maderensis* clade (clade III), and a second time, probably at the end of the Pleistocene by bats belonging to clade II. This colonization pattern remains to be confirmed by further studies relying on additional molecular markers. Indeed, adding new markers would allow to definitively exclude that the paraphyly of the two sympatric lineages in the Canary archipelago does not result from molecular bias like the sequencing of nuclear pseudogenes instead of the cytochrome *b*. Furthermore, the inclusion of Kuhl's pipistrelles from the North African Atlantic coast would help to assess the level of endemicity achieved in the Canary Islands. More generally,
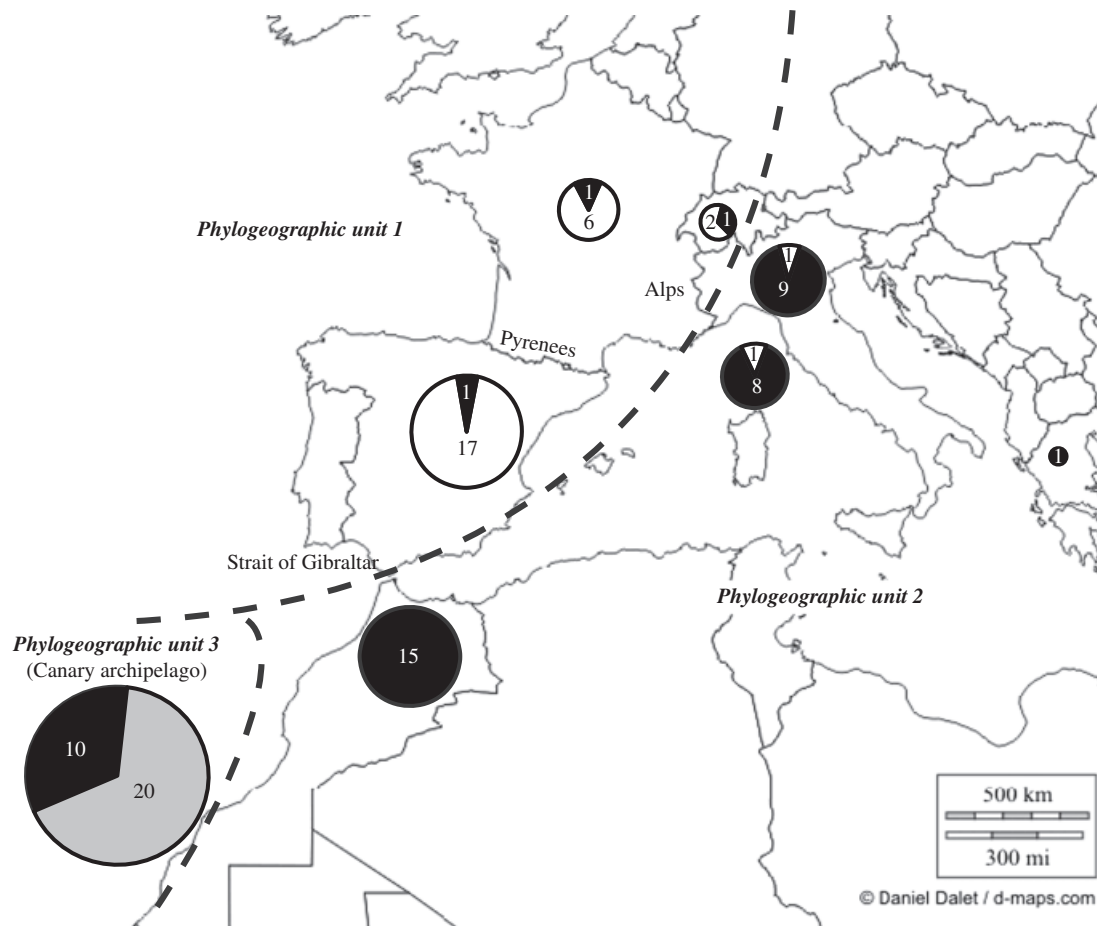
Figure 3 Phylogeographic units defined on the basis of the relative proportion of the three clades: 1 – western phylogeographic unit I for which the clade I (in black) is the most frequent; 2 – eastern phylogeographic unit 2 for which the clade II (in white) is the most frequent; 3 – Canarian phylogeographic unit 3 for which the clade III (in grey) corresponding to *P. maderensis* is the most frequent.

as mitochondrial DNA is maternally transmitted, it reflects only a part of the genetic structure of species. This is especially true in the case of bats, in which males and females often exhibit contrasting dispersal patterns (Ruedi & McCracken, 2009). Adding nuclear markers would thus be necessary to gain a better understanding of the evolutionary history of Kuhl's pipistrelles in the western Palaearctic.

Two highly divergent clades overlapped throughout most of the continental part of the western Palaearctic. Clade I is found in western Europe (Spain, Switzerland, continental France, Corsica and Italy), whereas clade II extends eastwards up to Greece and southwards up to North Africa. The clade endemic to Europe (clade I) shows a very low genetic diversity that could result from a drastic reduction in population size that might have occurred during the late Pleistocene glaciations. With regard to the geographic localization of the haplotype diversity, which is the highest in Spain, we suggest that this lineage has rapidly and recently colonized the western Europe from an Iberian refuge by a west to east colonization road. Clade II was found in Morocco (García-Mudarra *et al.*, 2009) and was

observed preferentially in the eastern part of our study area. Such a geographical distribution raises the possibility that Europe was colonized from a quaternary oriental or a North African refuge. Knowing that these two clades are highly divergent (K2P distance = 5.8%, i.e. a genetic distance typically found between sister species in bats, see Ruedi & McCracken, 2009) and largely overlap across Europe, they could represent two distinct species. However, members of clades I and II were found in a same breeding colony in Corsica, which might suggest potential gene exchanges between these two lineages. Clearly, further studies including nuclear molecular markers as well as acoustic, ecological and morphological approaches are needed before a possible reappraisal of the number of species of Kuhl's pipistrelle-like bats inhabiting Europe. In the same way, additional studies are necessary to be more specific about the biological and taxonomic status of *P. maderensis* with respect to *P. kuhlii,* these two taxa being sympatric in the Canary Archipelago. Furthermore, thanks to molecular studies, two additional taxa recently discovered belong to the *P. kuhlii* complex namely *Pipistrellus cf. lepidus* Blyth, 1845 (Mayer

et al., 2007) from Turkey, Israel, Syria and Iran, and *Pipistrellus deserti* Thomas, 1902 (Mayer *et al.*, 2007), which has been found in Morocco and Libya. This last taxon, whose range is restricted to desert areas, is closely related to the Moroccan clade (Fig. 1, node G) as it was demonstrated previously by García-Mudarra *et al.* (2009). According to these recent molecular studies and the present study, it appears that the taxonomy of Kuhl's pipistrelle-like bats should be revised to synchronize the nomenclature with the species complex phylogeny. However, as mentioned above, further studies are necessary to soundly choose among alternative nomenclatural proposals.

Our results suggest that Corsica was colonized at least twice by *P. kuhlii*. Within the eastern-southern clade II, the most common haplotype found in Corsica diverges from the ancestral haplotype by only one mutation. As this haplotype is also found in Italy, we may hypothesize that Corsica was recently colonized from the Liguria peninsula. The increase in commercial and touristic exchanges between Italia and Corsica as well as between continental France and Corsica might have facilitated the anthropogenic colonization of this island by the Kuhl's pipistrelle. Our results nevertheless underline a significant geographical structure in the genetic variation between Italy and Corsica that suggests limited current gene flow between continental and insular populations. Within this clade, Corsica and continental France share a common haplotype never found elsewhere that may reflect past and/or present exchanges between this continental region and the island. Furthermore, the most frequent haplotype, typical of the western clade I, has also been detected in Corsica.

Overall, our genetic results favour the hypothesis of multiple colonization events of Corsica by continental Kuhl's Pipistrelles belonging to highly divergent lineages and coming from different geographic localities, among which Italy has probably played a major role. By contrast, a North African origin has been suggested for the two other bat species studied to date in Corsica namely *M. punicus* (Castella *et al.*, 2000; Evin *et al.*, 2008) and *P. pipistrellus* (Hulva *et al.*, 2007), which moreover exhibit a higher insular genetic differentiation than *P. kuhlii*.

Together, these results highlight that the Corsican bat assemblage exhibits a complex biogeographical pattern by mixing species from different geographic origins that are more or less differentiated from their continental counterparts. Further studies devoted to other aspects of natural history would nevertheless be essential to fully appreciate the morphological or the ecological characteristics that might distinguish insular Kuhl's pipistrelles. Overall, our mitochondrial DNA results clearly suggest that contrary to what can be observed in the Canary Islands, no endemic lineage has evolved in Corsica in the case of the Kuhl's pipistrelle. This is in accordance with studies conducted for other flying organisms like birds, which highlight a high level of endemism at the species level in the Canary archipelago (e.g. Dietzen, Witt & Wink, 2003) in contrast to the presence of only one endemic bird species in Corsica, the Corsican Nuthatch *Sitta whiteheadi* (Pasquet, 1996). How-

ever, it is worth noting that the level of endemism for other bat species inhabiting Corsica is as yet unknown.

On a European scale, our results show that French, Swiss and Spanish populations are clustered in the same phylogeographic unit in which the western clade I is the most frequent while the eastern clade II is represented at a lower frequency. A reverse and similar proportion of these two clades is observed in the second phylogeographic unit that comprises Italy and Corsica as well as Morocco and Greece, where only the clade II is known to date (Fig. 3). These two phylogeographic units are found on both sides of the Alps, which constitute moreover a well-known suture zone for other European bat species like *Myotis myotis* (Ruedi *et al.*, 2008) as well as for other organisms (Taberlet *et al.*, 1998; Hewitt, 1999). In contrast with the Alps, the Pyrenees do not seem to constitute a suture zone in the case of the Kuhl's pipistrelle. Such a phylogeographic pattern has already been found for *M. myotis* (Ruedi *et al.*, 2008). French and Spanish individuals belong to the same phylogeographic unit probably because, as suggested by our genetic results, France was mainly colonized by the Kuhl's Pipistrelle thanks to the northward spatial expansion of the Spanish population.

Moreover, our results confirm that the Straits of Gibraltar constitute an efficient geographical barrier that limit gene flow between Spain and North Africa, which do not belong to the same phylogeographic unit (Fig. 3). The oceanic Canary Archipelago, which includes seven oceanic islands that lie 100 km from the African coast, and 1000 km from Spain, seems to have been colonized independently by two lineages. With regard to the Kuhl's Pipistrelle, the Atlantic Ocean did not prevent the colonization of the Archipelago, but likely favoured the evolution of insular lineages by limiting gene flow with the continent.

To conclude, this study raises several taxonomic and evolutionary questions. On a European scale, it would be of primary interest to investigate whether the two highly divergent lineages belong to the same biological species or not. Regarding the Kuhl's pipistrelles of Corsica, several questions remain to be explored in order to properly assess their taxonomic status and their conservation values, including their level of morphological distinctiveness from their continental counterparts and the potential current gene flow with nearby continental regions assessed with nuclear markers.

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Supporting information

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

Table S1. Information on mitochondrial data analysed in this study. ID refers to the identification code of haplotypes used by authors who provided the sequences.

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