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Dynamics of haplogroup frequencies and survival rates in a contact zone of two mtDNA lineages of the lizard *Lacerta vivipara*

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The analysis of contact zones between lineages that were previously isolated in allopatry can lead to important insights on evolutionary processes such as selection and adaptation. In this paper we conducted a comparative demographic study of two mitochondrial DNA (mtDNA) lineages of the lizard *Lacerta vivipara* in the western Pyrénées to provide detail on the dynamics of their contact zone. By surveying haplogroup frequency across the contact area, we revealed the existence of a stable and very narrow contact zone between two parapatric lineages, which we infer to demonstrate a role for selection in the maintenance of this contact zone. We suggest these two lineages evolved in allopatry after retreating to different refugia during the Pleistocene glaciations, and subsequently came into secondary contact after the last glacial maximum. Although haplogroup frequencies were stable over time, we found significant age and environment (temperature) dependent survival differences between mtDNA haplogroups in one contact population sampled yearly from 2002 to 2009. Therefore, temperature-induced demographic differences between the two mtDNA lineages may be responsible for the stability of this narrow contact zone. This is one of the first demographic studies conducted under natural conditions indicating the possibility of selection on mtDNA.

Over the past two decades, mitochondrial DNA (mtDNA) variation has been widely used as a tool to explore the evolutionary and phylogeographic history of populations. The majority of these studies have assumed that variation in mtDNA within a species is selectively neutral. However, accumulating evidence shows that selection acting on the mitochondrial genome might be more common than previously thought (review in Ballard and Kreitman 1995; see also Ballard and Rand 2005, Meiklejohn et al. 2007, Dowling et al. 2008). A direct approach to test the neutrality of mtDNA variation involves evaluating whether variation in the relative frequencies of distinct, but sympatric mtDNA lineages is temporally predictable (Ballard and Rand 2005). In addition to experimental approaches (e.g. on artificial laboratory populations), it is also possible to examine the neutrality prediction (stability of haplotypes frequencies) under natural conditions by surveying contact zones between mtDNA lineages. The direct measurement of natural selection on haplotype variation by following cohort survival has rarely been done (Ballard and Rand 2005).

We previously documented geographic variation of a maternally inherited nuclear marker (the female sex-linked alleles of the MPI enzyme) among the oviparous populations of the lizard *Lacerta vivipara* distributed in southwestern France (Aquitaine and Pyrénées) and northern Spain (Pyrénées and Cantabrian Mountains) (Guillaume et al. 2000). We described two parapatric groups that displayed either a slow or a fast migrating allele at the MPI locus. Only three populations were identified with a mixture of both alleles in southwestern France (Guillaume et al. 2000). Another study, also based on the use of a maternally inherited genetic marker (sequence of mtDNA Cytochrome b gene), provided similar results (Surget-Groba et al. 2001). The mtDNA data confirmed the existence of two sub-clades (lineages OF and OC) whose geographic distributions and contact zones corresponded exactly to those described from the allozyme data (Surget-Groba et al. 2001). Therefore, both the allozyme and mtDNA data corroborated the identification of two distinct phylogeographic groups that have come into contact in a few populations. However, because the methods employed were labour intensive (allozyme) or expensive (mtDNA sequencing), our previous studies were based on a relatively limited number of individuals ($n = 175$ for allozymes, and $n = 34$ for mtDNA).

In this study, we describe and apply a simple method to rapidly assign individuals to their mitochondrial lineage (haplogroup). This method allowed us to screen a large

number of specimens in order to: 1) refine the description of the geographic range and particularly of the contact zone between the two lineages and 2) evaluate whether there was variation in the relative frequencies of these two lineages over time in three contact zone populations. In one of these contact zone populations we also performed a capture–mark–recapture study from 2002 to 2009 allowing us to compare the survival rates of the two lineages.

Methods

The species

Lacerta vivipara is a Eurasian lizard that is characterized by distinct viviparous (VU, VB, VH, PA) and oviparous (OS, OC, OF) haplogroups. The distribution of these haplogroups is presented in Fig. 1. Detailed accounts on the evolutionary and phylogeographic history of these haplogroups have previously been published (Heulin et al. 1999, Surget-Groba et al. 2001, 2006). In the present study we focus on the contact zone of the oviparous haplogroups (OC and OF) living in the south-westernmost part of the species' range.

Samples

Samples were collected from 77 populations (Table 1). We used a non-invasive sampling method (tail autotomy) to collect tail fragments (ca 1 cm) that were preserved in 95% ethanol. All lizards were immediately released at their point of capture. For the analyses we used the samples (n = 34) that were already sequenced by Surget-Groba et al. (2001), and new samples (n = 2869) that were collected between 2000 and 2009. We sequenced a 427 bp mtDNA fragment (cytochrome b gene) for 200 new individuals, and we applied a new procedure of assignment to haplogroups (amplification with lineage-selective primer) to all (n = 2903) individuals.

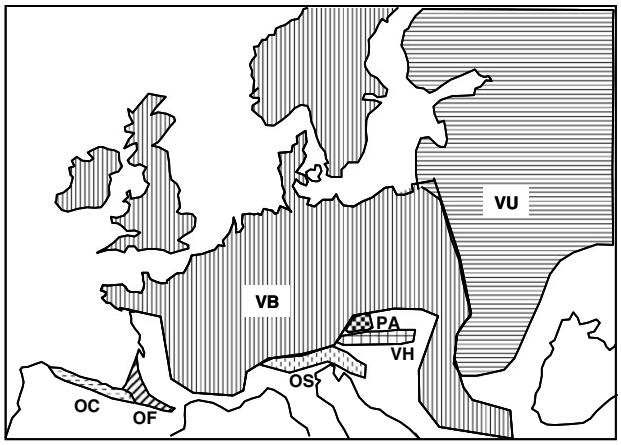

Figure 1. Spatial distribution of the haplogroups of *Lacerta vivipara* in Europe. The present study focus on the southwestern OC and OF haplogroups (Fig. 3).

MtDNA sequencing and phylogenetic inference

Total DNA was chelex extracted according to Estoup et al. (1996) from small amounts of tail. A 427 bp fragment of mtDNA was amplified using primers MVZ04 and MVZ05 (Smith and Patton 1991), and sequenced as previously described (Surget-Groba et al. 2001). The new haplotypes identified in this study were aligned by eye with previously described haplotypes. A haplotype network was first reconstructed using the statistical parsimony method (Templeton et al. 1992) as implemented in TCS ver. 1.21 (Clement et al. 2000). We then used a Bayesian-based inference (BI) method for inferring phylogenetic relationships among haplotypes. The best-fit model of nucleotide substitutions was selected prior to BI analyses using MrAIC ver. 1.4.2 (Nylander et al. 2004). The HKY model was selected using the Akaike information criterion and was incorporated into MrBayes ver. 3.1.2 for BI analyses. We ran two independent analyses with four chains each (one cold and three heated) for 10 000 000 generations. Trees were sampled every 1000 generations. Posterior probabilities were obtained from the 50% majority-rule consensus of trees sampled after discarding the first 2 000 000 generations. Convergence was checked by plotting the parameters against generations, and using the diagnostic tools available in MrBayes. Haplotypes belonging to the other clades of *Lacerta vivipara* (OS1, VH1, PA1, VB1 and VU1) were used as outgroups in the phylogenetic analyses (Surget-Groba et al. 2006).

Design of lineage-specific primers and procedure of haplogroup assignment

Nucleotide variation among the nine sequences already known (haplotypes OC1 to OC5 and OF1 to OF4) was surveyed along the entire length (427 bp) of the fragment to define conserved segments and sites showing differences between the two lineages. The two haplogroups only differ by single nucleotide polymorphisms (SNP) at 3 sites: in position 168 (with a C for the haplogroup OF and a T for the haplogroup OC), in position 201 (A for OF and T for OC) and position 267 (C for OF and T for OC). This allowed us to design three primers for amplification of fragments internal to MVZ04 and MVZ05 (Fig. 2). One primer SNPR (5′ ATACTTCAGGTTTCAGTATAAATATAGG 3′) was determined for a fully conserved region among the sequences of the two haplogroups. One lineage selective primer SNP1 (5′ CCTTATTATTCAAATTATTACAGG***C** 3′) was designed to match to positions 144 to 168, exclusively for the haplogroup OF. The other lineage-selective primer SNP2 (5′ GCTATACACTATACCGCAGATGT***T** 3′) was designed to match to positions 178 to 201, exclusively for the haplogroup OC. To insure a very high specificity of the allele-specific PCR, we used a chemical modification (LNA = locked nucleic acid, symbolized by * above) for the last base of the lineage-specific primers. LNA are nucleic acid analogues that display a very high affinity with its complementary DNA and significantly increase the thermal stability of oligonucleotide (Petersen and Wengel 2003). The use of a LNA as the last base of an

Table 1. Populations and samples. No.: population identification number. Populations: name, latitude, longitude, altitude. Haplogroups: number of individuals belonging to the OF and/or OC haplogroups. Sequences: number and haplotypes determined by sequencing the 427 bp fragment. We sequenced the 427 bp mtDNA fragment for 234 individuals and we applied our new procedure of assignment to haplogroups to all (n = 2903) individuals.

No.	Populations	Haplogroups		Sequences
		OF	OC	
1	Sierra de Ancares, 42.76444°N–6.90008°W, 1750 m		3	1 OC4
2	Puerto de Letariegos, 42.99889°N–6.38528°W, 1530 m		1	1 OC4
3	Tarna, 43.08361°N–5.21778°W, 1490 m		1	1 OC3
4	Alto de los Tornos, 43.14472°N–3.44833°W, 900 m		2	2 OC2
5	Alto de Barazar, 43.01583°N–2.71250°W, 600 m		1	1 OC2
6	Roncesvalles, 43.01972°N–1.32417°W, 1060 m		30	1 OC9
7	Arradoy, 43.1879°N–1.2262°W, 454 m		5	1 OC2
9	Iraty, 43.04694°W–1.07306°W, 1010 m		2	2 OC1
10	Col d'Inharpu, 43.09557°N–1.03068°W, 1013 m		12	1 OC8
11	Pagolle, 43.2437°N–1.0170°W, 284 m		15	3 OC1
12	Espiute, 43.3492°N–0.9340°W, 200 m		5	1 OC1
13	La Rhune, 43.31585°N–1.62798°W, 528 m		3	2 OC2
14	Mondarrain, 43.31060°N–1.43279°W, 460 m		52	
15	Hasparren, 43.35709°N–1.33982°W, 267 m		1	1 OC1
16	Moura de Montrol, 43.51303°N–1.29636°W, 10 m	3	2	1 OC5, 2 OF1
17	St Raphael, 44.97333°N–0.79556°W, 45 m	2		2 OF1
18	Salles, 44.57464°N–0.82239°W, 55 m	1		1 OF1
19	Belin-Beliet, 44.4703°N–0.77822°W, 46 m	1		1 OF1
20	Saint-Magne, 44.54333°N–0.66528°W, 64 m	1		1 OF1
21	Belhade, 44.35345°N–0.68397°W, 58 m	1		1 OF1
22	Labouheyre, 44.21747°N–0.88997°W, 78 m	1		1 OF1
23	Trensacq, 44.23594°N–0.71822°W, 84 m	1		1 OF1
24	Plateau de Ger, 43.22472°N–0.06056°W, 430 m	1		1 OF3
25	Col des Palomières, 43.05693°N–0.19296°E, 817 m	41		3 OF1
26	Etang de Lers, 42.80778°N–1.37361°E, 1272 m	40		
27	Pinet-Bélesta, 42.87444°N–1.98083°E, 880 m	6		3 OF4
28	Estaing-Lac, 42.90980°N–0.22923°W, 1163 m	51		
29	Plaa d'Aste, 42.89019°N–0.26956°W, 1400 m	25		1 OF1
30	Louvie, 43.09304°N–0.38039°W, 378 m	100		14 OF1, 3 OF2
31	Aubisque, 42.97254°N–0.34547°W, 1590 m	101		8 OF1
32	Col de l'Ours, 42.89791°N–0.38302°W, 1900 m	13		1 OF1
33	Soussouéou bas, 42.90139°N–0.36673°W, 1425 m	120	10	1 OC1, 4 OF1
34	Soussouéou haut, 42.88224°N–0.33895°W, 1556 m	11		2 OF1
35	Hourcq, 42.9113°N–0.4357°W, 836 m	31		1 OF1
36	Aydius, 42.994°N–0.5074°W, 1079 m	14		3 OF1
37	Gloutaret, 43.02429°N–0.47512°W, 1178 m	1		1 OF1
38	Benou, 43.06322°N–0.45656°W, 822 m	27		1 OF2
39	Aran- Rigassou, 43.04700°N–0.52374°W, 1295 m	24		2 OF1, 6 OF2
40	Aran-Cardouet, 43.04684°N–0.52235°W, 1310 m	19		1 OF1, 1 OF2
41	Col de Marie-Blanque, 43.07075°N–0.50787°W, 1039 m	16		1 OF1, 6 OF2
42	Moulin, 43.07367°N–0.54011°W, 688 m	3		1 OF2
43	Barescou, 43.07716°N–0.59873°W, 350 m	29		3 OF2
44	Berguery-Laguns, 43.1162°N–0.5492°W, 345 m	5		1 OF2
45	Serre, 43.10003°N–0.61099W, 390 m	32		1 OF2
46	Ponsuzou Est, 43.03528°N–0.60436°W, 365 m	21		1 OF2
47	Sabatte-Gey, 43.0337°N–05942°W, 390 m	9		1 OF2
48	Bedous, 43.011118°N–0.60136°W, 412 m	16		1 OF1
49	Col de Bergout, 42.97693°N–0.566°W, 1183 m	1		
50	Cette, 42.9351°N–0.5806°W, 670 m	5		
51	Ayguebère, 42.89613°N–0.44424°W, 1440 m	3	10	2 OC1, 1 OF1
52	Gabas, 42.89672°N–0.42367°W, 1160 m	373	912	42 OC1, 18 OF1
53	Sud Sagette, 42.8944°N–0.3960°W, 1754 m	23	8	4 OC1, 2 OF1
54	Artouste Lac, 42.86338°N–0.33594°W, 1947 m		6	2 OC1
55	Pourtalet, 42.79530°N–0.40384°W, 1700 m		73	6 OC1
56	Brousset, 42.85125°N–0.38935°W, 1300 m		69	8 OC1
57	Fabrege Lac, 42.8679°N–0.3946°W, 1254 m		8	3 OC1
58	Bious, 42.8719°N–0.4484°W, 1320 m		25	3 OC1
59	Biscau, 42.89846°N–0.43321°W, 968 m		6	3 OC1
60	Somport, 42.79871°N–0.53144°W, 1560 m		18	2 OC1
61	Baralet, 42.87020°N–0.58232°W, 1117 m		6	1 OC6
62	Cirque de Lescun, 42.90117°N–0.64748°W, 1125 m		28	1 OC6
63	Laberouat, 42.94946°N–0.66441°W, 1422 m		9	1 OC6
64	Col de Bouesou, 43.00468°N–0.66954°W, 1000 m		6	1 OC6
65	Osse, 43.01151°N–0.60645°W, 406 m		30	1 OC6
66	Ponsuzou Ouest, 43.03206°N–0.60595°W, 405 m		20	1 OC6
67	Rachou, 43.04797°N–0.61001° E, 400 m		25	1 OC6
68	Bosdapous, 43.05911°N–0.61905°W, 850 m		5	1 OC7

Table 1. Continued.

No.	Populations	Haplogroups		Sequences
		OF	OC	
69	Sarrances, 43.0639°N–0.6013° W, 322 m	22	125	1 OF2, 2 OC6
70	Apouns, 43.0683°N–0.6076°W, 397 m		17	6 OC6
71	Pacq, 43.07869°N–0.6170°W, 420 m		58	5 OC6
72	Bugangue, 43.1361°N–0.6383°W, 300 m		5	1 OC6
73	Col de Lie, 43.06025°N–0.69357°W, 600 m		10	1 OC6
74	Issarbe-Léphelcé, 43.0310°N–0.7970W, 1140 m		29	1 OC6
75	Lanne Barétous, 43.0987°N–0.7697°W, 372 m		5	1 OC6
76	Madeleine, 43.14639°N–0.83911°W, 719 m		5	1 OC6
77	Gurs, 43.2752°N–0.7375°W, 151 m		29	1 OC6

allele-specific primer drastically reduces the probability of amplifying an alternative allele.

PCRs were carried out in 20 µl volumes containing 0.30 µl (20 µM) of each of the three primers, 8 µl of the Diamond DNA polymerase-500 master-mix (Bioline), 9 µl of ultra-pure water, and 2 µl of template DNA. The PCR conditions were an initial denaturation step at 94°C (5 min) followed by 35 cycles of 95°C (20 s), 50°C (20 s), 72°C (20 s) and a final extension phase at 72°C (2 min).

Since the three primers generated two possible PCR products of different sizes (139 bp for the OC haplogroup vs 172 bp for the OF haplogroup), we used electrophoresis to assign each sample to one of the two haplogroups. We loaded 4 µl of PCR product of each sample on a 2.5% agarose gel and that was run for 55 min at 100 V. PCR bands were stained with ethidium bromide and visualized under UV light. Samples with the slowest migration (i.e. longer fragment) were assigned to the OF haplogroup and samples with the fastest migration (i.e. shorter fragment) were assigned to the OC haplogroup. Among the 20 samples loaded for each electrophoresis, we always included two controls from each haplogroup. These controls were PCR products obtained from individuals whose haplotypes were previously determined by sequencing.

Survey of contact zone populations

We evaluated the stability of haplogroup frequencies over time in three contact populations: Soussouéou (pop. 33 in Table 1 and Fig. 3) sampled in 2003 and 2007, Sarrances (pop. 69) sampled in 2006 and 2009, and Gabas (pop. 52) sampled every year from 2002 to 2009. The Gabas population was followed by capture–mark–recapture to estimate the annual survival rates of the two haplogroups OC and OF from 2002 to 2009. The study plot, 72 × 71 m (0.51 ha), was located in a peatbog. A detailed description of the site and of the demography of this population (period 1990–1994) can be found in Heulin et al. (1994, 1997). During each sampling period (May–June), lizards were captured by hand and released after noting their identity. At first capture, each lizard was individually marked by toe clipping (for future recognition) and a piece of its tail collected and kept in 95% ethanol (for genetic analysis). To estimate survival rates we considered three distinct cohorts: adult males, adult females, and subadults. Subadults were individuals born during the preceding summer (July–August) and for which it was not possible to determine the sex during the sampling periods (i.e. the spring following their birth). The matrix of encounter histories of individuals marked as subadults, and the modeling of these data focused solely on their first year survival rate referred to as age class a(1). Any individual marked as subadult (unknown sex) and recaptured the second year, referred to as age-class a(2), was subsequently included in one of the two adult data sets (either as male or female), together with the other unmarked adults captured at the same sampling session.

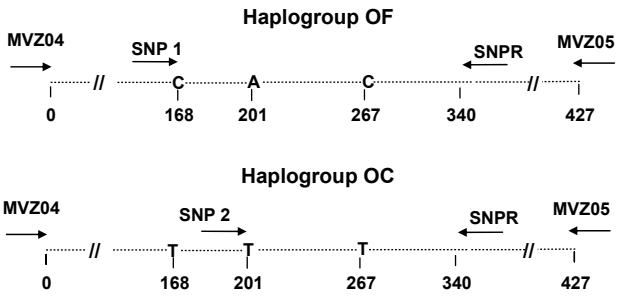

Figure 2. Locations of the diagnostic sites and of the primers used in the haplotypes of haplogroups OF and OC.

Prior to modeling the data, we performed goodness-of-fit tests (test for transience-age effect and test for trap-dependence) with the U-CARE 2.2 software (Choquet et al. 2005), in order to define an appropriate general (starting) model for each of our three data sets. We then used the M-SURGE 1.8 software (Choquet et al. 2004, 2006) to model the survival rates and recapture rates (proportion of individuals actually captured at a given session, among all the marked individuals known to be present at this session). This software, using maximum likelihood methods, was used to fit our data (matrix of individuals' capture–recapture histories) to single state models derived from the Cormack–Jolly–Seber (CJS) approach (Lebreton et al. 1992). For each model tested, M-SURGE provides the estimates (and 95% confidence limit) of recapture rates and survival rates, the relative deviance, and the Akaike information criterion (AIC = relative deviance of the model + 2N, where N is the number of parameters estimated). Starting from a general model, we selected more satisfactory (i.e. more parsimonious) models when they presented lower values of AIC than the initial model. We also performed log likelihood ratio tests (LRT: χ^2 values calculated as the difference in deviance between

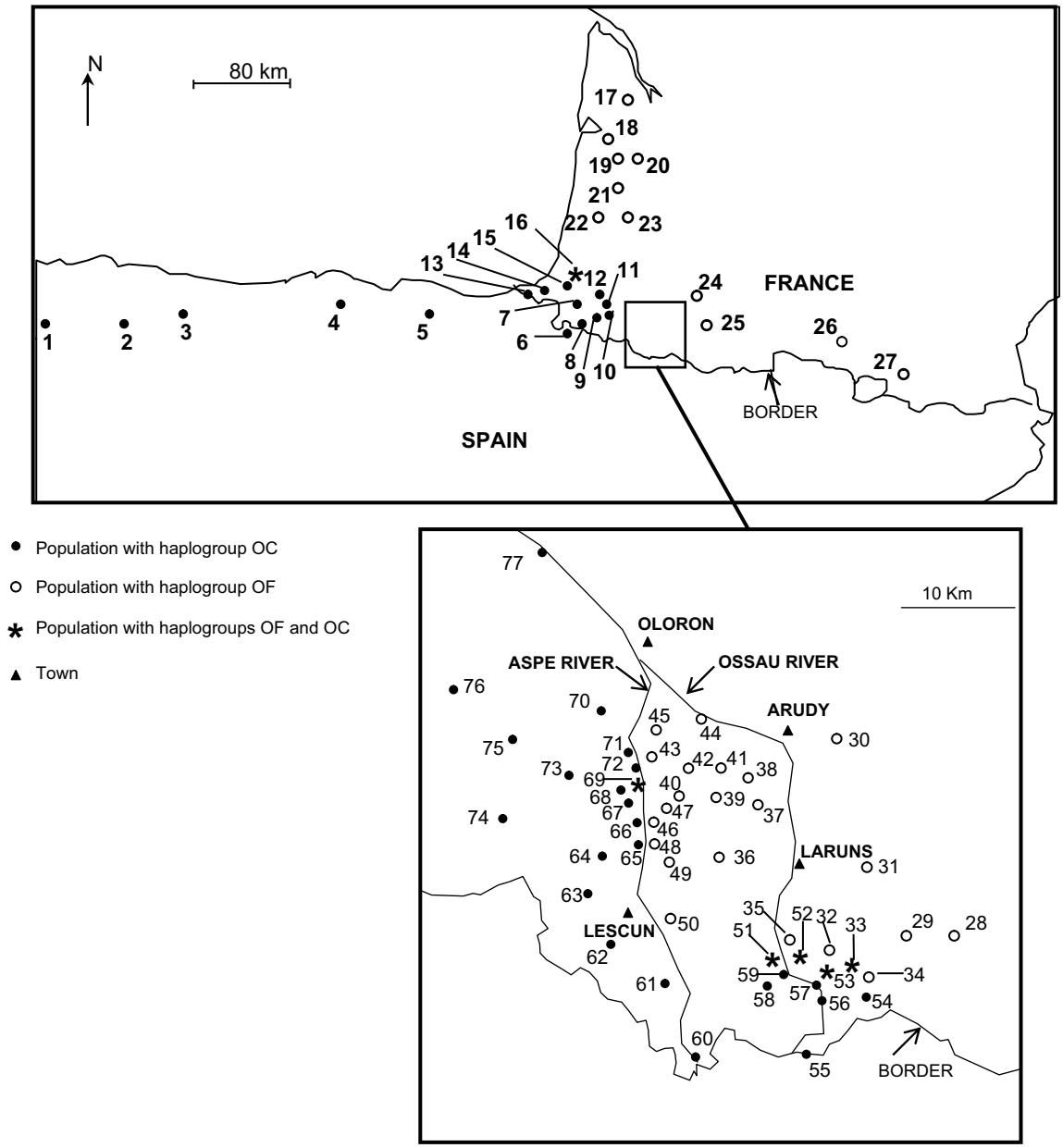

Figure 3. Populations sampled and geographic distribution of the two haplogroups OC and OF. The assignment of populations to haplogroups was done by typing all the samples with our lineage-selective primers method and by sequencing the complete mtDNA fragment of 234 individuals (see samples sizes in Table 1). The different haplotypes identified by sequencing were OC1 (in populations 8, 9, 11, 12, 15, 33, and 51–60); OC2 (in pop. 4, 5, 7, 13); OC3 (in pop. 3); OC4 (in pop. 1, 2); OC5 (in pop. 16); OC6 (in pop. 61–67 and 69–77.); OC7 (in pop. 68); OC8 (in pop. 10); OC9 (in pop. 6); OF1 (in pop. 16–23, 25, 29–37, 39–41, 48, 51–53); OF2 (in pop. 30, 38–47, 69); OF3 (in pop. 24); OF4 (in pop. 27).

2 models) in order to check whether the AIC-selected models were statistically acceptable.

After simplifying the recapture rates we tested several models of survival with simple haplogroup (g) or simple time (t) effects, combined effect with interaction (g.t), additive effect without interaction (g+t), or constant rate. In parallel to these tests of structure simplification, we also used M-SURGE to test whether one could simplify the initial model by expressing the survival as a function of external explanatory variables (e.g. climate). In the present study we tested models in which the survival rate is expressed as a function of temperature, either with two distinct relationships (one for each haplogroup) or with a single relationship (i.e. identical for the two haplogroups). The variables tested were the mean temperature (mean of monthly means) during the activity season of the lizards (May–October: variable T_A), during the following hibernation period (November–April: variable T_H) and for the whole year (variable T_Y). These variables were obtained from the database of the National Meteorological Centre (climatic data recorded within 1 km of our study site, and at the same altitude).

Results

Haplotypes and haplogroups

Sequencing of the 427 bp fragment of mtDNA from 200 new individuals allowed us to identify four new haplotypes (haplotypes OC6 to OC9, Genbank accession numbers GU227619-GU227622) in addition to the nine previously described haplotypes in this area. According to the phylogenetic analyses, these four new haplotypes all belong to the OC haplogroup (Fig. 4). In order to validate the procedure of haplogroup assignment (with lineage specific primers) we applied it to the 234 individuals (200 from this study and 34 from Surget-Groba et al. 2001) with known (sequenced) haplotypes, and we obtained 100% correct assignment. This haplogroup assignment procedure was then applied to the other samples (populations and sample sizes given in Table 1).

The haplogroups OC and OF have parapatric distributions and we only found six contact populations (pop. 16, 33, 51, 52, 53 and 69) in which the two haplogroups are present (Fig. 3). The altitudinal distributions of the haplogroup OC (10–1950 m), and of haplogroup OF (10–1900 m) are very similar, and populations where the two haplogroups co-exist are found both at low (10–322 m, in pop. 16, 69) or high (1160–1754 m in pop. 33, 51, 52, 53) altitudes (Table 1, Fig. 3.). The relative frequency of the haplogroup OF in the contact populations ranged from 15 to 92% (Fig. 5). We detected no significant temporal variation in haplogroup frequencies in the three contact populations that were sampled multiple times ($\chi^2 = 1.302$, DF = 7, p = 0.99 in Gabas, pop. 52; $\chi^2 = 0.041$, DF = 1,

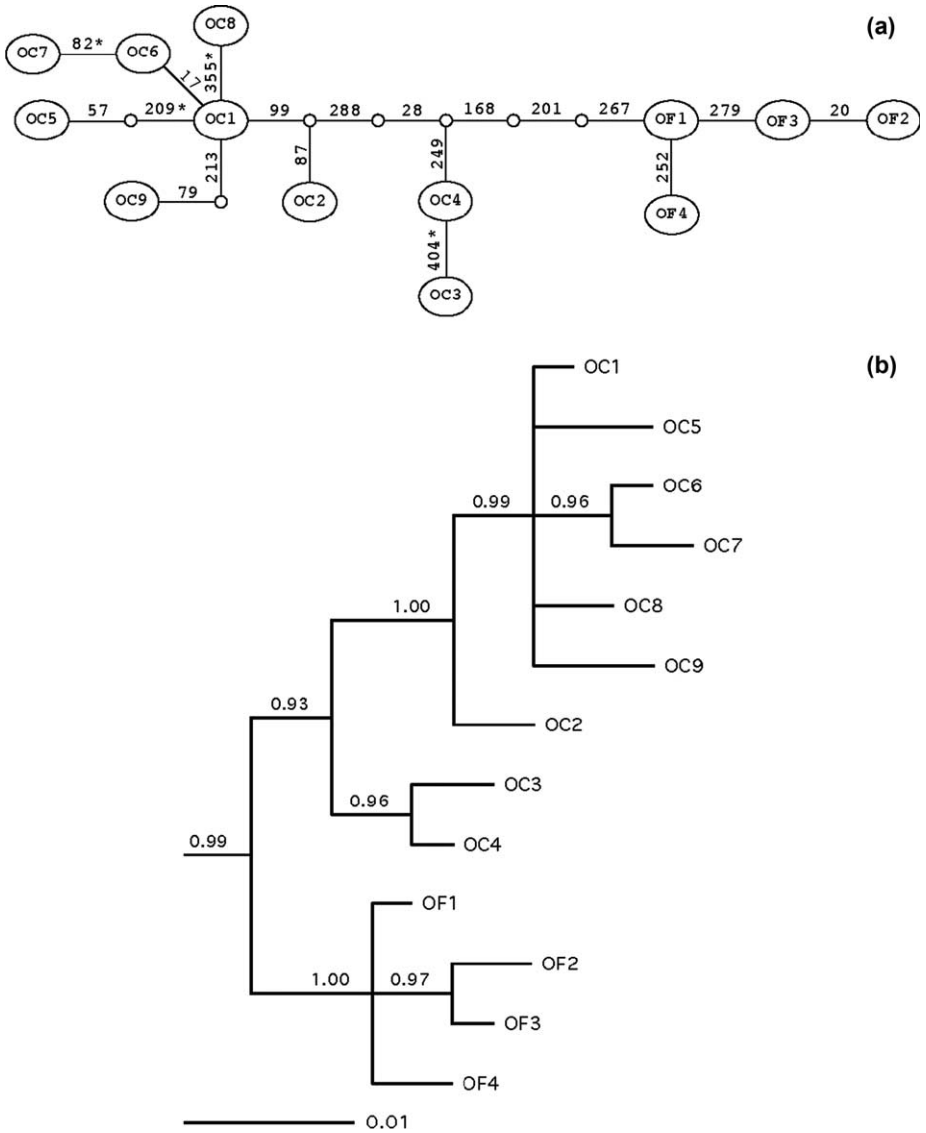

Figure 4. Phylogenetic position of the haplotypes (OC and OF) of the 427 bp mtDNA fragment sequenced in the populations of the southwestern oviparous clade of *Lacerta vivipara.* (a) Haplotype network reconstructed by statistical parsimony method. Each segment represents one mutation. Numbers are positions of the mutation on the fragment. * indicates mutations resulting in one amino-acid change. (b) Phylogenetic tree obtained by Bayesian inference. The numbers represent the posterior probability of the nodes. Outgroups are not represented on this tree.

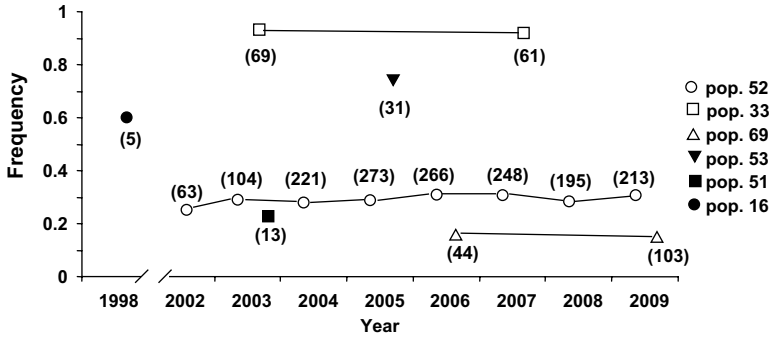

Figure 5. Frequencies of the haplogroup OF in the six contact populations. Numbers between brackets designate sample sizes.

$p = 0.84$ in the Soussouéou, pop. 33; $\chi^2 = 0.044$, DF = 1, $p = 0.83$ in Sarrances, pop. 69).

Sequencing of 60 individuals from Gabas (Table 1) indicated that, in this population, the haplogroup OC is probably exclusively represented by the haplotype OC1 (found in 42 individuals) and the haplogroup OF is probably exclusively represented by the haplotype OF1 (found in 18 individuals). Hence, though we cannot discard the possibility that other rare haplotypes could also exist in this population, one can reasonably consider that the following comparisons of survival rates between haplogroups in Gabas mainly corresponded to a comparison of haplotypes OC1 vs OF1.

Survival rates of the two haplogroups in the contact population of Gabas

Starting models

The first component of the U-CARE goodness of fit test indicated that there was no 'transience' (or age) effect in the adult data sets (two-tailed test with $p = 0.54$ for females and $p = 0.44$ for males, one-tailed test with $p = 0.73$ for females and $p = 0.22$ for males) whereas this effect was clearly significant in the subadult data set (two-tailed test with $p = 0.0034$, one-tailed test with $p = 0.0017$). This significant effect found in the subadult data set corresponds to a very common demographic pattern (lower survival rate of subadults vs adults), which led us to select a starting model in which the survival rates of two age classes a(1) and a(2) were distinguished. On the contrary, no age effect was considered in the starting models built for the adult male and adult female data sets (Table 2, 3). The trap-dependent component of the U-CARE goodness of fit test was not significant in adult females ($p = 0.3658$) or in subadults ($p = 0.3008$), but this test could not be performed for the adult males, because of insufficient data. Nevertheless, in subsequent analyses we assumed no trap dependent effects in adult males, since there were no such effects in the two other data sets.

Model selection for adult survival

Log-likelihood ratio tests (LRT), performed by comparing models no. 0–1, 0–2, and 1 (Table 2), revealed no difference in recapture rate between the haplogroups OC and OF in the two adult data sets ($\chi^2 = 2.905$, DF = 7, $p = 0.89$ for adult males, $\chi^2 = 6.535$, DF = 7, $p = 0.48$

Table 2. Selection of the models of survival and recapture rates in adult males and adult females. Simplification of recaptures rates (models 0 to 1); structure simplification of survival rates (models 1 to 5); expression of survival rates as functions of climatic variables differing between the two haplogroups (models 6–1, 7–1, 8–1) or identical for two haplogroups (models 6–2, 7–2, 8–2). The three climatic variables (T_A, T_H, T_Y) respectively correspond to the mean temperatures calculated for the activity period, for the hibernation period, and for the whole year. N – number of parameters of the model; AIC – Akaike information criterion; P-Mod1 – probability value of the Likehood ratio test of the comparison of a model x to the model l, with $N_x - N_1$ degrees of freedom. The AIC value of the best supported model is shown in bold. The model notation follow the model definition language used in M-Surge: haplogroup effect (g), time effect (t), time and group effects including their interaction effect (g.t), additive time and group effects without interaction (g+t), constant (i), expression as a function of an external variable X ([i+t×X], where i symbolize the intercept of the function).

Model no.	Survival	Recapture	N	AIC males	P-Mod1 males	AIC females	P-Mod1 females
0–1	g.t	g.t	28	371.786	0.8096	718.879	0.2630
0–2	g.t	t	21	360.691	0.4701	711.414	0.1620
1	g.t	i	15	354.285	/	708.626	/
2	g+t	i	9	345.221	0.817	701.918	0.507
3	g	i	3	350.851	0.057	702.423	0.122
4	t	i	8	**343.426**	0.872	**699.918**	0.624
5	i	i	2	349.185	0.075	700.444	0.165
6–1	g.[i+t×T_A]	i	5	352.475	0.052	704.975	0.090
6–2	i+t×T_A	i	3	351.031	0.054	702.043	0.135
7–1	g.[i+t×T_H]	i	5	354.649	0.026	705.753	0.072
7–2	i+t×T_H	i	3	351.009	0.055	701.783	0.144
8–1	g.[i+t×T_Y]	i	5	353.757	0.035	705.319	0.081
8–2	i+t×T_Y	i	3	351.175	0.052	701.783	0.144

Table 3. Selection of the models of survival and recapture rates in subadults. Survival corresponding to the subadult period (first year) is associated to the age symbol a(1) and was modeled as explained in Table 2. The subsequent survival of individuals recaptured as adults (second year and later) is associated with the age symbol a(2). We kept this second term as being simply time-dependent (in accordance to the result presented in Table 2, and see text). The AIC value of the best supported model is shown in bold.

Model no.	Survival	Recapture	N	AIC	P-Mod1
0–1	a(1).g.t+a(2).t	g.t	34	996.334	0.9643
0–2	a(1).g.t+a(2).t	t	27	986.554	0.9761
1	a(1).g.t+a(2).t	i	21	975.768	/
2	a(1).[g+t]+a(2).t	i	15	976.013	0.056
3	a(1).g+a(2).t	i	9	973.269	0.044
4	a(1).t+a(2).t	i	14	974.785	0.072
5	i+a(2).t	i	8	972.025	0.052
6–1	a(1).g.[i+t × T_A]+a(2).t	i	11	**970.976**	0.125
6–2	a(1).[i+t × T_A]+a(2).t	i	9	973.957	0.035
7–1	a(1).g.[i+t × T_H]+a(2).t	i	11	976.245	0.025
7–2	a(1).[i+t × T_H]+a(2).t	i	9	973.046	0.046
8–1	a(1).g.[i+t × T_Y]+a(2).t	i	11	975.206	0.035
8–2	a(1).[i+t × T_Y]+a(2).t	i	9	973.791	0.037

for adult females) and no variation over time of these recapture rates ($\chi^2 = 5.594$, p = 0.47 for adult males; $\chi^2 = 9.212$, DF = 6, p = 0.16 for adult females). This allowed us to select our two initial models of adult survival rates (model no. 1 in Table 2, Fig. 6), with a constant recapture rate of 93% (confidence limit 65–99%) for adult males, and of 76% (CL 65–85%) for adult females. All other (simpler) models of survival were then compared to this initial (no. 1) model (Table 2). AIC values and LRT both indicate that model no. 4 (i.e. simple time-variation of survival) had the most satisfactory fit both for adult females and males. Although some models including the effect of climatic variable are statistically acceptable for adult females, none of them has an AIC value lower than those of model 4. Hence one can conclude that the most appropriate model of adult survivorship simply involved time variation of survival rates, without difference between haplogroups, and without effect of the climatic variables T_A, T_H or T_Y.

Model selection for subadult survival

Patterns of subadult recapture rates mirrored the trends for adult lizards. LRT revealed no difference in recapture rate between the haplogroups OC and OF in the subadult data set ($\chi^2 = 4.22$, DF = 7, p = 0.75) and no variation over time of these recapture rates ($\chi^2 = 1.214$, DF = 6, p = 0.98).

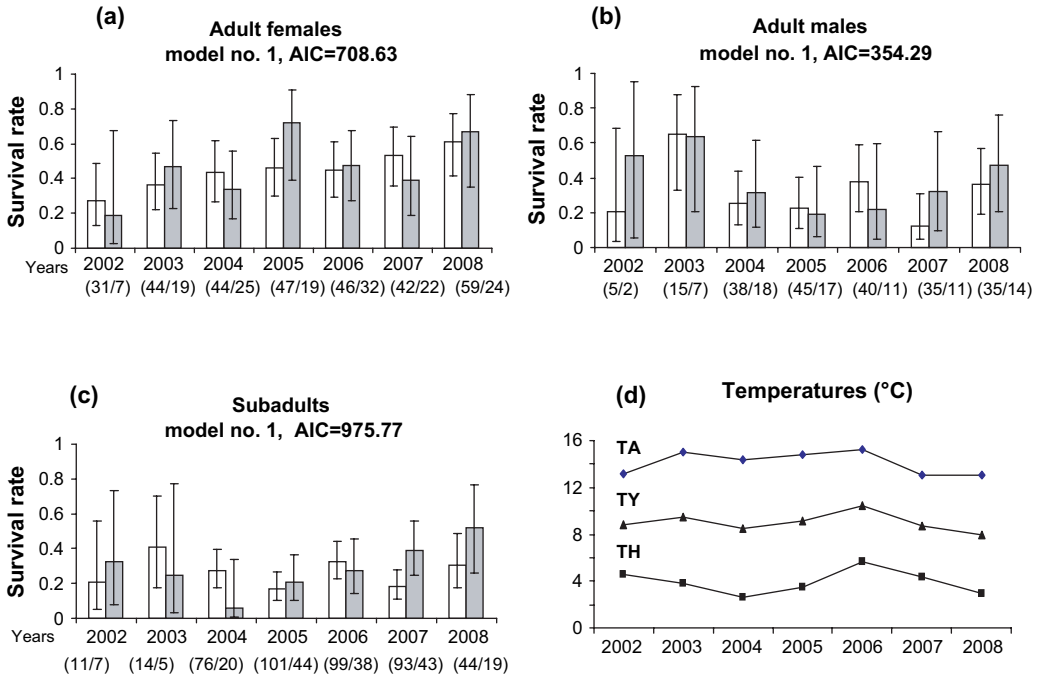

Figure 6. Estimates of survival rates (a, b, c) and climatic data (d) in the population of Gabas. Survival estimates of the haplogroup OC (in white) and of the haplogroup OF (in grey) corresponding to the survival model no. 1 (as in Table 2, 3). Values are presented with their 95% confidence limits. Sample sizes are given between bracket (OC/OF) below the histograms. AIC: value of the Aikaike information criterion. Mean temperatures: T_Y (annual), T_A (May–October) and T_H (November–April).

Our model 1 for subadults (Table 3, Fig. 6) corresponds to a constant recapture rate of 77% (CL 66–86%). In the modelling of the survival rates of subadults we exclusively focused on the first year survival rate [i.e. the true subadult period referred to as a(1)], whereas according to our previous analysis (preceding paragraph), we kept survival during subsequent periods [adult period referred as a(2)] as being simply time variable (Table 3). Compared to model 1 of survival (including the time × haplogroup interaction effect), the model 2 (without this interaction) has a higher AIC, and the LRT is significant at $p = 0.056$ (Table 3). This pattern clearly contrasts with what was observed in adult males and females (Table 2). This suggests that there may exist differences of survival rates between the two haplogroups in subadults and that these differences vary (in intensity, sign, or both) from year to year. With regard to this, it is worth noting that the most satisfactory model for the survival of subadults (model 6–1, Table 3: effect of T_A temperature during activity period) may effectively account for time-variation in the differences between the two haplogroups: the two relationships involved in this model, $S = 0.032\ T_A - 0.201$ for the survival (S) of haplogroup OC and $S = -0.082\ T_A + 1.463$ for survival of the haplogroup OF, revealed a difference in the sensitivity to temperature between the two haplogroups of subadults (positive slope for OC/negative slope for OF).

Discussion

General findings

The existence of suture zones between genealogical lineages, races, subspecies, or sister species in the Pyreneo–Iberian area has been documented for a variety of animal and plant taxa (Hewitt 1988, Mossakowsky et al. 1990, Lazare 1992, Salomon and Hemin 1992, Alcobendas et al. 1996, Garcia-Paris et al. 2003, Rodríguez-Muñoz et al. 2007, Deffontaine et al. 2009). Suture zones have been related to the pattern of range contractions/expansions during the Pleistocene glaciations, to refugia during warm periods (Pyreneo–Cantabric mountains) as well as during cold periods (Iberian and French lowlands), and to the existence of geographic barriers (east-west oriented montane glaciers). These barriers favoured temporary vicariance and allopatric differentiation during glacial advances, but allowed secondary contact during the interglacial periods (for general discussion on these subjects see Hewitt 1996, 2000, Taberlet et al. 1998). Our own data on *Lacerta vivipara* provides an additional observation consistent with this general biogeographic scenario. In addition, the comparative demography approach suggests the existence of temporal variation in age-specific survival rates between the two mitochondrial lineages. Maintenance of a narrow contact zone may be a consequence of age-specific differences (subadults vs adults) in natural selection arising from temperature variation between the two haplogroups. Furthermore, our study, together with several recent studies (review in Ballard and Kreitman 1995, Ballard and Rand 2005) tends to reject the classical null model of neutrality of the mtDNA variation.

Biogeography and evolutionary scenario

Our mtDNA investigations (present study, Surget-Groba et al. 2001) revealed that the oviparous clade of *L. vivipara* inhabiting the lowlands of Aquitaine and along the Pyreneo–Cantabric range consists of two sub-clades showing distinct, parapatric distributions: a southwestern haplogroup (haplotypes OC1 to OC9) distributed along the Cantabrian mountains and in the western part (Basque region) of the Pyrénées and a northeastern haplogroup (haplotypes OF1 to OF4) distributed in the lowlands of Aquitaine and in the eastern and central parts of the Pyrenean range. This pattern is similar to that found in a previous allozyme study (Guillaume et al. 2000). Compared to our previous mtDNA study based on a limited sample (Surget-Groba et al. 2001), the present study based both on sequencing and a new method of haplogroup assignment (i.e. increasing sample size) strongly reinforces and extends our knowledge of mtDNA variation in this oviparous clade of *L. vivipara*. Indeed, we identified four new haplotypes (OC6 to OC9) and four new contact populations (33, 51, 53, 69 in Fig. 3, Table 1).

The phylogeographic pattern observed in this oviparous clade of *L. vivipara* corresponds to category II of Avise et al. (1987) and is characteristic of recent secondary contact zones between populations that previously evolved in allopatry. Following this scheme, we hypothesize that two sub-groups of the oviparous clade of *L. vivipara* retreated to different refugia (one in southern France, OF and the other in northwestern Spain, OC) during the Pleistocene glaciations and subsequently came into secondary contact in the vicinity of the western part of the Pyrenean range during warmer post glacial periods. Indeed, previous estimates of divergence times indicate that the radiation of *L. vivipara* would have occurred during the Pleistocene (Surget-Groba et al. 2001). These divergence time estimates support the above scenario, despite the lack of data to infer whether the timing of divergence between haplogroups OF and OC occurred before (and subsequently reinforced during) the retreat, or strictly during the retreat (allopatric differentiation in the refugia).

Dynamics of haplogroup frequencies and survival rates

We did not find any significant temporal variation in the frequency of the haplogroups in the contact populations (pop. 33, 52 and 69) that were sampled two or more times (Fig. 5). The Gabas population (52), sampled every year from 2002 to 2009, exhibited limited variation (between 25 and 31%) in the frequency of haplogroup OF. Moreover, the capture–mark–recapture study did not reveal any difference of survival rate between adults of either haplogroups OF and OC. However, we found a significant interaction effect (haplogroup × time) on subadult survival rate. The most satisfactory model of subadult survival (T_A dependent model of Table 3) suggested that the sensitivity of survival to climatic effects might be expressed differentially between subadults of the two haplogroups. It is worth noting that the slope of the regression line relating subadult survival to temperature (variable T_A: temperature during

the activity season) is positive for the haplogroup OC and negative for the haplogroup OF. Hence there may exist a subtle difference of survival between the two haplogroups. Because this difference of survival varies with age (i.e. only detected in subadults) and time (e.g. as a function of yearly variation in temperature), there is an absence of strong and directional changes in haplogroup frequencies.

Since the pioneering work of Avise et al. (1987), the use of mtDNA markers has become an irreplaceable tool to investigate phylogeny and phylogeography, both at interspecific and intra-specific levels. However, as these studies rely on the assumption that most variation in mtDNA is selectively neutral, it is necessary to examine the validity of the assumption. Recent reviews present evidence that mtDNA variation could directly or indirectly (i.e. in interaction with nuclear genes) influence a variety of traits associated with fitness, such as bioenergetic performances, thermal adaptation, resistance to cold and starvation, growth, survival, fecundity and egg size, sperm performance, lifespan and ageing (reviews in Ballard and Rand 2005, Dowling et al. 2008). Indeed, a complex of enzymes encoded by both mitochondrial and nuclear genes are involved in oxidative phosphorylation, which is the metabolic pathway allowing the production of most of the energy in the eukaryotic cells. Therefore, genetic variation (of mitochondrial genes, nuclear genes, or both) affecting this process is predicted to also alter metabolic performance and, consequently, other traits linked to an individual's fitness (e.g. life-history traits, thermal adaptation). Ballard and Rand (2005) suggest that a test of the neutrality prediction that haplotype frequencies should not change in any predictable or repeatable way may be accomplished by assessing competition between mtDNA haplotypes. Our study generated data relevant to this question. On the one hand we did not find any evidence of strong and directional changes in haplogroup frequencies over the period considered (2002–2009), and there was no apparent difference in the climatic preference (at least in correlation with altitudinal distribution) of the two haplogroups. On the other hand, we detected a difference in survival between subadults of both haplogroups, and that this difference could vary over time with respect to some climatic (temperature) variables.

Mitochondrial DNA encodes numerous enzymes and enzymatic processes are temperature sensitive. Consequently, one prediction is that mtDNA evolves in response to selection imposed from the local thermal environment. Such thermal adaptation has been suspected in several studies (review in Ballard and Rand 2005, Dowling et al. 2008). Hence, we hypothesize that a difference of thermal sensitivity could account for the survival difference between subadults of the two haplogroups. This age-specific effect could be due to the fact that some variation in metabolic performances (e.g. altering the cost of growth) would more strongly affect survival in younger than in older individuals. Because, no recombination generally occurs in the mtDNA molecule, an advantageous mutation taking place at any point of this genome will lead to the selection of the whole molecule by genetic hitchhiking. Hence, the fact that the three mutations differentiating the two haplogroups within the cytochrome b fragment analysed here are synonymous (i.e. no amino-acid change: Fig. 4a) does not preclude the existence of selective effects due to a mutation situated elsewhere in the mitochondrial genome.

Narrowness of the contact zone

Despite the length of the putative contact front, likely extending from the Aquitaine coastline to the Aspe and Ossau valleys, contact populations containing the two haplogroups OC and OF were found in only a few localities (Fig. 3). The narrowness of the contact zone was obvious in the upper Ossau Valley, where the entire transition from northern populations (with haplotypes OF) to southern populations (with haplotypes OC) occurred within 5 km (see populations 28–36 and 51–59 in Fig. 3). In the lower part of the Aspe Valley, the two haplogroups are clearly separated by the Aspe River (haplogroup OF on the left bank vs haplogroup OC on the right bank), except for one population located on the right bank of the river and containing a small proportion (15%) of individuals belonging to the OF haplogroup (contact population 69, Fig. 3). Further west, it is difficult to infer the width of the OC-OF contact zone, since the lowland populations of Aquitaine are relatively rare and highly fragmented (restricted to discontinuous habitats such as peatbogs and wet heathlands: Heulin 1989, Heulin and Guillaume 1989, Heulin et al. 1993) and currently undergoing climate-forced extinctions (Sinervo et al. 2010).

Both the length of the contact front (150 km) and its narrowness (a few km) parallel the pattern frequently reported for hybrid zones after secondary contact of divergent populations (review in Barton and Hewitt 1985). In particular, according to the relation reported for a wide variety of animal taxa (Barton and Hewitt 1985), the width of the zone with mixed haplogroups (a few km; Fig. 2) matches the value expected from the dispersal capability of *Lacerta vivipara* (30–300 m; Van Nuland and Strijbosch 1981, Heulin 1985, Massot 1992, Strijbosch 1995). According to Barton and Hewitt (1985) such narrow interfaces between distinct populations could be maintained through a stable balance between dispersal (gene flow) and selection (spatially varying selection, or selection against hybrids). Our data do not allow us to distinguish among these possible mechanisms, because hybrids cannot be recognised with our genetic marker (maternally inherited mtDNA gene). However, there is some evidence from the literature that the disruption of lineage-specific co-adapted mito-nuclear gene complexes can sometimes result in selection against hybrids (Dowling et al. 2008). A third scenario emerging from our data is age-specific temporal variation in selection. Our survival data revealed evidence of opposing natural selection of annual temperature on subadults of each haplogroup. We hypothesize that the inter-annual fluctuations of temperature could be involved in the maintenance of a narrow contact zone.

Conclusions and prospects

To better understand the dynamics of contact zones, there is a need to develop novel and simple methods that allow the

screening of numerous samples at lower cost than large scale sequencing (Lindell and Murphy 2008). Our new procedure of haplogroup assignment based on lineage-specific PCR fulfils this objective. This method allowed us to refine our knowledge of the contact zone between two lineages of *Lacerta vivipara*, to develop new approaches (comparative demography of mtDNA lineages) and hence to address important evolutionary questions (e.g. neutrality of mtDNA variation). This study suggests that there are some differences of survival between mtDNA haplogroups that are age and temperature dependent. The addition of nuclear makers to this long-term survey of the contact zone will allow us to test more thoroughly this hypothesis. Indeed, the concomitant use of nuclear and mitochondrial genes may considerably improve the understanding of the phylogeographic history (Garcia-Paris et al. 2003, Godinho et al. 2008). Our future research, including the survey of pure (one lineage) and mixed (2 lineages in sympatry) populations, would enable us to evaluate whether the narrow contact zone identified in this study could be maintained through a stable balance between dispersal (gene flow) and selection (spatially varying selection, or selection against hybrids, Barton and Hewitt 1985; or as our data suggest, age-dependent temporally variable selection).

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