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Origin and genetic diversity of an introduced wall lizard population and its cryptic congener

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Abstract. The Common Wall Lizard (*Podarcis muralis*) has been introduced within large parts of Central Europe, the UK and parts of North America. In an introduced population of this species in Lower Saxony, Germany, we found in addition to mtDNA haplotypes of *P. muralis* also haplotypes of its congener *Podarcis liolepis*, a species that hitherto has never been recorded outside its native range. We therefore, (1) wanted to identify the geographic origin of the founder individuals of both non-native populations, (2) test for hybridization between introduced individuals of both species in Germany and (3) compare levels of genetic diversity between native and introduced populations. We sequenced a fragment of the mitochondrial cytochrome *b* gene and genotyped individuals of the introduced as well as native populations of both species at eleven microsatellite loci. Our results suggest that the founders presumably stem from a region in the eastern Pyrenees, where sympatric populations of *P. muralis* and *P. liolepis* are known. No evidence for gene flow between the two species was found in the introduced population. These results are consistent with behavioural observations indicating agonistic interactions of *P. muralis* towards *P. liolepis* rather than cross-species attraction. Compared to the native populations, high levels of genetic diversity have been retained in the introduced population of both species and no evidence for a genetic bottleneck was found. The effective population size was high in *P. muralis*, but substantially smaller in *P. liolepis*.

Keywords: bottleneck effect, effective population size, genetic variability, hybridization, invasive species, microsatellite, mtDNA.

Introduction

Globalization has favoured an exponential increase in the rate and spatial extent of alien species introductions worldwide. The ecological threat posed by alien invasive species is a severe problem in nature conservation (Strayer et al., 2006; Perrings et al., 2010). During recent decades, a considerable amount of research has been carried out to study contemporary evolutionary events in the process of biological invasions in order to determine which mechanisms drive invasions and to evaluate the impact of invasions. It is generally believed that genetic attributes like additive genetic variance, epistasis, heterosis, genetic drift and genomic rearrangements promote the success of invaders as they provide a buffer to respond to natural selection and allow adapting to new environments (reviewed in Lee, 2002). Several recent studies have shown that invasive populations often exhibit only minimal reductions in genetic diversity as a consequence of a large number of founders or multiple introductions (Holsbeek et al., 2008; Simberloff, 2009). Furthermore, admixture of genotypes from different source populations often boosts genetic diversity and therefore, may support the invasiveness of species (Kolbe et al., 2004; Pairon et al., 2010). As a consequence of genetic drift, selection and hybridization, a rapid genetic divergence of invasive populations from their ancestral source population is often observed (Bossdorf et al., 2005).

Due to the inevitable bias of nearly exclusively sampling successful invasive populations, a loss of genetic diversity associated with population bottlenecks during the invasion processes is reported less frequently (Kelly et al., 2006). In general, a loss of genetic diversity occurs in introduced populations that have been founded by a few closely related individuals, which only

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represent a subset of the genetic variability of a certain source population within the native range (so called founder effect). Although many studies found patterns of inbreeding and outbreeding in invasive populations (e.g. Huxel, 1999; Facon et al., 2011), a small number of founders, high inbreeding and low genetic variation does not necessarily lead to negative fitness consequences or extinction of invasive populations (Verhoeven et al., 2011).

The Common Wall Lizard (*Podarcis muralis*) is one of the few reptile species that has successfully colonized regions in north-western Europe and North America far outside its sub-Mediterranean native range. While determining the origin of 77 introduced wall lizard populations in Central Europe, we discovered one mitochondrial haplotype of the Catalonian wall lizard (*Podarcis liolepis*) at one location (Nörten-Hardenberg, Germany) together with two haplotypes of the Western France *P. muralis* Clade (see fig. 1; Schulte et al., 2012). Based upon information of local residents, the population stems from an intentional introduction and exist at least since the end of the 1980s (Schulte et al., 2011). Recently considered as a valid species within the *P. hispanicus* complex (Renoult et al., 2009, 2010), *Podarcis liolepis* is distributed in the northern Iberian Peninsula (Catalonia, the Ebro Valley, Basque Country, the northern Castilian Plateau southwards to Valencia) and in southern France up to the Rhone river (Carretero, Marcos and de Prado, 2006; Renoult et al., 2010; Kaliontzopoulou et al., 2011; fig. 2). Morphologically, *P. muralis* and *P. liolepis* are relatively difficult to distinguish (Gosá, 1985; Pérez-Mellado, 1998; Vacher and Geniez, 2010). Introduced populations might thus be overlooked, particularly as the latter species is usually not expected outside its native range.

In order to gain a deeper understanding of the rapid parallel establishment of these two non-native wall lizards at a single locality in Germany, we focused on their genetic architecture by using a combination of phylogeographic marker systems (mtDNA) and highly variable microsatellite markers. We specifically wanted to (1) identify the putative source region of the introduced populations of both species, (2) test for hybridization between introduced individuals of both species in Nörten-Hardenberg (Germany) and (3) compare levels of genetic diversity between native and introduced populations.

Figure 1. Lateral view of a male specimen (NOE14) from Nörten-Hardenberg (Germany) attributed to *Podarcis liolepis liolepis*. Photo: US (16.06.2010). This figure is published in colour in the online version.

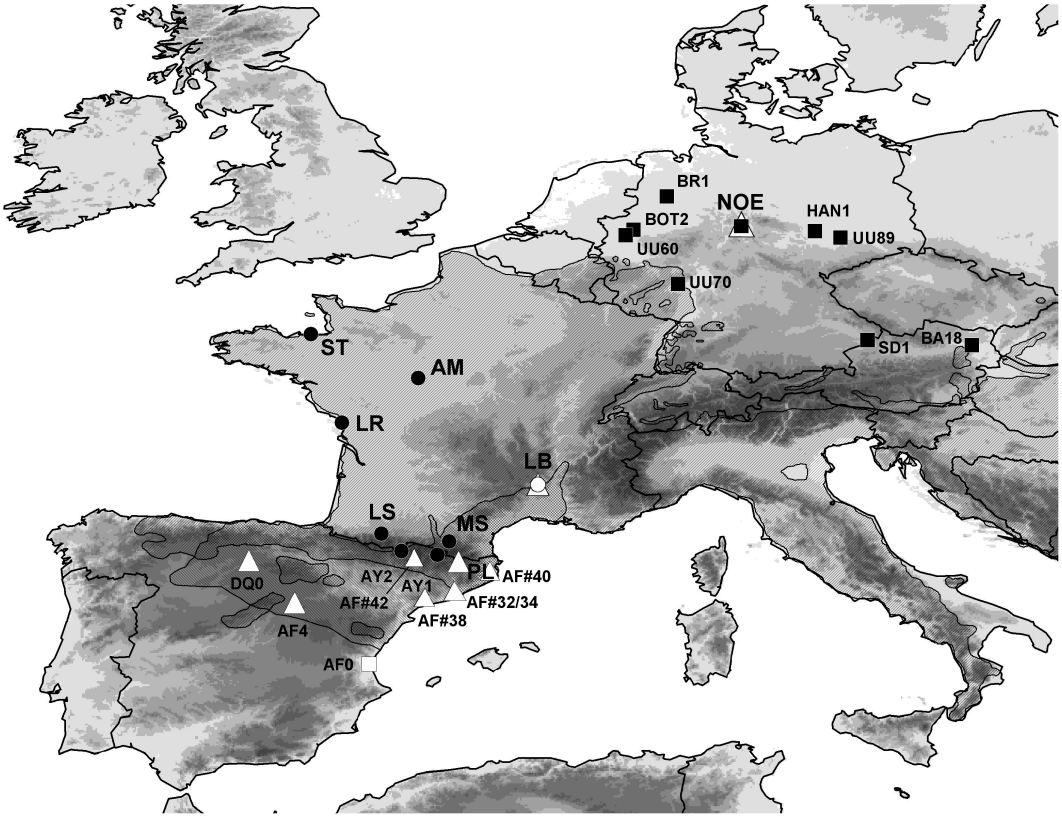

Figure 2. Location of the introduced population in Germany (NOE, Nörten-Hardenberg, Lower Saxony) and geographic range of *P. liolepis* (upward diagonal shaded area, from Renoult et al., 2010 and Kaliontzopoulou et al., 2011) and *P. muralis* (downward diagonal shaded area, from Schulte, 2008) in western Europe. Sampled localities within the native ranges correspond to symbols (black dots: *P. muralis* Western France Clade; white triangles: *P. liolepis*; white square: *P. hispanicus* sensu stricto). Black squares within Germany and Austria correspond to introduced *P. muralis* populations representing six different genetic lineages (see Appendix): BR1 = Bramsche; BOT2 = Bottrop; UU60 = Duisburg-Hüttenheim; UU70 = Mainz; NOE, Nörten-Hardenberg; HAN1 = Halle a. d. Saale; UU89 = Altenhain; SD1 = Schärding; BA18 = Klosterneuburg; LB, Labeaume; AM, Amboise; ST, St. Malo; LR, La Rochelle; LS, Lourdes; AY2, Benasque (AY234155); AF#42, Pyrenees (AF469442); AY1, Andorra (AY151908); MS, Montségur; PL, Planoles; AF#40, Girona (AF469440); AF#32/34, Barcelona (AF469432, AF469434); AF#38, Tarragona (AF469438); AF0, Valencia (AF052635); AF4, Medinaceli (AF469436); and DQ0, Burgos (DQ08114).

Materials and methods

Sampling

A total of 51 lizards (juveniles and adults of both sexes) were captured by hand or by noosing randomly from the introduced mixed population in Nörten-Hardenberg in July 2010 (Lower Saxony, Germany, figs 1 and 2). Lizards autotomized the tail tip after exerting light pressure and were immediately released afterwards. Tail tips were stored in 99.8% ethanol p.a. Additionally, 15 individuals were sampled at a locality in Labeaume (Département Ardèche, Southern France), where *P. muralis* and *P. liolepis* also occur in syntopy. We added 25 samples of *P. muralis* from Montségur ($n = 13$; Département Ariège), Lourdes ($n = 6$; Département Hautes-Pyrénées) and La Rochelle ($n = 6$; Département Charente-Maritime). For the mtDNA analyses we used samples of *P. muralis* from Amboise and Saint-Malo as well as a museum specimen of *P. liolepis* from Planoles (Spain, fig. 2).

Assignment of geographic origin

Sequence data were collected for ten morphologically variable specimens from the introduced German population, for ten samples from six native French populations (Labeaume, Montségur, Lourdes, La Rochelle, Amboise and Saint-Malo) and for one specimen from Planoles (Spain) (fig. 2). DNA was extracted from muscle tissue of autotomized tail tips or of the tongue (museum specimens) using the QIAGEN DNEasy Blood and Tissue Kit (QIAGEN, Hilden) following the manufacturers' protocol. For amplifications of

cytochrome *b* PCR fragments we used 50 μl reaction tubes containing: 27 μl purified water, 20 μl of *Taq* polymerase (QIAGEN Hotstar), 1 μl of each PCR primer and 1 μl of genomic DNA. Reaction conditions comprised an initial denaturation step for 15 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 43°C, 90 s at 72°C, and a final extension step of 10 min at 72°C. We sequenced a 656- to 887-bp fragment of the mitochondrial cytochrome *b* gene using the primers LGlulk (5′-AACCGCCTGTTGTCTTCAACTA-3′), Sicnt (5′-TTTGGATCCCTGTTAGGCCTCTGTT-3′) and HPod (3′-GGTGGAATGGGATTTTGTCTG-5′) (Podnar et al., 2007; Schulte et al., 2012). Sequencing was performed with the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich) for sequencing reactions run on a MegaBACE 1000 automated sequencer. DNA sequences were corrected and aligned by eye. Sequences were deposited in GenBank under the accession numbers JQ403287-JQ403304. For lineage assignment, the sequences were aligned to sequences from individuals sampled within the native range of *P. muralis* (Carranza, Arnold and Amat, 2004; Busack, Lawson and Arjo, 2005; Giovannotti, Nisi-Cerioni and Caputo, 2010) or within the invasive range, when the geographic origin of the introduced population was known (see Schulte et al., 2012). Therefore, we included twelve *P. muralis* sequences of a preliminary study (Schulte et al., 2012) representing six different genetic lineages of the species which have been introduced in Germany. Sequences of the *Podarcis hispanicus* species complex, including seven of *P. liolepis* from Tarragona, Barcelona, Girona, the Pyrenees, Burgos and Medinaceli (Castilla y León), one of *Podarcis vaucheri* from Morocco, one of *P. hispanicus* sensu stricto from Valencia as well as one sequence of *Podarcis siculus* as outgroup were obtained from GenBank (AF052633, AF052635; Castilla et al., 1998; AF469432, AF469434, AF469436, AF469438, AF469440, AF469442, Harris and Sá-Sousa, 2002; DQ081144; Pinho, Ferrand and Harris, 2006; FJ867396, Giovannotti, Nisi-Cerioni and Caputo, 2010; see figs 2 and 3). As we focused on detecting the geographic origin within the native ranges of *P. liolepis* and *P. muralis* (Western France Clade), we ignored additional sequences from other Spanish lineages or species (Kaliontzopoulou et al., 2011). In order to assign introduced haplotypes to intraspecific evolutionary lineages of *P. muralis* and *P. liolepis* and their respective geographic

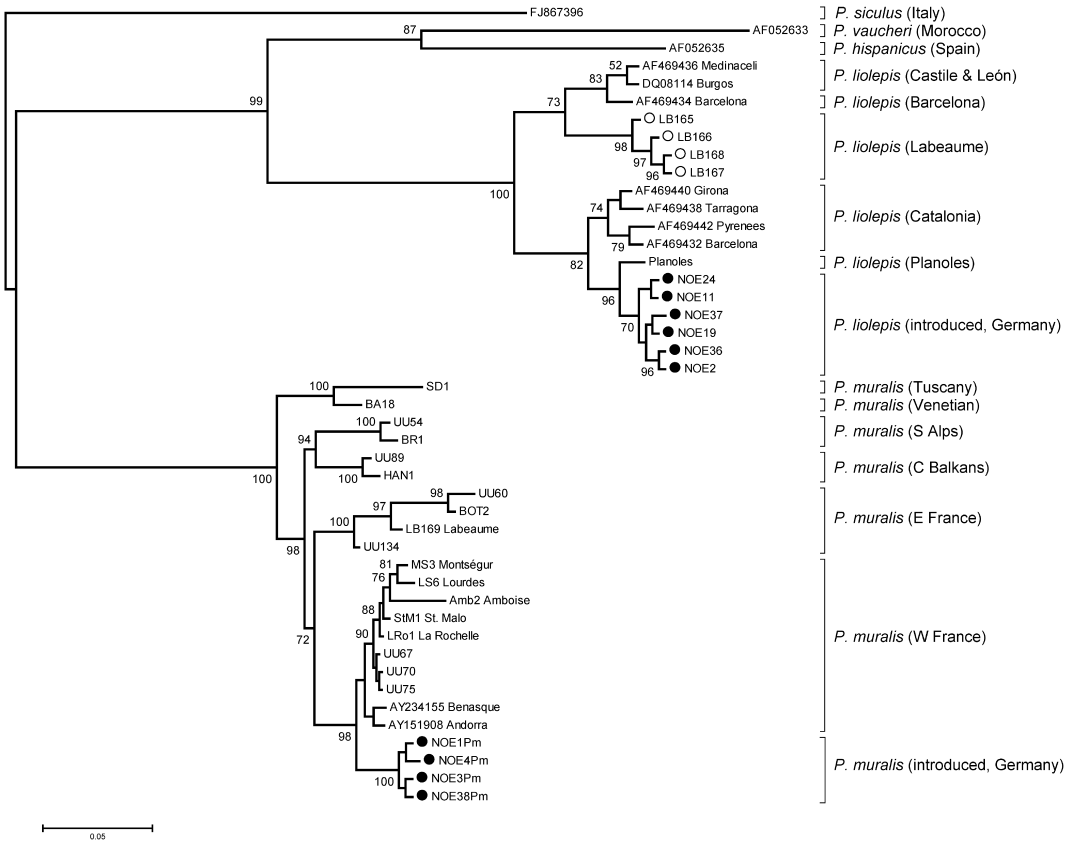

Figure 3. Bayesian consensus tree for the mitochondrial cytB gene for *Podarcis muralis* and *Podarcis liolepis*. Numbers are posterior probabilities. Filled circles represent samples from introduced populations in Nörten-Hardenberg (Lower Saxony, Germany), open circles represent *P. liolepis* samples from the native population in Labeaume (France) (for population names see Appendix).

range via a phylogenetic tree, we used Bayesian inference in MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). We applied the best-fit substitution model (GTR + I + G) suggested by MrModeltest 2.2 (Nylander, 2004). We ran four Monte Carlo Markov chains for one million generations each and sampled a tree every 100 generations. This was sufficient to let the average standard deviation drop below 0.01. We discarded 2500 trees as burn-in after checking for stationary and convergence of the chains. Support of the nodes was assessed with the posterior probabilities of reconstructed clades as estimated in MrBayes (Ronquist and Huelsenbeck, 2003).

Genotyping

We genotyped 51 individuals of the introduced wall lizard population, 14 individuals of the native *Podarcis liolepis* population from Labeaume and 25 *P. muralis* individuals from three native populations in south-western France. All individuals were genotyped at eleven microsatellite loci, six of which have been developed for *Podarcis muralis* (A7, B3, B4, B7, C8, C9; Nembrini and Oppliger, 2003), two for *Zootoca vivipara* (Lv-4-alpha, Lv-472, Boudjemadi et al., 1999) and three for *Podarcis bocagei* (Pb10, Pb50, Pb73; Pinho et al., 2004). Amplification was performed in a Multigene Gradient Thermal Cycler (Labnet) using the 2.5 × 5PRIME HotMasterMix (5PRIME). For each PCR we used 5 μl reaction mix containing: 1.2 μl genomic DNA, 2.2 μl HotMasterMix, 2.2 μl water and 0.1 μl of the forward and reverse primers. The PCR conditions were as recommended by the manufacturer, with locus-specific annealing temperature between 53°C and 61°C. The 5′-end of each forward primer was labelled with a fluorescent dye, either FAM, TAMRA or HEX. PCR products were run on a MegaBACE 1000 automated sequencer. Fragment lengths were determined using MegaBACE ET550-R size standard and MegaBACE Fragment Profiler (Amersham Biosciences).

Data analysis and descriptive statistics

We tested our data for the occurrence of null alleles with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) and for linkage disequilibrium with Fstat 2.9.3.2 (Goudet, 2001). STRUCTURE 2.3.3 (Pritchard, Stephens and Donnelly, 2000) was used to analyse for genetic structuring among subpopulations. The admixture model was used as we wanted to test for potential hybridization. We chose the correlated allele frequency model with a burn-in of 100 000 simulations followed by one million Markov chain Monte Carlo simulations. Tests were run for K = 1-10 with ten iterations per K. This range of values for K was chosen taken into consideration that we have sampled four *P. muralis* populations and two *P. liolepis* populations. Several methods have been proposed to infer the optimal K value from STRUCTURE runs. The method described by Pritchard, Stephens and Donnelly (2000) is known to sometimes lead to asymptotic convergence and tends to result in too high K values. The optimal K value suggested by Evanno, Regnaut and Goudet (2005) is based on the second order rate of change (ΔK) and tends to result in low K values (Hausdorf and Hennig, 2010; Campana et al., 2011). Recently, a new method (ΔF_{ST}) has been proposed by Campana et al. (2011). We compared all three methods in the CorrSieve package for R (Campana et al., 2011). However, based upon our sampling design, we expected that a biologically meaningful minimum value for K would be four (as we sampled two species and each from at least two very distant localities). The results obtained using ΔF_{ST} ($K = 2$) and ΔK ($K = 3$) both suggested values that appeared not biological meaningful. We suppose that this is caused by the strong differentiation at the species level. As our $\ln P(D)$ values showed no asymptotic convergence and K was biological meaningful, we used the K value with the highest average $\ln P(D)$ value as suggested by Pritchard, Stephens and Donnelly (2000).

We used GenAlEx 6.4.1 (updated from Peakall and Smouse, 2006) to calculate the number of alleles (N_A), the inbreeding coefficient (F_{IS}), as well as for expected and observed heterozygosities (H_E and H_O) for each locus and population. Fstat was used to calculate allelic richness (A_R). As traditional methods of population differentiation (F_{ST}, G_{ST}) have recently been strongly criticized, we calculated D_{EST} as an estimate of population differentiation (e.g. Jost, 2008; Gerlach et al., 2010) using the DEMEtics package for R (Gerlach et al., 2010). However, in our case F_{ST} and D_{EST} had a strong linear correlation ($R^2 = 0.91$). Therefore, we used F_{ST} in an AMOVA with 9999 iterations in GenAlEx with the genetic clusters suggested by STRUCTURE as populations and the two species as "regions". We estimated the effective population size (N_E) of clusters identified by STRUCTURE using ONeSAMP, which uses an approximate Bayesian computation for estimating N_E and 95% confidence limits (CL) (Tallmon et al., 2008). The program generates 50 000 simulated populations with N_E between a conservatively estimated lower and upper bound for N_E (for all four populations: 2-500). After executing ten iterations of estimating N_E we calculated the mean and standard deviation of N_E for each population.

To detect recent bottlenecks in the introduced populations, the program BOTTLENECK 1.2.02 was used (Cornuet and Luikart, 1996). Recent bottlenecks (0.2-4 N_E generations) can create a heterozygosity excess compared to populations at mutation-drift equilibrium, because rare alleles that have little impact on heterozygosity can be lost quickly. We calculated H_{EQ} using the two-phase model with a variance of 30 and a proportion of 70% of the step-wise mutation model in the two-phase model (Di Rienzo et al., 1994), as this is believed to be the most likely mutation model for microsatellites (Piry, Luikart and Cornuet, 1999). Statistical significance was assessed with a one-tailed Wilcoxon-test, since this test proved to be the best for less than 20 loci (Piry, Luikart and Cornuet, 1999). Analyses were performed with 1000 iterations.

Results

Geographic origin of the introduced populations

The introduced population of *P. muralis* in Nörten-Hardenberg belongs to the Western France mtDNA clade (fig. 3). This lineage differs substantially from seven other introduced *P. muralis* lineages found in Central Europe (Schulte et al., 2012), with an average p-distance of 0.049 to its sister clade (Eastern France clade). Three of four individuals shared one haplotype, while the fourth individual had a very similar haplotype (p-distance of 0.002). These haplotypes were most similar to haplotypes found in Andorra and Benasque (Carranza, Arnold and Amat, 2004; Busack, Lawson and Arjo, 2005) and differed substantially from another introduced population of this lineage in Germany (Mainz), which originated from the Atlantic coast of southern France. Therefore, the *P. muralis* population in Nörten-Hardenberg most probably originated from a region in the eastern Pyrenees. Six haplotypes that differed in two substitutions were found among the introduced *P. liolepis* individuals. These haplotypes confirmed an affiliation to the subspecies *P. l. liolepis* (Boulenger, 1905), which occurs at the north-eastern coast of Spain, in the Central and East Pyrenees as well as in departments Pyrenées-Orientales, parts of Aude and occasionally in Haute-Garonne (Geniez and Deso, 2009). In the phylogenetic tree the haplotypes from the introduced population form a strongly supported group with the haplotype from Planoles in the province of Girona (fig. 3, p-distance: 0.01). The haplotypes of *P. liolepis* from the native population sampled in France (Labeaume) were rather different from the introduced clade (p-distance: 0.038) and confirmed an affiliation to the subspecies *P. l. cebennensis* (Guillaume and Geniez, 1986), which occurs in south-western France up to the departments Drôme and Vaucluse east of the river Rhone (Geniez et al., 2008). One haplotype from Labeaume represented the Eastern France Clade of *P. muralis* (fig. 3).

Genetic structure

All microsatellite markers proved to be polymorphic for both species. We found evidence for null alleles at locus B3 in all populations and, therefore, excluded this locus from further analyses. There was no evidence for large allele drop-out or other scoring errors. All pairwise tests for linkage disequilibrium were non-significant ($p > 0.05$). The most likely number of genetic clusters (K) among all analysed populations revealed by model-based clustering in STRUCTURE was five (fig. 4). There was no indication for hybridization between both

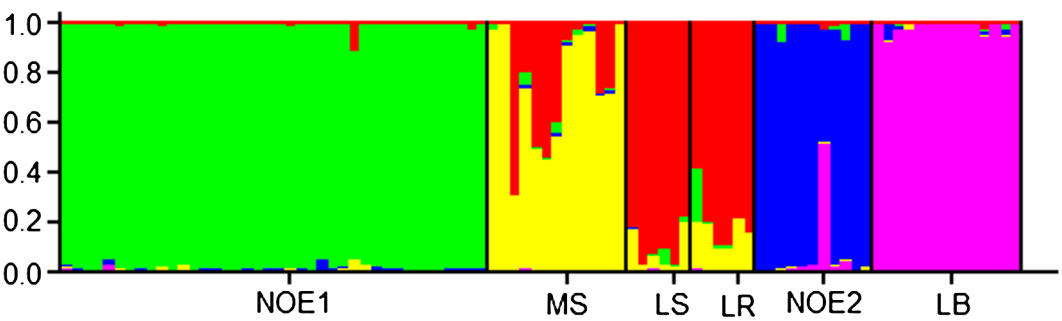

Figure 4. Genetic clusters obtained from the STRUCTURE analysis ($K = 5$) for all 90 samples. Each individual is represented by a single vertical line, divided into K colours. The coloured segment shows the individual's estimated proportion of membership to that genetic cluster. NOE1: *P. muralis*, introduced (Nörten-Hardenberg); MS: *P. muralis*, native (Montségur); LS: *P. muralis*, native (Lourdes); LR: *P. muralis*, native (La Rochelle); NOE2: *P. liolepis*, introduced (Nörten-Hardenberg); LB: *P. liolepis*, native (Labeaume). This figure is published in colour in the online version.

Table 1. Pairwise D_{EST} values (upper right part) and pairwise F_{ST} values (lower left part) between the native and introduced populations of *Podarcis muralis* and *Podarcis liolepis*. Population names: NOE, Nörten-Hardenberg; LB, Labeaume; LR, La Rochelle; LS, Lourdes; MS, Montségur.

	P. muralis (NOE, introduced)	*P. muralis* (LS/LR, native)	*P. muralis* (MS, native)	*P. liolepis* (NOE, introduced)	*P. liolepis* (LB, native)
P. muralis (NOE, introduced)		0.496	0.374	0.797	0.768
P. muralis (LS/LR, native)	0.142		0.131	0.771	0.669
P. muralis (MS, native)	0.098	0.048		0.780	0.680
P. liolepis (NOE, introduced)	0.268	0.268	0.263		0.496
P. liolepis (LB, native)	0.287	0.287	0.276	0.179	

Table 2. Comparison of genetic variability and effective population size (N_E) in introduced and native populations of *Podarcis muralis* and *Podarcis liolepis*; with n = number of samples, N_A = mean number of alleles, A_R = allelic richness, H_O and H_E = observed and expected heterozygosity, F_{IS} = inbreeding coefficient. Population names: NOE, Nörten-Hardenberg; LB, Labeaume; LR, La Rochelle; LS, Lourdes; MS, Montségur.

Species/origin	n	N_E	N_A	A_R	H_O	H_E	F_{IS}
Podarcis muralis (NOE, introduced)	40	89 ± 13.35	9	6.43	0.691	0.685	0.042
Podarcis muralis (LS/LR, native)	12	25 ± 3.4	6.9	6.72	0.695	0.668	−0.042
Podarcis muralis (MS, native)	13	32 ± 2	7.2	6.73	0.708	0.658	−0.081
Podarcis liolepis (NOE, introduced)	11	23 ± 3.69	6.6	6.70	0.564	0.648	0.138
Podarcis liolepis (LB, native)	14	30 ± 2.69	6	5.95	0.621	0.601	−0.029

species at lower numbers of genetic clusters. If a higher K was chosen, likelihood values decreased and new genetic clusters appeared with no individual having a high probability (using a strict threshold value of $q = 0.20$) of belonging to it. A clear separation of the introduced and native *P. liolepis* population as well as between the native and introduced *P. muralis* population was found. This result was confirmed by the AMOVA, which revealed that a significant portion ($p < 0.001$) of the genetic variation was explained by "species" (16%) and "populations" (11%). Differentiation between native and introduced populations was high and only exceeded by differentiation among species (table 1). The lowest D_{EST} and F_{ST} values were found between the two native populations of *P. muralis*.

Genetic diversity between native and introduced populations

Compared to the native populations of *P. muralis*, the introduced population had a lower allelic richness, but rather similar values of H_E and H_O (table 2). On the contrary, the introduced *P. liolepis* population had a higher allelic richness and expected heterozygosity than the native population. Only H_O was higher in the native than in the introduced population. Within the introduced populations, *P. muralis* had higher H_E and H_O values than *P. liolepis* (table 2). Native *P. liolepis* from southern France had the lowest H_E and H_O. The inbreeding coefficient (F_{IS}) was highest in the introduced *P. liolepis* population and lowest in the native *P. muralis* population from Montségur (table 2). Nevertheless, the introduced *P. liolepis* population exhibited a high genetic diversity.

The estimated N_E of the introduced *P. liolepis* was much smaller than that of the introduced *P. muralis* (23 ± 3.69 vs. 89 ± 13.35, table 2). Effective population size of the native *P. liolepis* population in Labeaume was 30 ± 2.69, whereas the native *P. muralis* populations had an estimated N_E of 32 ± 2 (Montségur) and 25 ± 3.4 (cluster Lourdes/La Rochelle, table 2). We found no evidence for a genetic bottleneck (heterozygote excess) in any of the analysed populations of either species. Neither of the introduced populations exhibited significant departures from Hardy-Weinberg equilibrium.

Discussion

Geographical origin of the introduced populations

Our results suggest that both non-native wall lizard species stem from a region in the eastern Pyrenees, where the native ranges of both species overlap (see fig. 2) and syntopic populations of *P. liolepis* and *P. muralis* are frequent (Geniez and Deso, 2009). Although the temporal course of introductions remains unknown, we hypothesize that both populations were introduced simultaneously, as it is rather unlikely that they have been transported two times independently from the same area to exactly the same locality in Germany. This represents the first record of the Catalonian wall lizard (*Podarcis liolepis*) as a non-native species in Germany. The pathway of the introduction remains unclear, but an intended introduction is most likely as more than 73% of all known introduced populations in Germany can be traced back to human-mediated introductions (Schulte et al., 2008, 2011). Based upon the information of local residents, the introduction took place at least in the 1980s.

Genetic structure and diversity within the native and invasive range

Even though the native *P. liolepis* population was not the source population of the introduced population in Nörten-Hardenberg and more populations need to be analysed for further comparisons, we compare both populations regarding their genetic diversity. The high allelic richness of the introduced *P. liolepis* population might be caused by its origin in the centre of the species' distribution (eastern Pyrenees), while the native *P. liolepis* population analysed occurs at the northern edge of the species' range in the department Ardéche in France (fig. 2). A reduced genetic diversity at a species' northern range margin is rather typical due to smaller population sizes, partial isolation, stronger genetic drift and higher selection pressure (Hewitt, 2001; Böhme et al., 2007). The effective population size of *P. liolepis* was rather small, while N_E in the introduced *P. muralis* population even exceeded the values found in the native populations. This might have been caused either by different founder numbers, different time of introductions or by an initial decrease in population size in the introduced *P. liolepis* population.

Our observation of a reduced allelic richness, but similar heterozygosity in the introduced *P. muralis* population compared to the native populations from Western France is in line with the expectation that allelic richness is more strongly affected by genetic drift than heterozygosity (Amos and Balmford, 2001). Compared to the available literature on genetic diversity within native *P. muralis* populations in Central Europe (Gassert, 2005; Altherr, 2007), heterozygosity and allelic richness of the native and introduced *P. muralis* populations were rather high. The Montségur population is located in the south-western part of the range, where Pleistocene glacial refugia may have existed. This might explain, why the population has conserved a higher genetic diversity than populations further north, such as in Switzerland (Altherr, 2007; Blondel and Aronson, 2010). The high genetic diversity of the in-

troduced population might also be influenced by its origin from a hotspot of genetic diversity. Compared to an introduced population in Cincinnati, Ohio (Lescano, 2010) and other introduced populations in Germany (Schulte et al., unpublished data), originating from northern Italy (a hotspot of genetic diversity for *P. muralis*) the genetic diversity of the introduced *P. muralis* population in Nörten-Hardenberg was much higher. We thus hypothesize that propagule pressure of both species must have been quite high, since no sign for a recent bottleneck was detected within the introduced populations. Indeed, introductions of numerous individuals might occur frequently among hobby herpetologists, as a high propagule size has for example been reported from a population in Linz (Austria, 130 introduced individuals; Schulte, 2008). It is possible that the high genetic diversity of both non-native populations has facilitated their establishment success. However, in Cincinnati *P. muralis* appears to be a successful colonizer despite originating from a small number of only twelve founders and multiple bottlenecks (Lescano, 2010). Inbreeding and a loss of genetic diversity, therefore, do not necessarily hamper the successful establishment and spread of introduced species (Lindholm et al., 2005; Schmid-Hempel et al., 2007; Ficetola, Bonin and Miaud, 2008).

Although Pinho, Harris and Ferrand (2008) suggested that *Podarcis* species take a long time of divergence to acquire complete reproductive isolation and detected gene flow between *P. muralis* and *P. liolepis*, we did not find evidence for hybridization among the introduced populations. In contrast, we observed occasionally aggressive and territorial interactions of both sexes of *P. muralis* towards *P. liolepis*, with matings occurring exclusively among conspecifics. Furthermore, we observed a microhabitat segregation between both species (*P. muralis*: widely distributed even within the moister talus, *P. liolepis*: restricted to vertical structures in rocky habitats with crevices), which is known from sympatric populations throughout the range (Salvador, 1986; Castilla and Bauwens, 1991; Martín-Vallejo et al., 1995; Carretero, Marcos and de Prado, 2006). In a recent study, Gabirot et al. (2010) suggest that chemical cues may reduce the occurrence of hybridization even between the genetically more closely related species *P. liolepis* from Columbretes islands and *P. hispanicus* (morphotypes 1 or 2) from Madrid. Hence, olfactory traits might also act as premating barriers between *P. muralis* and *P. liolepis* and it is likely that premating barriers are well developed considering the divergence times and overlapping distribution of both species (fig. 2).

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Appendix. *Podarcis muralis* and *Podarcis liolepis* populations sampled and used from GenBank with information on sample ID, species and clade affiliation, sampling locality, GenBank accession numbers and references.

Sample ID	Species (clade affiliation)	Sampling locality	GenBank accession number	Reference
NOE1	*P. muralis* (Western France Clade)	Nörten-Hardenberg	HQ652969	Schulte et al., 2012
NOE3	*P. muralis* (Western France Clade)	Nörten-Hardenberg	HQ652966	Schulte et al., 2012
NOE4	*P. muralis* (Western France Clade)	Nörten-Hardenberg	JQ403287	This study
NOE38	*P. muralis* (Western France Clade)	Nörten-Hardenberg	JQ403288	This study
LB169	*P. muralis* (Eastern France Clade)	Labeaume, France	JQ403289	This study
MS3	*P. muralis* (Western France Clade)	Montségur, France	JQ403290	This study
LS6	*P. muralis* (Western France Clade)	Lourdes, France	JQ403291	This study
LRo1	*P. muralis* (Western France Clade)	La Rochelle, France	JQ403292	This study
StM1	*P. muralis* (Western France Clade)	St. Malo, France	JQ403293	This study
Amb2	*P. muralis* (Western France Clade)	Amboise, France	JQ403294	This study
AY151908	*P. muralis* (Western France Clade)	Andorra	AY151908	Carranza et al., 2004
AY234155	*P. muralis* (Western France Clade)	Benasque, Spain	AY234155	Busack et al., 2005
BR1	*P. muralis* (Salps Clade)	Bramsche, Germany	HQ652960	Schulte et al., 2012
UU54	*P. muralis* (Salps Clade)	Bramsche, Germany	HQ652944	Schulte et al., 2012
HAN1	*P. muralis* (Central Balkan Clade)	Halle a. d. Saale, Germany	HQ652958	Schulte et al., 2012
UU89	*P. muralis* (Central Balkan Clade)	Altenhain, Germany	HQ652886	Schulte et al., 2012
UU60	*P. muralis* (Eastern France Clade)	Duisburg-Hüttenheim, Germany	HQ652880	Schulte et al., 2012
BOT2	*P. muralis* (Eastern France Clade)	Bottrop, Germany	HQ652955	Schulte et al., 2012
UU134	*P. muralis* (Eastern France Languedoc subclade)	Germany	HQ652908	Schulte et al., 2012
UU67	*P. muralis* (Western France Clade)	Mainz, Germany	HQ652893	Schulte et al., 2012
UU70	*P. muralis* (Western France Clade)	Mainz, Germany	HQ652894	Schulte et al., 2012
UU75	*P. muralis* (Western France Clade)	Mainz, Germany	HQ652896	Schulte et al., 2012
BA18	*P. muralis* (Venetian Clade)	Klosterneuburg, Austria	HQ652943	Schulte et al., 2012
SD1	*P. muralis* (Tuscany Clade)	Schärding, Austria	HQ652937	Schulte et al., 2012
NOE2	*P. liolepis*	Nörten-Hardenberg	HQ652946	Schulte et al., 2012
NOE11	*P. liolepis*	Nörten-Hardenberg	JQ403295	This study
NOE19	*P. liolepis*	Nörten-Hardenberg	JQ403296	This study
NOE24	*P. liolepis*	Nörten-Hardenberg	JQ403297	This study
NOE36	*P. liolepis*	Nörten-Hardenberg	JQ403298	This study
NOE37	*P. liolepis*	Nörten-Hardenberg	JQ403299	This study
Planoles	*P. liolepis*	Planoles, Spain	JQ403300	This study
LB165	*P. liolepis*	Labeaume, France	JQ403301	This study
LB166	*P. liolepis*	Labeaume, France	JQ403302	This study
LB167	*P. liolepis*	Labeaume, France	JQ403303	This study
LB168	*P. liolepis*	Labeaume, France	JQ403304	This study
AF469432	*P. liolepis*	Barcelona, Spain	AF469432	Harris and Sá-Sousa, 2002
AF469434	*P. liolepis*	Barcelona, Spain	AF469434	Harris and Sá-Sousa, 2002
AF469436	*P. liolepis*	Medinaceli, Spain	AF469436	Harris and Sá-Sousa, 2002
AF469438	*P. liolepis*	Tarragona, Spain	AF469438	Harris and Sá-Sousa, 2002
AF469440	*P. liolepis*	Girona, Spain	AF469440	Harris and Sá-Sousa, 2002
AF469442	*P. liolepis*	Pyrenees, Spain	AF469442	Harris and Sá-Sousa, 2002
DQ081144	*P. liolepis*	Burgos, Spain	DQ081144	Pinho et al., 2006
AF052635	*P. hispanicus* sensu stricto	Valencia, Spain	AF052635	Castilla et al., 1998
AF052633	*P. vaucheri*	Atlas, Maroc	AF052633	Castilla et al., 1998
FJ867396	*P. siculus* (outgroup)	Italy	FJ867396	Giovannotti et al., 2010