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Rapid genetic assimilation of native wall lizard populations (*Podarcis muralis*) through extensive hybridization with introduced lineages

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Abstract

The Common Wall Lizard (*Podarcis muralis*) has established more than 150 non-native populations in Central Europe, stemming from eight geographically distinct evolutionary lineages. While the majority of these introduced populations are found outside the native range, some of these populations also exist at the northern range margin in southwestern Germany. To (i) infer the level of hybridization in contact zones of alien and native lineages; and (ii) compare the genetic diversity among purebred introduced, native and hybrid populations, we used a combination of maternally inherited markers (mtDNA: cyt*b*) and Mendelian markers (microsatellites). Our results suggest a rapid genetic assimilation of native populations by strong introgression from introduced lineages. Discordant patterns of mtDNA and nDNA variation within hybrid populations may be explained by directed mate choice of females towards males of alien lineages. In contrast to previous studies, we found a nonlinear relationship between genetic diversity and admixture level. The genetic diversity of hybrid populations was substantially higher than in introduced and native populations belonging to a single lineage, but rapidly reaching a plateau of high genetic diversity at an admixture level of two. However, even introduced populations with low founder sizes and from one source population retained moderate levels of genetic diversity and no evidence for a genetic bottleneck was found. The extent of introgression and the dominance of alien haplotypes in mixed populations indicate that introductions of non-native lineages represent a serious threat to the genetic integrity of native populations due to the rapid creation of hybrid swarms.

Keywords: gene pool swamping, hybrid swarm, introgression, invasive species, microsatellites, mtDNA

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Introduction

The displacement of native biota by invasive taxa is a serious threat to biodiversity (Williamson 1997; Primack 2006). One important mechanism behind such displacement processes is reproductive interference, including hybridization (Rhymer & Simberloff 1996; Lee 2002; Gröning & Hochkirch 2008). For the native population, hybridization can have variable consequences ranging from negative fitness effects, such as the loss of locally adapted alleles, outbreeding depression and displacement by gene pool swamping (Arntzen & Thorpe 1999; Vorburger & Reyer 2003; Schmeller *et al.* 2005, Hochkirch & Lemke 2011; Sacks *et al.* 2011), to positive effects caused by hybrid vigour (Drake 2006; Fitzpatrick & Shaffer 2007; Pfennig 2007). While interspecific hybridization is often recognized as a threat to biota, interbreeding among subspecies or evolutionary lineages is less often seen as a threat to native species (Meyerson *et al.* 2010). Augmentation has even been successfully used to diminish negative effects from inbreeding in conservation management (e.g. Johnson *et al.* 2010).

However, in the context of biological invasions, both positive and negative fitness effects from hybridization

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are problematic. Positive fitness effects may enhance the invasiveness in terms of adaptive divergence through a creation of novel genotypes (Ellstrand & Schiernbeck 2000; Kolbe *et al.* 2004; Wolfe *et al.* 2007), whereas negative fitness effects may lead to outbreeding depression and thus threaten the native population (Huff *et al.* 2011). Furthermore, the displacement of the native gene pool by non-natives may lead to a loss of local adaptations and disruption of co-adapted gene-complexes (Allendorf *et al.* 2001). The displacement of the native gene pool ('gene pool swamping') is one of the most detrimental effects of hybridization (Avise *et al.* 1997; Riley *et al.* 2003; Hall *et al.* 2006). It is driven by asymmetric hybridization, which is caused by differences in population sizes or selective advantages of invaders, leading to differences in reproductive success (Wirtz 1999; Gröning & Hochkirch 2008). Hybridization also causes serious problems in conservation practice as it is complicated to develop useful management strategies for hybrids of endangered species (Allendorf *et al.* 2001). This is even more problematic in intraspecific hybridization events, as legislation is usually not differentiating between conspecific native and introduced evolutionary lineages (Schulte *et al.* 2011a).

The Common Wall Lizard (*Podarcis muralis*) has successfully colonized regions in northwestern Europe far outside its sub-Mediterranean native range. More than 150 self-sustaining populations have emerged mainly from intended introductions (Schulte 2008). Introduced populations in Central Europe have been assigned to eight geographically distinct evolutionary lineages (Schulte *et al.* 2012a): (i) Western France clade (native range: W France and parts of the Pyrenees); (ii) Eastern France clade (SE France to W Germany and S Netherlands); (iii) Southern Alps clade (NW Italy, S Alps and Inn valley); (iv) Venetian clade (NE Italy to NW Croatia); (v) Tuscany clade (Tuscany to N Campania); (vi) Romagna clade (NE Apennine); (vii) Marche clade (C Italy and W Istria); and (viii) Central Balkans clade (Balkan Peninsula to NE Austria). In addition to the introductions outside the native range, there is also an increasing detection of introduced populations at the northern range margin of the species. The high phenotypic variability of this species (Bellati *et al.* 2011) often hampers the detection of such introductions within the native range and makes it nearly impossible to detect hybridization based on a morphological basis.

The wall lizard represents an excellent model species for the study of genetic consequences of biological invasions. Due to the distribution pattern of the wall lizard in Central Europe, three major population types can be compared: (i) Purebred native populations at the northern range margin; (ii) purebred introduced populations outside the native range stemming from different source regions; and (iii) mixed populations between native and introduced wall lizards. This situation allows assessing the extent of intraspecific hybridization in mixed populations. Therefore, we first used a mtDNA marker (cyt*b*) to infer the geographic origin of introduced lineages in purebred introduced populations and their frequency in mixed populations. Second, we analysed the degree of differentiation among and within populations with different invasion histories (using 13 microsatellite loci) to test the hypothesis that populations stemming from similar source regions are less differentiated than those from different regions. Third, we wanted to examine whether admixture between non-native and native lineages occurs in mixed population. Finally, we tested the assumption that genetic diversity of introduced populations increases with the degree of admixture (Kolbe *et al.* 2008).

Methods

Sampling

A total of 566 lizards were captured by hand or by noosing from 10 populations in Germany (Fig. 1): (I) Mixed populations (=with both native and non-native mtDNA lineages): Freiburg Dreisam (FRD, $n = 52$), Freiburg Messe (FRM, $n = 22$), Lörrach/Inzlingen (LÖR, INZ, WÖL, $n = 85$), Mannheim (MAN, $n = 49$). (II) Purebred introduced populations (=with only one non-native mtDNA lineage): Bramsche (BRA, $n = 60$), Nörten-Hardenberg (NÖR, $n = 40$), Dresden (DRE,

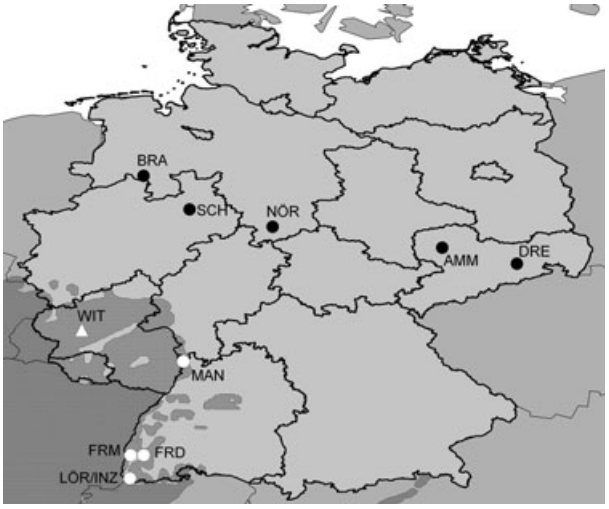

Fig. 1 Distribution of populations analysed in this study. White dots represent mixed populations, black dots represent purebred introduced populations and the white triangle corresponds to the native reference population (WIT). The dark shaded area in the southwest shows the natural range margin of *P. muralis*.

n = 63), Schloß Holte-Stukenbrock (SCH, n = 64), Ammelshain (AMM, n = 81), (III) Purebred native population (= with only native mtDNA haplotypes): Wittlich (WIT, n = 50). For all mixed populations, the occurrence of native Wall Lizard populations has been documented since the 19th century (Dürigen 1897; Table 1) and the introduction of alien individuals had been proven in a previous study except for FRM (Schulte *et al.* 2011a). The times of the first records of native and introduced wall lizards at the study sites are documented in Table 1. The population FRM was monitored in 2000 without any observations or suspicion of alien individuals (K Fritz & H Laufer, pers. comm. 2011) and was originally included in this study as a reference for native populations of *P. muralis*. However, this population turned out to be a mixed population as well (see results).

We collected DNA by noninvasively buccal swabbing each specimen using a diagnostic fine-tip dry swab (Medical Wire & Equipment, MW-100) (Schulte *et al.* 2011b). Samples were stored in sterile tubes at −20 °C until DNA extraction. DNA was extracted using the Qiagen DNEasy blood and tissue kit following the manufacturer's protocol (replacing ATL buffer by 400 μL PBS buffer as recommended in the supplementary protocol).

Assignment of geographic origin

Sequence data were collected for all 208 specimens sampled in the four mixed populations as well as for some specimens in the purebred introduced and native populations (n = 22). For amplifications of cytochrome *b* fragments, we used 50-μl reaction tubes containing: 27 μL purified water, 20 μL of HotStarTaq Master Mix (Qiagen Hotstar, including 0.4 U Taq polymerase, 90 mM KCl, 5 mM Mg^{2+}, 400 μM of each dNTP), 0.0625 pmol/μL of each primer and 2–10 ng 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-bp mtDNA fragment (cyt*b*) using the primers LGlulk (5′-AACCGCCTGTTGTC TTCAACTA-3′) and HPod (3′-GGTGGAATGGGATT TTGTCTG-5′) (Podnar *et al.* 2007; Schulte *et al.* 2012a). The PCR product was purified using the High pure PCR product purification kit (Roche) according to the manufacturers' protocol. Sequencing reactions were performed using the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich) and run on a MEGABACE 1000 automated sequencer. We corrected and aligned the sequences by eye. Ambiguous data from the beginnings and ends of the fragments were not included in the analyses. All sequences were deposited in GenBank under the accession numbers (JX065611–JX065629). For lineage assignment, the sequences were aligned with sequences from individuals with known origin (AY234155, Busack *et al.* 2005; DQ001023, DQ001024, DQ001028, DQ001029, DQ001032, Podnar *et al.* 2007; FJ867393, FJ867389, Giovannotti *et al.* 2010; HQ652963, HQ652952 (FRD); HQ652920, HQ652921 (LÖR); HQ652918, HQ652919 (INZ); HQ652960, HQ652874 (BRA); HQ652966, HQ652969 (NÖR); HQ652884 (DRE); HQ652905 (MAN); HQ652876, HQ652973 (SCH); HQ652885, HQ652886, HQ652887 (AMM), HQ652901, Schulte *et al.* 2012a,b,c; Schweiger *et al.* unpublished data) and fitted into a phylogenetic tree using *P. siculus* and *P. melisellensis* as outgroups (HQ154646, AY185097, Podnar *et al.* 2004). We used Bayesian inference to infer a phylogeny as implemented in MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003), applying the parameters of the substitution model (GTR+I+G) suggested by MrModeltest 2.2 (Nylander 2004). We ran the Monte Carlo Markov chain for two million generations, sampling every 2000 generations. We discarded 500 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 & Huelsenbeck 2003). This approach allowed us to assign introduced haplotypes to intraspecific evolutionary lineages of *P. muralis* and their respective geographic range (see also Schulte *et al.* 2012a). We used TCS 1.21 (Clement *et al.* 2000) and DnaSP 5 (Librado & Rozas 2009) to obtain haplotype frequencies.

Genotyping

All 566 individuals were genotyped at 13 microsatellite loci, seven of which were developed for *Podarcis muralis* (A7, B3, B4, B6, B7, C8, C9; Nembrini & Opplinger 2003), three for *Zootoca vivipara* (Lv-319, 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 Qiagen Multiplex Mastermix or 5PRIME HotMasterMix. We used multiplexed PCR protocols for a combination of three or two loci with variable annealing temperatures (C9/B4/Pb73: 57 °C; B3/Pb10/Lv319: 56 °C; Lv472/Pb50: 53 °C; A7/Lv4alpha: 60 °C A7/B7: 60 °C). Multiplex PCRs were performed in 10 μL reaction mix containing: 2–10 ng genomic DNA, 5.5 μL MultiplexMasterMix, 2.0 μL water and 0.1 μM of each primer. PCR conditions were used as recommended by the manufacturer. For primers C8 and B6, we used singleplex PCRs in a 5 μL reaction mix containing: 2–10 ng genomic DNA, 2.2 μL 5Prime MasterMix, 2.2 μL water and 0.0625 pmol of the forward and reverse primers at the locus-specific annealing temperature of

Table 1 Native and non-native records of wall lizards within sampled populations (with information of number of founders and source region in some cases). Origin and genetic variability among mixed, purebred introduced and purebred native populations of *Podarcis muralis*; with mtDNA lineage frequency; Hapl, recorded haplotypes; *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 (*significant departure from HWE); Bottleneck: *P* values of the test for genetic bottlenecks using the TPM mutation model

Population	First native record	First non-native record	Origin (mtDNA lineage)	*Hapl.*	*n*	n_a	A_r	H_o	H_e	F_{IS}	Bottleneck
Mixed populations											
FRD	Dürigen (1897)	>1960 (Laufer *et al.* 2007; Fritz pers. comm.)	Southern Alps (81%) Tuscany (17%) Eastern France (2%)	SA3/6 TU1 EF3	52	8.00	5.40	0.709	0.740	0.051	0.12
FRM	Dürigen (1897)	<2010 (this study)	Tuscany (59%) Eastern France (36%) Southern Alps (5%)	TU1/2 EF3 SA6	22	7.00	5.59	0.689	0.733	0.084	0.23
INZ	Dürigen (1897)	<1998 (Deichsel pers. comm.)	Romagna (72%) Venetian (21%) Southern Alps (7%)	RO2 VE1 SA5/6	14	6.62	5.82	0.708	0.718	0.052	0.27
LÖR	Dürigen (1897)	>1998 (Schulte *et al.* 2011a)	Southern Alps (78%) Romagna (22%)	SA2 RO1/2/3	9	6.31	6.00	0.761	0.727	0.013	0.75
WÖL	Dürigen (1897)	>1998 (Schulte *et al.* 2011a)	Eastern France (95%) Southern Alps (5%)	EF2/3 SA2	62	10.46	6.16	0.657	0.735	0.114*	0.88
MAN	Dürigen (1897)	<2006 (Schulte 2008)	Venetian (86%) Southern Alps (14%)	VE1 SA4	49	8.39	5.55	0.626	0.697	0.113*	0.71
Purebred introduced populations											
BRA	—	1982 (16 founders from Lago Maggiore) (Schulte *et al.* 2011a)	Southern Alps	SA1	60	6.92	4.18	0.533	0.577	0.085*	0.83
NÖR	—	>1980 (from E Pyrenees) (Schulte *et al.* 2012b)	Western France	WF1/2	40	9.08	6.15	0.641	0.678	0.067	0.14
DRE	—	<1900 (from Bologna-Modena region) (Schulte 2008)	Venetian	VE1	63	5.23	3.69	0.562	0.607	0.082	0.19
SCH	—	1964 (10 founders) (Hallau pers. comm..)	Eastern France	EF3/4	64	6.15	3.95	0.572	0.596	0.047	0.42
AMM	—	>1980 (from Hungary) (Richter 1994; Schulte *et al.* 2011a)	Central Balkans	CB1/2	81	7.92	4.37	0.499	0.587	0.157*	0.66
Purebred native population											
WIT	Dürigen (1897)	—	Eastern France	EF1	50	5.46	4.00	0.541	0.584	0.083	0.04*

57 °C. The 5′-end of each forward primer was labelled with a fluorescent dye, either FAM, TAMRA or HEX. PCR products were run on an MEGABACE 1000 automated sequencer. Fragment lengths were determined using Fragment Profiler 1.2 (Amersham Biosciences).

Population admixture analysis and descriptive statistics

As null alleles are often affecting microsatellite analyses, we tested our data in Micro-Checker 2.2.3 (van Oosterhout *et al.* 2004) for the occurrence of null alleles. We used Fstat 2.9.3.2 to test for linkage disequilibria among loci (Goudet 2001), including also a test for linkage disequilibrium among mtDNA lineages and microsatellite genotypes. STRUCTURE 2.3.3 (Pritchard *et al.* 2000) was used to analyse for genetic structuring within and among populations. The admixture model was used, because it is more powerful in detecting potential hybridization. The admixture proportion of each individual Q, as an estimate of an individual's proportion of ancestry from each of the clusters, was obtained by STRUCTURE to separate hybrids from purebred parental individuals within populations (Vähä & Primmer 2006). We chose a conservative threshold value of $Q = 0.20$–0.80 for hybrid detection, because values outside this range tended to detect hybrids even in purebred populations (see also Randi 2008; Sacks *et al.* 2011).

Within STRUCTURE, 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 $K = 1$–15 with 10 iterations per K. To find the optimal K value, we calculated the second-order rate of change (ΔK) as suggested by Evanno *et al.* (2005) using the CorrSieve package for R 2.13 (Campana *et al.* 2011). As the highest ΔK (at $K = 12$) suggested a finer population substructure in LÖR/INZ, we divided this population into three geographically defined subpopulations: LÖR, WÖL and INZ. As our sampling consisted of several levels of differentiation (distantly and closely related lineages, populations, hybrids within populations), we expected that ΔK would tend to find an optimal K between populations, but might fail to detect hybrids within populations, which might be part of such Hardy–Weinberg populations during a late stage of admixture. Therefore, we ran the analyses until the Q values for the next cluster dropped below 0.9 in all individuals ($K = 15$). We also performed STRUCTURE runs independently for single hybrid populations. The pattern of within-population structure of these runs for single populations remained identical compared with the complete data set at $K = 14$.

In our special case, different timescales (evolutionary lineages/populations) might play a role for population differentiation, making the choice of an ideal measure of differentiation difficult. R_{ST} (Slatkin 1991) might be an appropriate measure for the highly divergent evolutionary lineages in different non-native populations, as these lineages might have accumulated a high number of stepwise mutations during evolution. In contrast, F_{ST} might be more appropriate for population processes that have already reached Hardy–Weinberg equilibrium (HWE) (Balloux & Lugon-Moulin 2002). On the other hand, the use of F_{ST} as a measure of population differentiation has recently been strongly criticized (e.g. Jost 2008; Gerlach *et al.* 2010). However, a recent analysis showed that F_{ST} performs well under certain conditions (Meirmans & Hedrick 2011). We, therefore, calculated in addition to F_{ST} also R_{ST} using GenAlEx 6.4 (updated from Peakall & Smouse 2006) and D_{EST} using the DEMEtics package for R (Gerlach *et al.* 2010). We ran a F_{ST}-based and a R_{ST}-based AMOVA with 9999 iterations in GenAlEx using the genetic clusters inferred according to the maximum ΔK in STRUCTURE.

We used Fstat to calculate the number of alleles (n_a), allelic richness (A_r) and the inbreeding coefficient (F_{IS}). Expected and observed heterozygosities (H_E and H_O) for each locus and population as well as deviations from HWE were calculated in GenAlEx. We calculated an ANOVA in R 2.14.0 to test for significant differences in H_E between different admixture levels (i.e. number of lineages). In order to find an optimal function to describe the relationship between within-population genetic diversity (H_E) and the number of source populations (mtDNA clades), we used a curve fitting approach in Lab Fit 7.2.47 (Silva & Silva 2009).

To detect recent bottlenecks within introduced populations, the program BOTTLENECK 1.2.02 was used with allele frequency data from a single temporal sample (Cornuet & Luikart 1996). Recent bottlenecks (0.2–4 N_E generations) can create a heterozygosity excess compared with populations at mutation-drift equilibrium, because rare alleles that have little impact on heterozygosity can be lost quickly. We calculated H_{EQ} (expected heterozygosity corrected for sample size) using the two-phase mutation model (TPM, Di Rienzo *et al.* 1994), as this is the most likely mutation model for microsatellites (Piry *et al.* 1999). Statistical significance was assessed with a one-tailed Wilcoxon test, because this test proved to be the best for microsatellite data with fewer than 20 loci (Piry *et al.* 1999). Analyses were performed with 1000 iterations.

Results

Haplotype diversity (mtDNA)

In total, we found 20 haplotypes belonging to seven different evolutionary lineages of *P. muralis* in our sample

(Table 1, Fig. 2). The posterior probabilities of the lineages were high (≥ 99) and only some internodes had a lower support. TCS obtained five nonconnected haplotype networks with a maximum of six different haplotypes (Appendix S1, Supporting information). Although all mixed populations in southwestern Germany were located in the native range of the Eastern France clade, this lineage was completely missing in three populations (INZ, LÖR and MAN) and only one native haplotype was found in the FRD population. Even the FRM population, which was initially sampled as a native reference population turned out to contain only a small fraction of identical Eastern France haplotypes as in FRD (36%). Only in the WÖL population, the native Eastern France haplotype dominated (95%). In INZ, LÖR and MAN, we found exclusively mtDNA lineages stemming from Italy (Table 1). Haplotype sharing among populations was relatively low, except for some adjacent populations (Appendix S1, Supporting information) and the Venetian haplotype, which was found

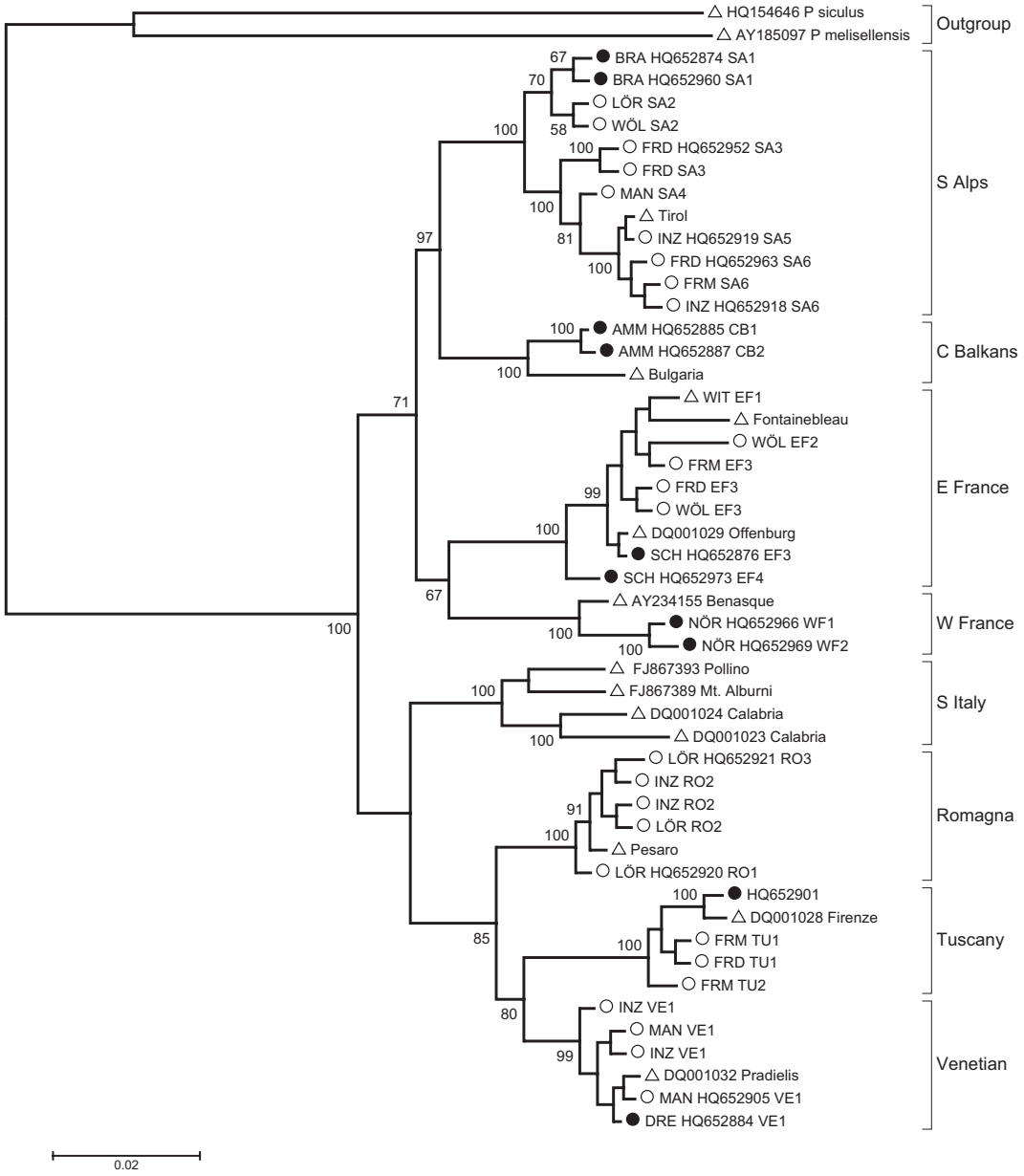

Fig. 2 Bayesian consensus tree for the mitochondrial cyt*b* gene for *Podarcis muralis*. Numbers are posterior probabilities. Filled circles represent samples from purebred introduced populations, open circles represent samples from mixed populations, open triangles represent samples from native wall lizard populations. Mixed populations = FRD, FRM, INZ, LÖR, WÖL, MAN; purebred introduced populations = BRA, NÖR, DRE, SCH, AMM; purebred native population = WIT. Haplotype abbreviations are given in Table 1.

in DRE, MAN and INZ. The highest non-native haplotype diversity was found for the Southern Alps lineage (six different haplotypes).

Genetic structure and differentiation

Within the populations INZ and LÖR, we found no evidence for null alleles, whereas the other populations showed evidence for null alleles at 1–4 loci. However, no locus showed evidence for null alleles across all populations and nearly all Oosterhout values were below 0.2. Furthermore, in introduced populations, deviations from HWE may be caused by small founder sizes, increasing the rate of inbreeding. Hence, we did not exclude any locus from further analyses. All pairwise tests for linkage disequilibrium were nonsignificant ($P > 0.05$). In some loci, allele size ranges seemed to be specific for lineages. The locus A7 had two separate allele size ranges (152–200 and 390–412). The longer lengths were only found in populations with founders of the Venetian, Romagna and Tuscany clades. In locus B4, allele sizes >135 were only found in populations with founders belonging to the Southern Alps clade. For locus C9, allele sizes >190 were only found in the NÖR population (Western France origin).

The most likely number of genetic clusters (K) among all analysed populations revealed by model-based clustering in STRUCTURE applying the method of Evanno *et al.* (2005) was 12 (Fig. 3). In contrast to our initial sampling, the LÖR population was geographically separated into three clusters: LÖR, WÖL and INZ. A stepwise increase in K revealed the differentiation of nuclear DNA variation among populations and enabled us to identify lineage-specific genotypes regardless of the haplotype frequencies (see Appendix S2, Supporting information for $K = 1$–14). At $K > 12$ intrapopulation genetic structure occurred, probably caused by hybridization (Fig. 3). From $K = 1$–11, the native population (WIT) always clustered together with the mixed population WÖL, which was predominantly composed of native mtDNA haplotypes (95%). At the maximum ΔK ($K = 12$), a nearly complete separation of all populations was found.

The strong differentiation among all populations was confirmed by the AMOVA, which revealed that a significant portion ($P < 0.001$) of the genetic variation occurred among populations (24% for F_{ST}-based, 28% for R_{ST}-based AMOVA). Levels of differentiation between all populations were high and significant, with F_{ST} ranging from 0.113 to 0.364 (Table 2), R_{ST} ranging from 0 to 0.588 and D_{EST} ranging from 0.366 to 0.819 (Table 3). For all three measures the lowest differentiation between the native Wittlich and a non-native population was found for the mixed WÖL population

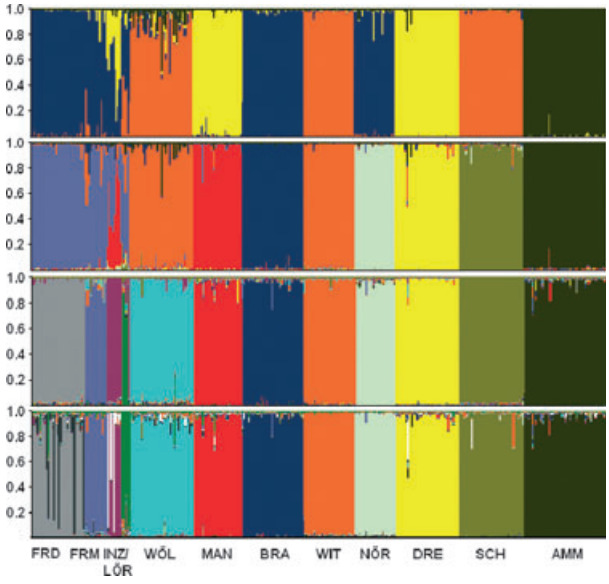

Fig. 3 Genetic clusters obtained from the STRUCTURE analysis for all 566 samples ($K = 4$, 8, 12 and 14). The optimal K according to ΔK was found at $K = 12$. Each individual is represented by a single vertical line, divided into K colours. The coloured segment shows the individual's estimated proportion of membership to the genetic cluster. Mixed populations = FRD, FRM, INZ, LÖR, WÖL, MAN; purebred introduced populations = BRA, NÖR, DRE, SCH, AMM; purebred native population = WIT.

(F_{ST} = 0.163, R_{ST} = 0.03, D_{EST} = 0.551) and the SCH population (F_{ST} = 0.168, R_{ST} = 0.011, D_{EST} = 0.399). All these populations were dominated by Eastern France haplotypes (Table 1). The highest differentiation based upon an infinite alleles model was found between the native WIT and the introduced purebred AMM population (F_{ST} = 0.364), which is close to the maximum F_{ST} value possible in this data set (F'_{ST} = 0.88), according to Meirmans & Hedrick (2011). R_{ST} values correlated stronger with the haplotype data, with the lowest values found between populations with similar mtDNA lineage composition (e.g. FRD/FRM: R_{ST} = 0.0).

Population-specific levels of hybridization

In the mixed populations, we found multiple mtDNA haplotypes, which were mostly not concordant with the genetic clusters obtained from the STRUCTURE analysis. We found different mtDNA lineages within clearly separated STRUCTURE clusters as well as identical mtDNA haplotypes across different clusters, suggesting that large parts of the mixed populations represent completely admixed hybrid swarms.

The population FRD was composed of three genetic clusters (Fig. 1). The most common cluster was found in 77% ($n = 40$) of the individuals with high Q values

Table 2 Pairwise F_{ST} values between analysed populations of *Podarcis muralis* (all P values < 0.001)

	FRD	FRM	INZ	LÖR	WÖL	MAN	BRA	WIT	NÖR.	DRE	SCH
FRM	0.123										
INZ	0.143	0.140									
LÖR	0.113	0.122	0.126								
WÖL	0.166	0.159	0.178	0.141							
MAN	0.188	0.156	0.125	0.207	0.218						
BRA	0.209	0.174	0.224	0.238	0.251	0.246					
WIT	0.188	0.236	0.257	0.189	0.163	0.273	0.323				
NÖR.	0.186	0.198	0.183	0.196	0.216	0.208	0.288	0.240			
DRE	0.237	0.240	0.195	0.238	0.241	0.186	0.311	0.330	0.284		
SCH	0.229	0.229	0.251	0.198	0.196	0.268	0.327	0.168	0.190	0.315	
AMM	0.267	0.251	0.252	0.277	0.226	0.251	0.307	0.364	0.297	0.272	0.346

Table 3 Pairwise R_{ST} values (lower left part) and D_{EST} values (upper right part) between analysed populations of *Podarcis muralis*

	FRD	FRM	INZ	LÖR	WÖL	MAN	BRA	WIT	NÖR.	DRE	SCH	AMM
FRD		0.708	0.682	0.766	0.456	0.510	0.470	0.589	0.664	0.565	0.470	0.625
FRM	0.000		0.783	0.652	0.718	0.569	0.697	0.726	0.508	0.756	0.754	0.772
INZ	0.030	0.000		0.818	0.638	0.719	0.512	0.555	0.796	0.742	0.366	0.524
LÖR	0.117	0.062	0.001		0.707	0.699	0.779	0.589	0.649	0.704	0.819	0.749
WÖL	0.180	0.103	0.097	0.036		0.569	0.524	0.551	0.576	0.475	0.614	0.725
MAN	0.175	0.228	0.270	0.402	0.465		0.524	0.688	0.488	0.586	0.687	0.614
BRA	0.283	0.264	0.198	0.145	0.078	0.588		0.559	0.733	0.672	0.461	0.641
WIT	0.230	0.175	0.155	0.062	0.030	0.528	0.157		0.729	0.642	0.399	0.690
NÖR.	0.279	0.243	0.238	0.226	0.118	0.546	0.394	0.212		0.656	0.751	0.715
DRE	0.130	0.153	0.187	0.280	0.367	0.001	0.442	0.400	0.430		0.710	0.707
SCH	0.227	0.166	0.142	0.049	0.040	0.501	0.114	0.011	0.140	0.404		0.613
AMM	0.226	0.169	0.100	0.038	0.097	0.480	0.073	0.091	0.250	0.390	0.082	

(>0.8), while only four individuals (8%) were assigned to the second cluster (with $Q > 0.8$) and only low fractions of a third cluster appeared (maximum $Q = 0.36$). Using a threshold Q-value of 0.2, four individuals of the population were assigned as hybrids between these three clusters (8%). Only one hybrid individual carried the native Eastern France mtDNA haplotype, whereas the other hybrids had Southern Alps clade haplotypes. Within the nearby population FRM, 86.4% of the individuals ($n = 19$) belonged to one cluster ($Q > 0.8$). Two of the three individuals with Q values below 0.8 showed admixed genotypes with low fractions of the native cluster found in the population WIT ($Q = 0.12$–0.13). These two individuals carried also native Eastern France mtDNA haplotypes. In the populations INZ, LÖR and WÖL, a total of four genetic clusters occurred. Four individuals were assigned as hybrids between these clusters (4.7%). All other populations were composed of separate genetic clusters, with only three individuals assigned as potential hybrids (0.73%).

Genetic diversity among populations

Purebred introduced and native *P. muralis* populations had a significant lower genetic diversity (expected heterozygosity H_E) than mixed populations originating from two or more lineages (ANOVA, $F_{2,9} = 24.4$, $P < 0.001$, Fig. 4). The increase in H_E was not linear and an admixture of a third lineage had nearly no effect on the genetic diversity. The optimal function to describe this

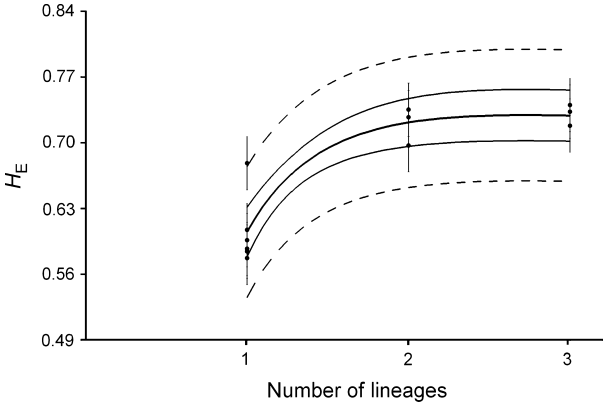

Fig. 4 Correlation between genetic diversity (expected heterozygosity H_E) and number of source lineages. The optimal function to describe the correlation is defined by $H_E = (0.605 \times n \text{ lineages})^{(0.509/n \text{ lineages})}$ ($r^2 = 0.84$). Upper and lower solid lines show 95% confidence bands, upper and lower dashed lines show predict bands.

correlation was a geometric modified model, with $H_E = 0.605 \times n^{(0.509/n)}$, where n is the number of lineages ($r^2 = 0.84$). Allelic richness (A_R), H_O and H_E were lower in the native population and purebred introduced populations with low founder numbers than in mixed populations that consisted of two or three lineages (Table 1). However, the purebred introduced population NÖR, which retained a high genetic diversity, represents an exception. The lowest values for H_E and H_O were found in the purebred introduced populations AMM and BRA as well as in the native population WIT, which is located at the northern range margin in Rhineland-Palatinate. The inbreeding coefficient (F_{IS}) significantly departs from HWE within the mixed populations WÖL ($F_{IS} = 0.114$) and MAN ($F_{IS} = 0.113$) as well as within the purebred introduced populations BRA ($F_{IS} = 0.085$) and AMM ($F_{IS} = 0.157$). The lowest inbreeding coefficient was found in the mixed population LÖR ($F_{IS} = 0.013$) and the purebred introduced population SCH ($F_{IS} = 0.047$; Table 1). Applying the two-phase model (TPM), we found evidence for a genetic bottleneck in the native population ($P = 0.04$, Table 1) but not in the mixed or within the purebred introduced populations.

Discussion

Our results revealed extensive intraspecific hybridization between introduced wall lizard lineages from Italy and native *P. muralis* populations at the northern range margin. In some mixed populations, the mtDNA signal of the native lineage completely disappeared. In FRD, INZ, LÖR and MAN, we found no or only few specimens with native mtDNA haplotypes, while in FRM and WÖL, the native haplotypes were still common, but only one fully admixed genetic cluster (based on microsatellites) was found. The extent of introgression and the dominance of Italian haplotypes in mixed populations indicate that most mixed populations have rapidly reached late stages (nearly complete admixture) of a hybrid swarm (according to Brumfield 2010). Our results confirm the hypothesis that the degree of admixture and the source region influence the genetic diversity of introduced populations (Kolbe *et al.* 2004). Altogether, it is reasonable to state that these introductions represent a serious threat to the genetic integrity of native lineages due to the creation of hybrid swarms.

Genetic population structure

Our results confirm a strong genetic differentiation among all populations, regardless of their origin (mtDNA lineage). Even between the mixed population FRD and the nearby mixed population FRM (distance *c.* 5 km), which consisted of the same mtDNA lineages, a strong genetic differentiation was found. This is probably caused by the different frequencies of the lineages in these populations (Table 1). Due to the strong genetic structuring, we even had to split the mixed Inzlingen-Lörrach population into three geographically separated subpopulations (INZ/LÖR/WÖL). The strong genetic differentiation at the population level also hampered the use of the purebred native population (WIT) as a reference for detecting native genotypes in most of the mixed populations. Nevertheless, the mixed population WÖL (consisting to 95% of native haplotypes) clustered together with the native population until $K = 11$ and some individuals of the mixed populations also showed low fractions of the 'native cluster'. Although the inclusion of reference samples is not needed to detect hybrids (Vähä & Primmer 2006), such reference samples help to assign hybrids to the correct lineage.

The reasons for the high genetic structure among wall lizard populations remain unknown. In the case of introduced lineages, the different colonization histories, origins and admixture levels of the populations are probably major causes for increasing genetic differentiation (Kolbe *et al.* 2008). It is also likely that introduced populations are strongly influenced by genetic drift (including founder events) during establishment as well as during recent range expansion. Indeed, strong genetic structuring has also been found in invasive populations of the gecko *Hemidactylus mabouia* in Florida at very small spatial and temporal scales (Short & Petren 2011) as well as in other wall lizard populations (Cincinnati, Passau) stemming from a single founder event (NV Lescano & K Petren, unpublished data; Schulte unpublished data). Similar patterns can also be found during natural range expansion processes (Hochkirch & Damerau 2009). Therefore, it is possible that rapid genetic structuring due to founder events is a principle pattern of leading edge range extension processes (Hampe & Petit 2005). An additional factor influencing the high genetic structure in wall lizard populations might be found in the species' pronounced territoriality (Boag 1973; Edsman 1990).

Genetic diversity within populations

Compared with the purebred native and purebred introduced populations, the mixed populations exhibited the highest genetic diversity. This positive relationship between genetic diversity and the number of source populations (in our case lineages) in the process of admixture coincides with the pattern found in *Anolis sagrei* in Florida (Kolbe *et al.* 2008). However, our curve fitting approach detected a plateau of high genetic diversity, which was already reached at an admixture level of two. Indeed, a linear relationship between H_E

and the number of lineages is unrealistic as by definition $H_E \leq 1$. The high genetic diversity is probably caused by multiple introductions of founders belonging to four different mtDNA lineages originating from the Apennine Peninsula that interbreed with native populations. In contrast, the analysed purebred native population stems from the northwestern range margin. A reduced genetic diversity at the edge of range expansions is rather typical due to smaller population sizes, partial isolation, stronger founder effects, genetic drift and higher selection pressure (Hampe & Petit 2005; Böhme *et al.* 2007). Compared with native *P. muralis* populations near Basel, Switzerland (Altherr 2007), genetic diversity in the nearby hybrid populations (INZ, LÖR, WÖL) was rather high and might enhance the species invasiveness (Ellstrand & Schierenbeck 2000; Drake 2006). As the introduced founders stem from Italy, where multiple Pleistocene glacial refugia and a hotspot of genetic diversity for this species are found (Giovannotti *et al.* 2010; Bellati *et al.* 2011), these individuals might have further increased the genetic diversity by interbreeding with native populations. Levels of inbreeding were quite low except for the mixed populations WÖL and MAN and for the purebred introduced populations AMM and BRA, the latter of which is known to stem from only 16 founders (Table 1). Nevertheless, we only found a signal for a genetic bottleneck in the purebred native population WIT, but not in any of the purebred introduced populations. It is possible that the bottleneck of population WIT is a consequence of a founder event or stronger population fluctuations at the northern range margin.

In contrast to the mixed populations, four of the five purebred introduced populations had a rather low genetic diversity. However, compared with a purebred introduced population of *P. muralis* in Cincinnati, Ohio, the genetic diversity was higher in the German introduced populations (NV Lescano & K Petren, unpublished data). This was true even for BRA, which has a nearly identical invasion history as the Cincinnati population concerning propagule pressure and origin. This might either be explained by the slightly higher number of founders in BRA ($n = 16$) compared with Cincinnati ($n = 12$), or by a strongly unbalanced reproductive success of the founders in Cincinnati. However, despite the low genetic diversity and multiple bottlenecks in the Cincinnati population, *P. muralis* appears to be a successful colonizer even in North America (NV Lescano & K Petren, unpublished data). Inbreeding and a reduced genetic diversity do therefore not necessarily hamper the successful establishment and spread of introduced species (Schmid-Hempel *et al.* 2007; Ficetola *et al.* 2008).

The presence of different non-native haplotypes from the same lineages in the mixed populations FRD, FRM, INZ and LÖR (Tuscany, Romagna, Southern Alps; Table 1) suggests multiple independent introductions of individuals from Italy. In contrast, the populations INZ, DRE and MAN shared identical haplotypes of the Venetian clade (found in the Bologna-Modena region) with 13 non-native populations in Germany (Schulte *et al.* 2011a). We hypothesize that this may be caused by human-mediated secondary introductions, as the independent introduction of founders from the identical restricted source region in 15 cases seems rather unlikely. On the other hand, multiple introductions from different source populations have been found in 35% of the introduced wall lizard populations in which more than two individuals had been sampled (Schulte *et al.* 2011a).

Discordant patterns of mtDNA and microsatellite variation

Our results confirm several recent studies on squamate reptiles in which the combination of nuclear and mtDNA markers revealed discordant patterns (Renoult *et al.* 2009; Zarza *et al.* 2011). One potential reason for this phenomenon is introgressive hybridization linked to sex-biased dispersal. In wall lizards, juvenile males are considered the major group of dispersers due to a greater pronounced territoriality of males (compared with females) towards their own sex (Barboult & Mou 1988; Schulte 2008). In fact, discordance of mtDNA variation and (nuclear coded) morphology has also been found at the boundaries of the natural ranges of the Tuscany clade of *P. muralis* and its neighbouring clades, suggesting male-biased gene flow (W Mayer, pers. comm., 2011). A second reason for cytonuclear discordance in genetic structure may be found in the different effective population sizes of mitochondria, which are only transferred by the females and only available in one copy. Hence, in diploid organisms, mitochondrial N_E is only one-fourth of the nuclear N_E (Hedrick 2009). In mixed populations, native mtDNA structure might thus erode four times faster than nDNA.

In our case of anthropogenic intraspecific hybridization, two other hypotheses may also explain the incongruence of mtDNA and nDNA variation: (i) directed sexual selection for males stemming from Italy south of the Po river (Venetian clade, Tuscany clade, Romagna clade); or (ii) asymmetric interbreeding success (Wirtz 1999). Although the reasons remain unknown, the first hypothesis would fit well with the pattern observed at the native range boundaries of the Tuscany clade. Males belonging to the Venetian, Tuscany and Romagna clades are larger in size and more colourful (Boag 1973; Schulte 2008). Thus, they might have an advantage in territoriality and mate acquisition. The ventral colour of

wall lizards correlates with the immune response and is an honest signal of fitness and important during mate choice (López & Martín 2005; Sacchi *et al.* 2007; Caalsbeck *et al.* 2010).

Hybridization and its implications for conservation

Problems in distinguishing introduced species or subspecies morphologically from native ones may facilitate introgressive hybridization. As a result, the invader remains cryptic until it is abundant, and its eradication becomes almost impossible. This scenario is plausible for *P. muralis*, a species that exhibits a high phenotypic variability in colour pattern at both the intraspecific and intrapopulation level (Caalsbeck *et al.* 2010; Bellati *et al.* 2011). The only lineages that are relatively easy to distinguish from native wall lizards in Germany by means of their dorsal coloration are the lineages from Central Italy that display partial green dorsal colorations (Schulte *et al.* 2011a). In fact, even for local field-herpetologists, it came as a surprise that so many alien haplotypes were found in the mixed population FRM, which we initially had sampled as a purebred native reference population.

Hybridization between introduced and native lineages of species is known to be a serious threat for the genetic integrity and persistence of native species (Dowling & Childs 1992; Rhymer & Simberloff 1996). Local adaptations may get lost through intraspecific hybridization (Allendorf *et al.* 2001) and result in outbreeding depression (Huff *et al.* 2011). This is particularly important for populations at the range border, because they may have developed even stronger local adaptations to cope with episodes of unfavourable environmental conditions (e.g. wet and cold early summers in *P. muralis*, Strijbosch *et al.* 1980). It is obvious that a removal of hybrids from mixed populations is impossible. Therefore, conservation activities should primarily focus on the prevention of further human-mediated introductions. As the Common Wall Lizard is listed on appendix IV of the EU habitats directive, it is strongly affected by conservation actions. However, as the budget for nature conservation is limited, money should not be wasted in conservation of introduced or mixed populations, even though they belong to the same species and as such should profit from legislation. In cases where compensatory wall lizard translocations are mandatory (as happened in the mixed populations MAN and FRM), genetic analyses will help to avoid the further spread of alien lineages. Rather, it is necessary to focus conservation action on maintaining and expanding the remaining native not hybridized populations in urban environments.

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This study is part of the PhD thesis of U.S., who is interested in evolutionary consequences of biological invasions and conservation biology with a focus on reptiles and amphibians in Central Europe. M.V. is interested in the phylogeography, phylogeny and conservation of European fauna, with special emphasize on amphibians and bats of the Mediterranean region. A.H. is interested in biodiversity research, including evolutionary biology, phylogenetics, ecology, population genetics and conservation biology. He is particularly interested in the ecological significance of species interactions and their importance for nature conservation.

Data accessibility

DNA sequences: NCBI GenBank Accession nos JX065611–JX065629. Information about populations, individual ID's, haplotypes and GenBank Accession nos, microsatellite data and a readme.txt file: doi:10.5061/dryad.t5952

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Minimum spanning network for the cytochrome *b* haplotypes found in sampled wall lizard populations.

Appendix S2 Development of genetic clusters obtained from the STRUCTURE analysis for all 566 samples for $K = 2$ to $K = 14$.

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