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ORIGINAL PAPER

Human introductions create opportunities for intra-specific hybridization in an alien lizard

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Abstract Introduction of individuals from multiple sources could create opportunities for hybridization between previously isolated lineages, which may impact on the invasion process. Identifying the phylogeographic origin of introduced populations is therefore an important task to further test the causes and consequences of human-mediated translocations. The common wall lizard (*Podarcis muralis*) shows a strong phylogeographic structure as a result of past isolation in glacial refugia, but it has also been commonly introduced outside of its native range. Here we analysed 655 base pairs (bp) of the cytochrome *b* sequence from 507 individuals from 23 introduced populations of *P. muralis* in England. We identified 12 unique haplotypes in the introduced populations that were nested into five native geographically distinct clades with genetic divergences ranging from 2.1 to 5.7 %. Multiple clade origin was common within populations, with a maximum of three different haplotype clades being represented within a single population. The genetic data are consistent with a scenario whereby initial establishment was a result of translocation of animals from their native range, whereas more recent establishment (i.e. since the mid-1980s) is the result of translocations of animals from previously established non-native populations. However, this requires further study. Overall, our results show that human introductions have created substantial opportunities for hybridization between genetically and phenotypically distinct lineages, which may have important consequences for the establishment success and long-term viability of introduced wall lizard populations.

Keywords Invasive species · mtDNA · Phylogeography · Hybridization · Admixture · Lizard

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Introduction

Natural processes, such as the coming and going of ice ages and rising and subsiding of ocean levels have repeatedly isolated populations from each other, effectively setting the stage for further differentiation and eventually speciation (Mayr 1963; Hewitt 2004). This is exemplified by the presence of genetically distinct sister species, subspecies and races in mainland Europe, which are believed to have originated during isolation in glacial refugia (Hewitt 1996). For example, the three European clades of brown bears

(*Ursus arctos*) can be traced to three different Quarternary refugia (reviewed in Taberlet et al. 1998; Davison et al. 2011). Similar scenarios of population divergence have been documented in a wide range of species, including insects, amphibians and reptiles (Taberlet et al. 1998; Lunt et al. 1998; Palo et al. 2004; Joger et al. 2007).

Human activities are increasingly modifying the outcome of these processes by creating new barriers to gene flow or eliminating barriers among previously allopatric taxa (Storfer et al. 2010; Crispo et al. 2011). For example, humans may affect the rate or distance of dispersal, which can bring into contact populations that were previously isolated. When this involves several distinct genetic lineages (e.g., sub-species or species) it provides an opportunity for hybridization. Although hybridization has traditionally been considered of minor importance in animal evolution (e.g., Mayr 1963), it is increasingly acknowledged that it is common in animals and that hybrids are not universally unfit (Mallet 2005; Arnold and Martin 2010). This suggests that hybridization could result in collapse of evolutionary lineages (e.g., Rhymer and Simberloff 1996; Seehausen et al. 2008; Vonlanthen et al. 2012), contribute to novel phenotypic and genotypic variation (Grant et al. 2005; Stelkens et al. 2009; Nadachowska-Brzyska et al. 2012), and thus facilitate adaptive evolution (reviewed in Arnold 1997; Seehausen 2004; Arnold and Martin 2010). Consequently, it is important to understand the extent to which human activities bring previously isolated groups into contact and the consequences thereof (Estoup and Guillemaud 2010; Crispo et al. 2011).

To assess the potential for hybridization in a human-mediated introduction we used a phylogeographic approach to establish the distribution of native clade haplotypes within and among 23 non-native populations of the common wall lizard (*Podarcis muralis*) in England.

Materials and methods

Study species and sampling

The common wall lizard (*P. muralis*) is a small (up to 75 mm snout to vent length) diurnal lizard. It is typically saxicolous and is strongly associated with modified or artificial habitats (e.g., brick and stone walls) throughout its native range, which covers much of Western and Southern Europe (Fig. 1; Schulte 2007). *P. muralis* show a strong phylogeographic structure with several genetically and geographically distinct clades (Giovannotti et al. 2010; Schulte et al. 2012, see below) that likely originated during isolation in glacial refugia (Giovannotti et al. 2010). The taxonomy of the species is debated (Gruschwitz and Böhme 1986; Schulte 2007; Glandt 2010), but five or six sub-species are currently recognised, with many additional insular types described (Gruschwitz and Böhme 1986). However, more recent molecular analyses have revealed that morphologically distinct sub-species classifications are not fully congruent with genetic lineages, at least not with respect to insular forms (Bellati et al. 2011). In addition to its large native distribution, the species has also been introduced to many regions, including Germany, the United Kingdom (UK), and North America (Allan et al. 2006; Schulte et al. 2012; Gleed-Owen 2004; Burke and Deichsel 2008). In the UK alone, about 50 introductions are known, with more than 25 extant populations (one in Wales and the remaining ones in England; see below). The species has been common in herpetological collections ever since the nineteenth century and accordingly many of the introductions are the result of escapees or deliberate release of captive animals or their offspring (Frazer 1964; Lever 1977; Uller and While unpublished). However, some introductions may also have been mediated via the nursery trade or as cargo stow-away.

We collected tissue samples from 23 lizard populations throughout southern England between the years 2009–2011 (Fig. 2, Supplementary Table 1). Although sea cliffs, railways, stone walls and other human-made habitats enable dispersal from the original site of introduction, the large majority of the populations sampled in this study are currently separated by ecological, physical, or distance barriers that prevent natural dispersal. However, as populations continue to grow and expand, some may sooner or later come into contact (e.g., the populations along the Dorset coast, and the two populations in the Ventnor region; Fig. 2). We only included populations with recorded breeding, presence of juveniles and an estimated population size of at least ten adults, (all estimates are based on repeated visits and mark-recapture of individuals that could be reliably identified based on assigned codes from toe clipping or photos), with a single exception (Bristol, which may

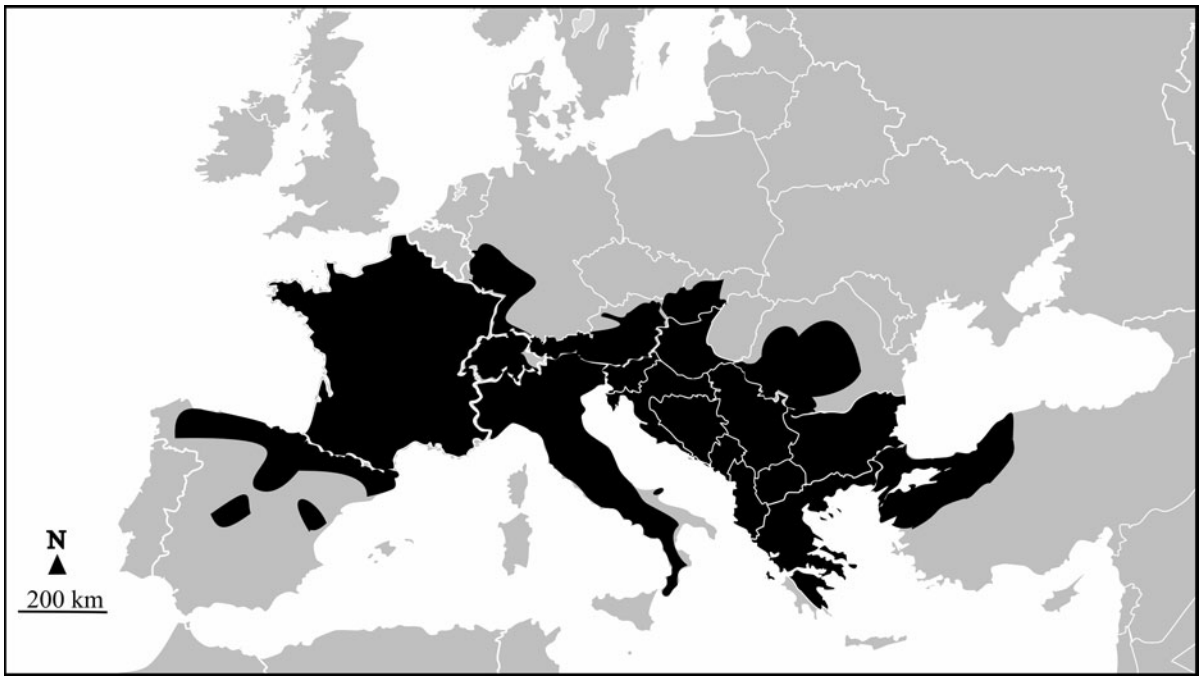

Fig. 1 Distribution of *Podarcis muralis* in the native range

have had fewer that ten adult lizards at the time of sampling). We collected tissue from adults by removing the tip of the tail or one or several toes (the latter in populations that are being subject to mark-recapture studies). For one population (Corfe Castle), we obtained samples collected by Natural England during an attempt to eradicate the population.

DNA isolation and sequencing

To identify native-range sources of the introduced populations, we extracted genomic DNA from tail tissue preserved in ethanol (90 %) from 507 individuals with DNeasy 96 plate kit (Qiagen, Valencia, CA) following manufacturer's instructions (with overnight lysis). We amplified a region of mitochondrion cytochrome *b* gene by polymerase chain reaction (PCR) using the primer pair LGlulk (5′-AACCGCCTGTTGT CTTCAACTA-3′) and Hpod (3′-GGTGGAATGGGA TTTTGTCTG-5′) (Deichsel and Schwiger 2004; Podnar et al. 2007; Schulte et al. 2012). Amplifications were carried out in a total volume of 15 µl consisting of 2 µl template DNA, 0.45 µl 8 pm of each primer (Eurofins), 0.6 µl 50 mM $MgCl_2$ (Invitrogen), 0.6 µl 10 mM dNTPs (Invitrogen), 0.06 µl Platinium Taq Polymerase (Invitrogen), 1.5 µl 10× PCR Buffer (Invitrogen) and 9.34 µl PCR grade H_2O. PCR conditions were as follows: an initial denaturation step at 94 °C for 1 min, followed by 35 cycles at 94 °C for 1 min, 53 °C for 45 s and 72 °C for 1 min and a final extension step at 72 °C for 10 min. PCR products were purified using the MinElute 96 UF PCR Purification Kit (Qiagen).

Sequencing reactions were carried out with BIG-Dye Terminator v3.1 Ready Reaction kit (Applied Biosystems, Warrington, UK) in both directions. Products were precipitated in isopropanol and analysed on an ABI 3130 automated capillary sequencer (Applied Biosystems, Warrington, UK). Mitochondrial DNA sequences from both directions were corrected by eye and aligned to obtain a consensus sequence. Accepted sequences were then aligned using the MAFFT algorithm (Katoh et al. 2002) implemented in Geneious Pro 5.5.6 (Drummond et al. 2011) in G-INS-i mode and trimmed into a uniform length of 655 base pairs (bp). We translated the sequenced cyt *b* region to amino acid sequences, in Geneious, to verify that no premature stop codons disrupted the reading frame. Unique haplotypes present in the introduced range of *P. muralis* were submitted to GenBank under the accession numbers in Supplementary Table 2.

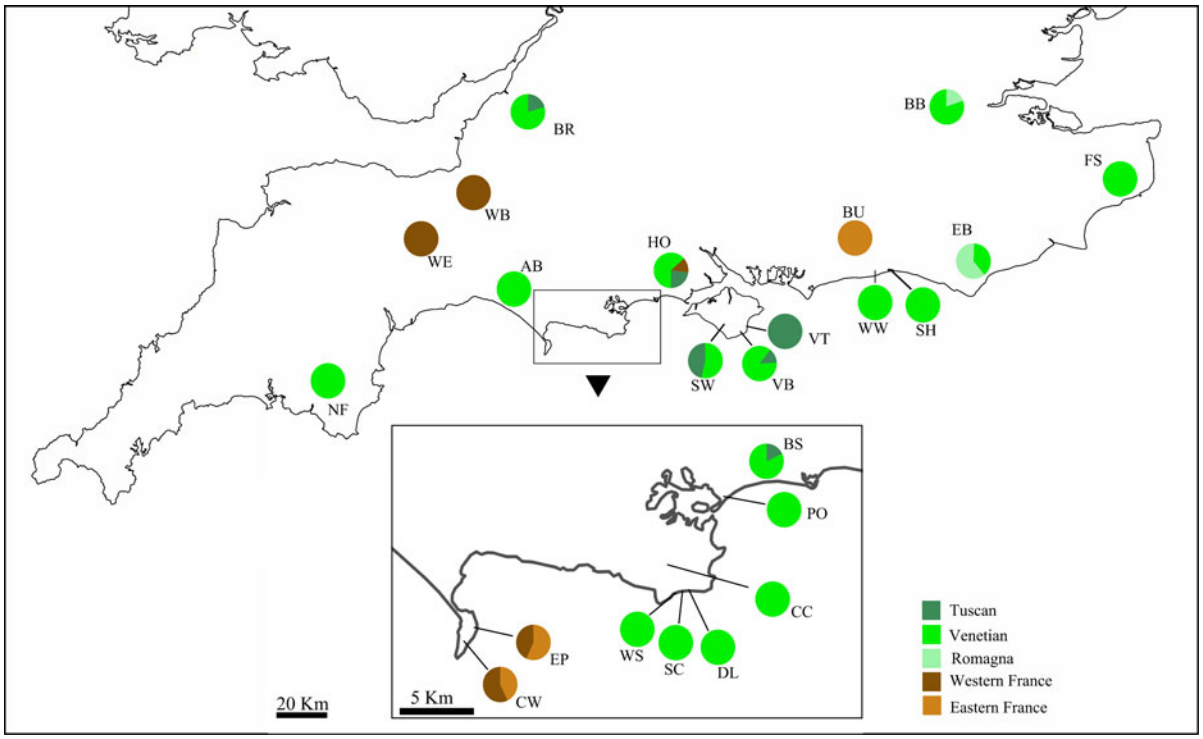

Fig. 2 Distribution of the 23 introduced populations in England. *Pie charts* indicate the percentage of sampled individuals matched to a specific clade from the native range. For populations abbreviations see Table 1

Phylogenetic and population-genetic analyses

We used the phylogenetic tree approach to resolve the origin of haplotypes sampled in the introduced populations. We combined the unique sequences from UK populations with 175 published sequences (of varying lengths) covering almost the entire native distribution of the species (Poulakakis et al. 2003, 2005a; Podnar et al. 2005, 2007; Giovannotti et al. 2010; Bellati et al. 2011; Schulte et al. 2012). Three cyt b sequences belonging to *P. siculus* (Podnar et al. 2005) and *P. liolepis* (Schulte et al. 2012) were used as outgroups in the phylogenetic analyses using Maximum Likelihood (ML) and Bayesian Inference (BI). The ML was conducted in MEGA 5.0 (Tamura et al. 2011) under the GTR+G+I nucleotide substitution model as selected by the best-fit model applying the Akaike Information Criterion, corrected for small sample sizes. We implemented BI analyses in MrBayes (Huelsenbeck and Ronquist 2001) also under the GTR+G+I nucleotide substitution model. The analysis was run with four chains of 2,000,000 generations and sampling every 100 trees. We discarded (burn-in-length) the first 20 % of the trees after checking for convergence of the chains and the posterior probability branch support was estimated from the 50 % majority-rule consensus tree.

We calculated nucleotide divergence among clades under the Tamura-Nei model of evolution (Tamura and Nei 1993), in MEGA. Divergence times of selected nodes were estimated using the equation $D_A = 2\mu T$, in which μ is the average substitution rate per nucleotide, T is the divergence time, and D_A is the net number of nucleotide differences between populations (Nei and Li 1979). To provide an estimate of the divergence time, we used the published evolutionary rate for *Podarcis peloponnesiaca* and *P. erhardii* (1.55 % per million years; Poulakakis et al. Poulakakis et al. 2005b) for our calculations (i.e., the analysis assumes that evolutionary rates are similar within *Podarcis*; Avise 1994).

We calculated the number of haplotypes (Nh), haplotype diversity (h) and nucleotide diversity (π) for each population. Pairwise genetic differentiation among the UK populations was computed as Φ_{ST} values and the level of genetic diversity within and

among populations was tested by hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992). All calculations were performed in ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010) and statistical support was estimated by 10,000 randomised permutations. In order to visualise the genetic relationships between the populations, a multidimensional scaling (MDS) analysis based on pairwise sequence divergence among populations (calculated in MEGA) was carried out using R (R_Development_Core_Team 2011).

To investigate evolutionary relationships between our samples, we constructed a parsimonious phylogenetic network using a median-joining algorithm in Network v.4.5.10 (Bandelt et al. 1999). This method uses median vectors as a hypothetical ancestral sequence required to connect existing sequences within the network with maximum parsimony. Samples sharing the same haplotype will group together, and the diameter of the pie (for each haplotype) will correspond to the number of samples sharing that haplotype.

Finally, we used reported historical information of the introduced populations (see Supplementary Table 1) to infer the relationships between the timing of introduction, individual haplotypes, and within-population genetic diversity.

Results

The mtDNA sequencing of 507 individuals from 23 introduced populations of the common wall lizard in England revealed 12 unique haplotypes with 59 informative sites and an overall haplotype diversity of 0.87 (Table 1). The reconstruction of the haplotype network, including all 507 samples from our study, identified 5 haplogroups (Fig. 3). The phylogenetic tree approach nested these haplotypes within 5 distinct clades from the native range of the species (Fig. 4; referred to as Venetian, Tuscan, Romagna, Western France and Eastern France clades, Schulte et al. 2012). The average pairwise genetic divergence between these clades range from 2.1 to 5.7 % (Supplementary Table 3). Under the assumption that the rate of divergence is similar to the congeneric species *P. erhardii* and *P. peloponnisiaca*, this suggest that the divergence between the French and Italian clades occurred at least 3.5 mya, whereas the three native Italian clades present in England (Venetian, Tuscan, and Romagna) diverged from each other between 2 and 2.5 mya (Supplementary Table 4, see also Bellati et al. 2011).

Overall, the most common form of wall lizards in England is the Venetian clade native to northern Italy, which was found in 16 of the 23 populations (70 %). The least common clade was Romagna, which could only be verified from two populations (9 %). We could verify multiple origins for nine populations, one of which contained haplotypes from three different clades (two native to Italy and one native to Western France). An Analysis of Molecular Variance (AMOVA) revealed that 77 % ($p < 0.005$) of the total variance in England was distributed among populations (Table 2).

The MDS analysis (Fig. 5) showed two main clusters; populations with French only haplotypes and populations with Italian only haplotypes. One population (HO), showed in the middle, exhibits both French and Italian haplotypes. Two minor groups (shown in ellipses) contain populations with mixed French or mixed Italian clades. There was no significant correlation between haplotype diversity and the year of introduction ($r = 0.286$, $p = 0.18$; Fig. 6).

Discussion

Our results show that the origin of the common wall lizard in England can be traced to at least five geographically and genetically distinct lineages spanning a large part of the species' native range. The taxonomy of *P. muralis* is subject to debate, but under the current classification these five clades are likely to include at least three subspecies whose morphology is consistent with that observed in introduced populations in England (*P. m. brogniardi* (Western France clade), *P. m. merremius* (Eastern France clade) and *P. m. nigriventris* (Tuscan clade); Gruschwitz and Böhme 1986; Schulte 2007). Regardless of the taxonomic status of the different clades, our analyses reveal that human introductions of wall lizards into England involve lineages with sequence divergences similar to that of other species and sub-species complexes of Lacertid lizards in Europe (e.g., *Lacerta agilis* Kalyabina et al. 2001; *P. hispanica* Harris and Sá-Sousa 2002; *L. bilineata/viridis* complex Böhme et al. 2007; reviewed in Joger et al. 2007). Although the Italian clades are likely to have diverged in ice age refugia (Giovannotti et al. 2010), the split between

Table 1 Introduced populations and abbreviations, number of individuals sampled, number of haplotypes (Nh), Haplotype diversity (Hd), π (nucleotide diversity)

Population	Abbr.[a]	Sample size	Nh	Hd (±SD)	π (±SD)	Haplotype name (number of individuals)	Clade
Abbotsbury	AB	25	2	0.0800 (0.0722)	0.000244 (0.000386)	$UKH4_{(1)}$, $UKH10_{(24)}$	Venetian
Birdbrook	BB	13	2	0.4615 (0.1096)	0.006342 (0.003790)	$UKH4_{(9)}$, $UKH5_{(4)}$	Venetian, Romagna
Boscombe	BS	25	3	0.5067 (0.0993)	0.007959 (0.004440)	$UKH8_{(4)}$, $UKH10_{(17)}$, $UKH11_{(4)}$	Tuscany, Venetian
Bristol	BR	5	2	0.4000 (0.2373)	0.009771 (0.006517)	$UKH11_{(1)}$, $UKH10_{(4)}$	Tuscany, Venetian
Bury	BU	20	2	0.1895 (0.1081)	0.001157 (0.000991)	$UKH2_{(18)}$, $UKH3_{(2)}$	E. France
Cheyne Weare	CW	25	3	0.6533 (0.0517)	0.015735 (0.008280)	$UKH3_{(8)}$, $UKH6_{(12)}$, $UKH12_{(5)}$	E. France and W. France
Corfe Castle	CC	25	3	0.4400 (0.0950)	0.001802 (0.001333)	$UKH4_{(1)}$ $UKH8_{(19)}$, $UKH10_{(5)}$	Venetian
Dancing Ledge	DL	25	2	0.2200 (0.0995)	0.001008 (0.000895)	$UKH8_{(21)}$ $UKH10_{(4)}$	Venetian
Eastbourne	EB	5	2	0.6000 (0.1753)	0.008244 (0.005587)	$UKH4_{(2)}$ $UKH5_{(3)}$	Venetian, Romagna
East Portland	EP	25	3	0.5267 (0.0836)	0.016763 (0.008787)	$UKH3_{(16)}$, $UKH6_{(2)}$, $UKH12_{(7)}$	E. France and W.France
Folkestone	FS	21	2	0.0952 (0.0843)	0.000291 (0.000428)	$UKH4_{(20)}$ $UKH10_{(1)}$	Venetian
Holmsley	HO	25	5	0.7667 (0.0535)	0.019919 (0.010341)	$UKH1_{(6)}$, $UKH4_{(3)}$, $UKH6_{(4)}$ $UKH8_{(2)}$, $UKH10_{(10)}$	Venetian, Tuscany, W. France
Newton Ferrers	NF	25	1	0	0	$UKH4_{(25)}$	Venetian
Poole	PO	25	3	0.5567 (0.0471)	0.002372 (0.001633)	$UKH4_{(1)}$, $UKH8_{(11)}$, $UKH10_{(13)}$	Venetian
Seacombe	SC	18	2	0.2092 (0.1163)	0.000319 (0.000455)	$UKH4_{(2)}$, $UKH8_{(16)}$	Venetian
Shoreham	SH	25	1	0	0	$UKH4_{(25)}$	Venetian
Shorwell	SW	25	3	0.5600 (0.0444)	0.012824 (0.006844)	$UKH1_{(12)}$, $UKH4_{(12)}$, $UKH8_{(1)}$	Venetian, Tuscany
Ventnor botanical garden	VB	25	3	0.5400 (0.0886)	0.006351 (0.003641)	$UKH1_{(3)}$, $UKH4_{(16)}$, $UKH10_{(6)}$	Venetian, Tuscany
Ventnor town	VT	25	1	0	0	$UKH1_{(25)}$	Tuscany
Wembdon	WB	25	1	0	0	$UKH6_{(25)}$	W. France
Wellington	WE	25	2	0.3333 (0.0978)	0.000509 (0.000586)	$UKH6_{(20)}$, $UKH7_{(5)}$	W. France
Winspit	WS	25	4	0.5767 (0.0661)	0.002372 (0.001633)	$UKH4_{(1)}$, $UKH8_{(14)}$, $UKH9_{(1)}$ $UKH10_{(9)}$	Venetian
West worthing	WW	25	1	0	0	$UKH9_{(25)}$	Venetian

[a] These abbreviations are used in all figures and tables

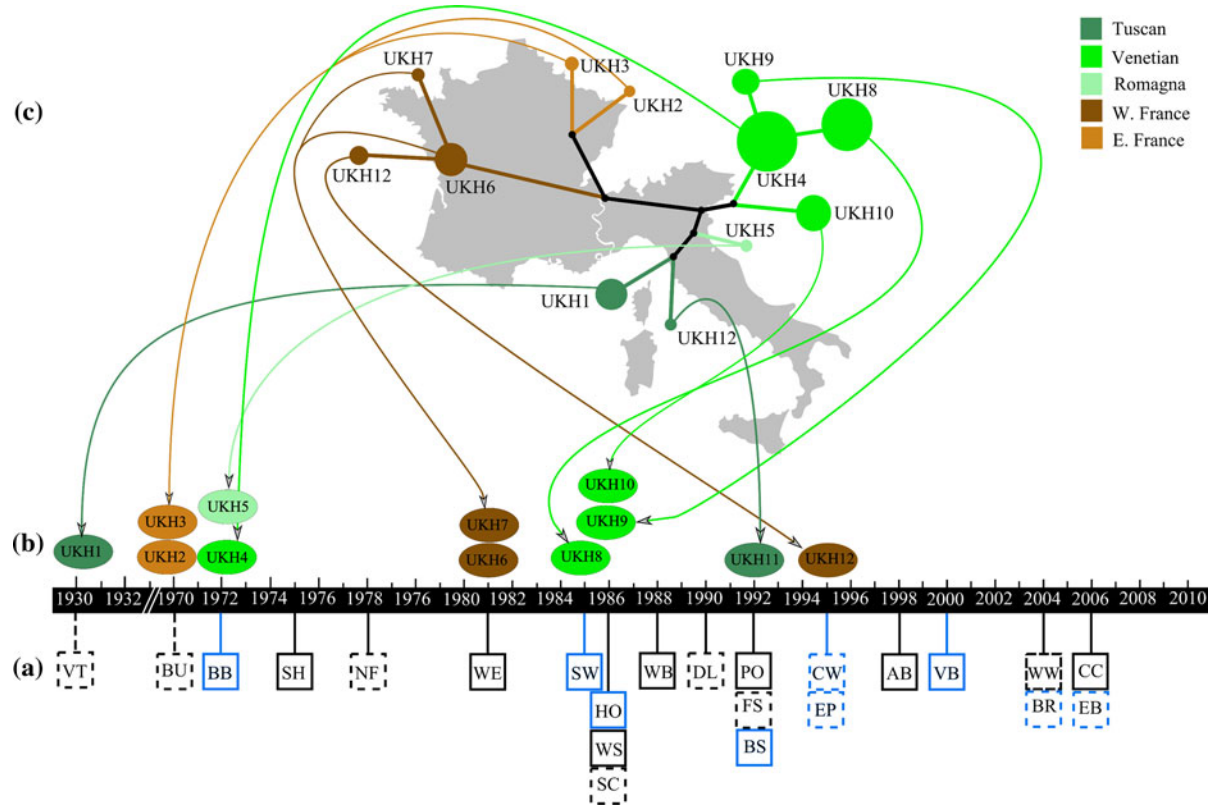

Fig. 3 Haplotype origins and history of introduction of wall lizards in England; **a** The timeline shows introduction* events of the UK populations (*blue squares* populations of confirmed mixed origin; *dotted squares* introduction date uncertain and approximated from first sighting or record of wall lizards in the area). **b** The first appearance of a unique haplotype is noted above the timeline. **c** The haplotype network of the introduced populations. The *circle* represents a single haplotype and its diameter is proportional to the number of individuals sharing the same haplotype. For populations abbreviations see Table 1. * See also Supplementary Table 1 for details on introduction dates. (Color figure online)

them and the two French clades may predate the Pleistocene.

Importantly, we found haplotypes from more than one lineage in nine out of the 23 introduced populations. This demonstrates that introductions of the wall lizard into England and/or subsequent translocations have created opportunities for intra-specific hybridization. A similar pattern is seen in Germany, where at least 25 % of introduced populations exhibit multiple geographic origins; see also Kolbe et al. 2012 for an analyses of four introduced *P. siculus* populations in the USA). Indeed, multiple introductions are increasingly recognised as a common feature of species introductions (Roman and Darling 2007). For example, Kolbe et al. (2004) showed that there have been at least eight separate introductions of the brown anole (*Anolis sagrei*) in Florida, and similar patterns have been found in other lizards (Chapple et al. 2012; Kolbe et al. 2012). Multiple introductions can have substantial impact on genetic and phenotypic variation of introduced populations relative to those in the native range (Dlugosch and Parker 2008; Uller and Leimu 2011), and may even increase the ability of introduced species to adapt to local conditions (e.g. Lavergne and Molofsky 2007). Since the wall lizard clades introduced into England originate from a wide geographic range and differ substantially both genetically and phenotypically, clade origin(s) may have important implications for inter-population phenotypic divergence and establishment success in the UK. Indeed, a divergence time similar to that estimated here for the major clades for *P. muralis* has been associated with reduced hybrid fitness in crosses between species in the genus *Lacerta* (Rykena 1991; 1996) and for intra-specific lineages of the sand lizard *L. agilis* (Olsson et al. 2004; Rykena 1991; see also Rykena 1996).

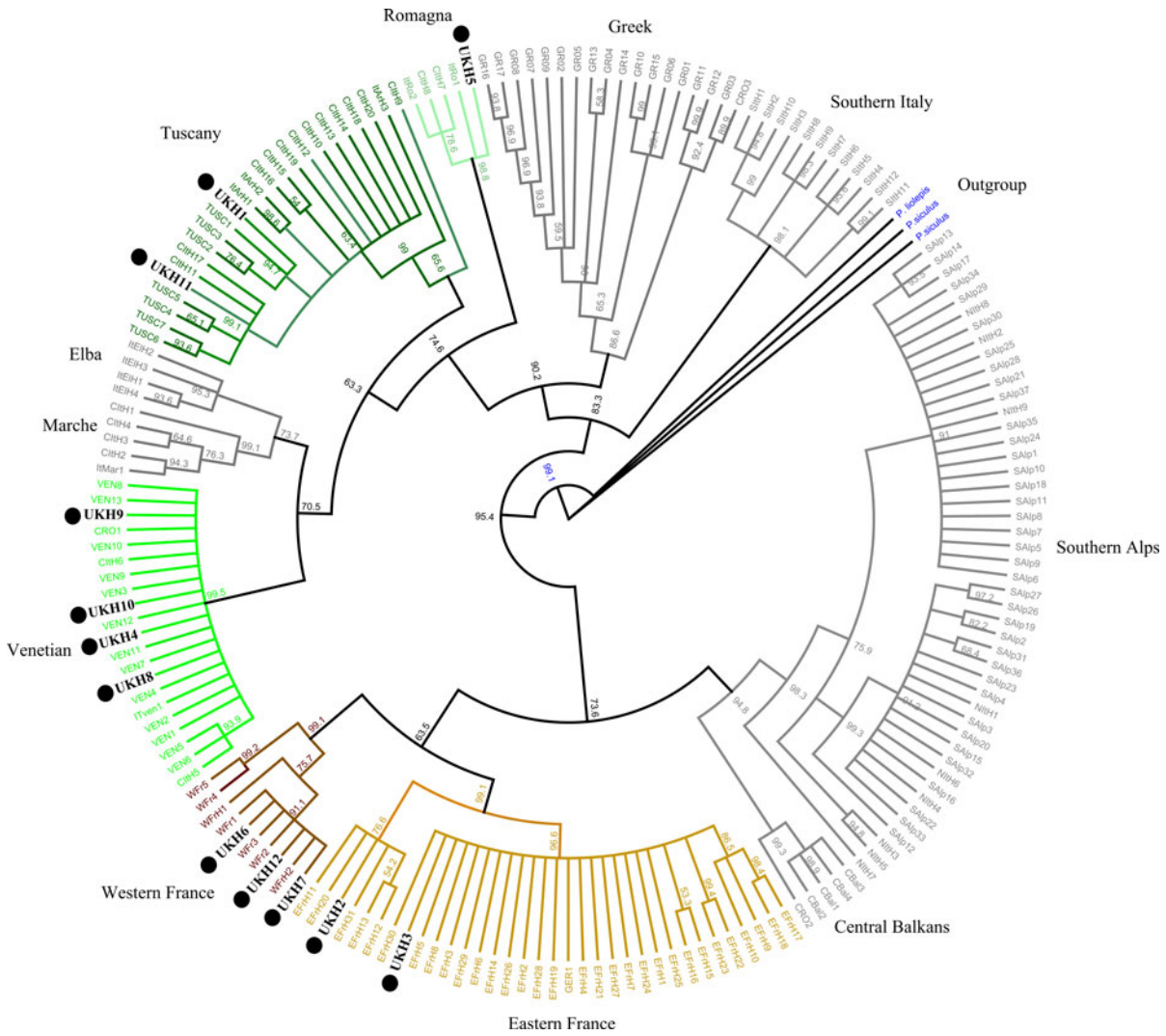

Fig. 4 Bayesian analysis consensus tree derived from mitochondrial cytochrome b sequences of *P. muralis* from 175 published sequences and 12 unique sequences from this study. Bootstrap values are indicated above nodes. *Black dots* indicate the UK haplotypes that are nested within clades/geographic regions (highlighted with different coloration) from the distribution of the species. (Color figure online)

Table 2 Analysis of molecular variance (AMOVA) showing distribution of genetic variation among and within introduced populations

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*P* value
Among populations	22	2,613.8	5.33	77.50	<0.0001
Within populations	484	749.9	1.55	22.50	
Total	506	3,363.7	6.89		

In contrast to the situation in Germany (Schulte et al. 2012), there are only a few populations of Eastern France origin in England and we did not find any evidence of the Southern Alps clade being present. The Eastern France clade represents the northernmost native distribution of the species, and hence should show a better tolerance than southern European clades to the relatively cool summers in England. However,

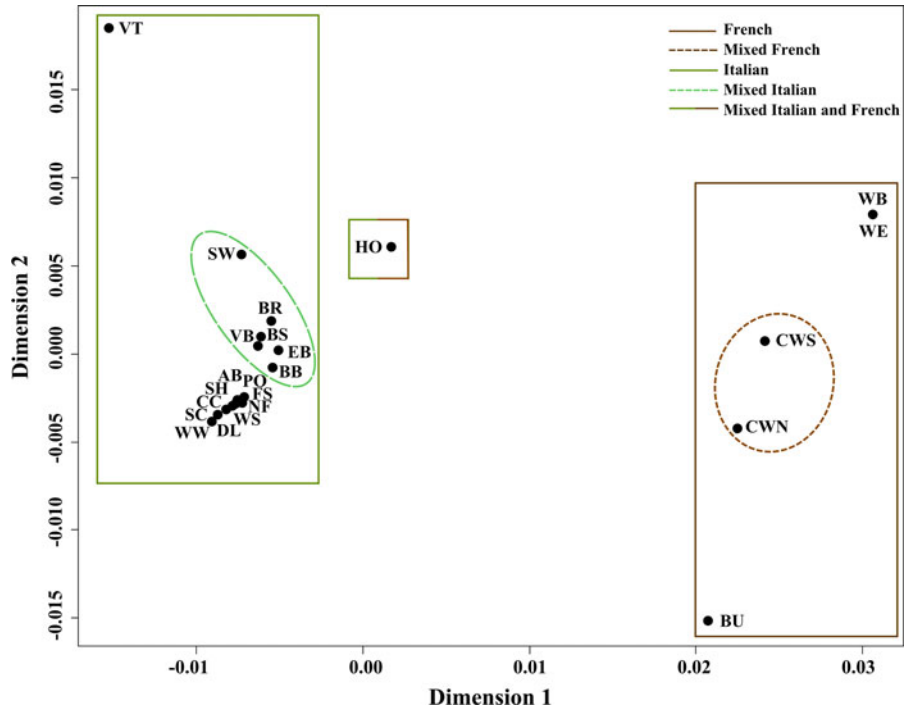

Fig. 5 Multidimensional scaling analysis based on pairwise sequence divergence among populations. The analysis revealed two main clusters; populations with French only haplotypes and populations with Italian only haplotypes. Within each cluster, ellipses indicate populations with multiple clade origin. One population (HO) had mixed French and Italian haplotypes. For populations abbreviations see Table 1

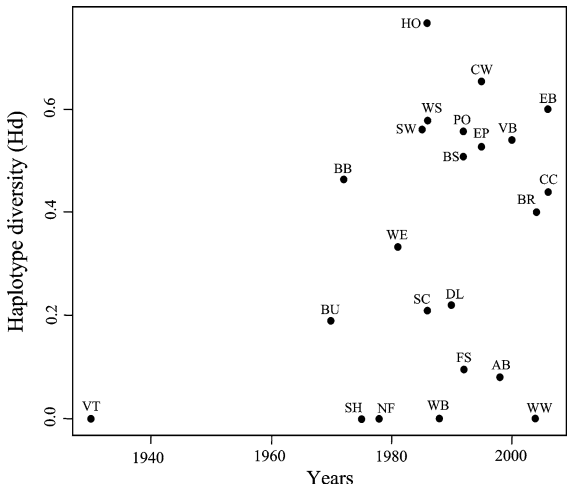

Fig. 6 Year of introduction and haplotype diversity (Hd). There is no evidence for a relationship between the age of a population and Hd. For populations abbreviations see Table 1

the climatic conditions of the introduced locations in England actually show a poor match with the environmental niche for all five clades (Schulte et al. 2012). The match is particularly poor for the most common Italian origins, which shows that lizard species from relatively warm climates also can survive in substantially cooler climates. This supports previous conclusions that the fundamental climatic niche is poorly represented by the realised niche in the native range for this species (Schulte et al. 2012). However, we caution that the long-term survival of introduced populations in England is uncertain as cool summer temperatures severely reduces recruitment due to hatching failure (Stumpel 2004; Uller and While, personal observation).

Although approximately half of the extant populations were established less than 25–30 years ago, deliberate attempts to establish wall lizard populations in the UK go back much further (Lever 1977). For example, wall lizards were apparently introduced into a garden in Abbotsbury in 1890 and at Farnham Castle in Surrey in 1932. Many of the populations have since gone extinct, and few extant populations in England are more than 40 years old (the extant population in Abbotsbury in Table 1 is a more recent introduction subsequent to the extinction of the first population in the 1960s; see Supplementary Table 1.). Although the presence of five different clades in England shows that there has been a wide range of sources for the introductions, the combination of genetic and historical records indicates that some of the older populations served as sources for more recent populations (Fig. 3). For example, the most common haplotype in

England (UK4) is from the Venetian clade, which first appeared in 1972 as a result of escapees from a private breeding colony of lizards obtained from a pet shop (Supplementary Table 1). The high occurrence of this haplotype across multiple introduced populations, despite high haplotype diversity in this part of the native range (Giovannotti et al. 2010) makes it likely that this well-established population, or the Shoreham population established in 1975 that has the same haplotype, has served as a source for later introductions. In fact, the use of Shoreham as a source population has been confirmed by interviews of those involved in the introductions to Ventnor Botanical Garden and Shorwell (Uller, unpublished). This pattern is not surprising considering that many of the populations have been founded by escaped or released pets which often may have been originally collected from local populations. However, a reconstruction of the colonization history of the species will require nuclear genetic markers and further sampling of the native range to enable statistical evaluation of different scenarios (Estoup and Guillemaud 2010; Lombaert et al. 2010). Importantly, our mtDNA data is likely to underestimate the actual number of sources. Thus, the true extent of admixture within populations is likely to be greater than the minimum estimates reported here. Detailed reconstruction of colonization routes using a combination of mtDNA and nuclear genetic markers will allow tests of how important particular introduced populations have been for the gradual, human-assisted, colonization history in England and provide further opportunities to establish the causes and consequences of admixture in the introduced range.

In summary, deliberate and accidental introductions of common wall lizards in England involve at least five genetically and geographically separated lineages from the native range. The presence of haplotypes from two or more native clades within 40 % of the introduced populations suggest potential scope for admixture, and the rate at which new populations are established could exacerbate intraspecific hybridization in the future.

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