MOLECULAR ECOLOGY

Molecular Ecology (2015) doi: 10.1111/mec.13206

Widespread primary, but geographically restricted secondary, human introductions of wall lizards, *Podarcis muralis*

SOZOS N. MICHAELIDES,* GEOFFREY M. WHILE,*† NATALIA ZAJAC* and TOBIAS ULLER*‡
*Department of Zoology, Edward Grey Institute, University of Oxford, South Parks Rd, Oxford OX1 3PS, UK, †School of Biological Sciences, University of Tasmania, PO Box 55, Hobart, Tas. 7001, Australia, ‡Department of Biology, Lund University, Sölvegatan 37, SE 223 62 Lund, Sweden

Abstract

Establishing the introduction pathways of alien species is a fundamental task in invasion biology. The common wall lizard, *Podarcis muralis*, has been widely introduced outside of its native range in both Europe and North America, primarily through escaped pets or deliberate release of animals from captive or wild populations. Here, we use Bayesian clustering, approximate Bayesian computation (ABC) methods and network analyses to reconstruct the origin and colonization history of 23 non-native populations of wall lizards in England. Our analyses show that established populations in southern England originate from at least nine separate sources of animals from native populations in France and Italy. Secondary introductions from previously established non-native populations were supported for eleven (47%) populations. In contrast to the primary introductions, secondary introductions were highly restricted geographically and appear to have occurred within a limited time frame rather than being increasingly common. Together, these data suggest that extant wall lizard populations in England are the result of isolated accidental and deliberate releases of imported animals since the 1970s, with only local translocation of animals from established non-native populations. Given that populations introduced as recently as 25 years ago show evidence of having adapted to cool climate, discouraging further translocations may be important to prevent more extensive establishment on the south coast of England.

Keywords: approximate Bayesian computation, colonization pathways, lizard, microsatellites, mitochondrial DNA, networks

Received 21 January 2015; revision received 3 April 2015; accepted 15 April 2015

Introduction

Retracing the routes of colonization is a fundamental goal of invasion biology (Estoup & Guillemaud 2010). By establishing the origin and pathways of introduction, it may be possible to put in place effective management regimes to control or prevent further spread of non-native species (Mack *et al.* 2000). Furthermore, as the introduction history of a population will affect its genetic diversity through founder effects (Dlugosch & Parker 2008), understanding the pathways of introduction may help predict the ecological and evolutionary responses of non-native populations and ultimately their potential to establish and invade (Lee 2002; Sax *et al.* 2007).

Species are transported to new locations by different means, the importance of which varies taxonomically, geographically and temporally (Ruiz & Carlton 2003; Hulme 2009; Wilson *et al.* 2009). Once species have successfully arrived at an introduction site, they have the potential to persist and expand. Such populations can subsequently be used as source populations for second-

Correspondence: Sozos N. Michaelides and Tobias Uller, Fax: +44 1865 310447; E-mails: sozos.michaelides@zoo.ox.ac.uk and tobias.uller@zoo.ox.ac.uk

ary introductions (e.g. stepping-stone populations; Suarez *et al.* 2001; Kolbe *et al.* 2004; Miller *et al.* 2005; Floerl *et al.* 2009). Identification of stepping-stone populations is of particular interest to both evolutionary biologists and wildlife managers because successful populations may have evolved adaptations to the local environment (Keller & Taylor 2008) that make them particularly good sources for further range expansion ('bridgehead effects'; Lombaert *et al.* 2010). Despite this, evidence for the contribution of stepping-stone populations to the movement of non-native species is still very limited (Estoup & Guillemaud 2010; Lombaert *et al.* 2010).

Whereas many species are introduced accidentally, vertebrates such as birds and reptiles are often deliberately introduced, often via the pet trade (Long 1981; Kraus 2009). For example, in Florida, the pet trade is estimated to account for more than 80% of the ca 150 independent introductions of reptiles, many of which have resulted in established populations (Krysko *et al.* 2011). Because of changes in legislation, trade in reptiles increasingly relies on captive breeding or sourcing from introduced populations that are not protected. This increases the likelihood that even isolated non-native populations can contribute to, possibly geographically discontinuous, range expansion via human translocations.

The European wall lizard, *Podarcis muralis* (Laurenti, 1768), has a wide distribution across central and southern Europe with a complex phylogeographic structure (Salvi *et al.* 2013) and associated large geographic variation in morphology (Böhme 1986). The species has been successfully introduced to several locations in North America (Allan *et al.* 2006; Burke & Deichsel 2008) and to more than 140 locations in north-western Europe (Schulte 2008; Schulte *et al.* 2008, 2012a) including over 40 times to the United Kingdom (UK) (Gleed-Owen 2004; Michaelides *et al.* 2013). In the UK, the species has been common in herpetological collections ever since the 19th century (Lever 1977). Currently, there are more than 25 extant populations, the large majority in Southern England. Many of these introduced populations are known to be deliberate releases of captive animals and/or their offspring, while a few may have arrived via the nursery trade or as cargo stowaway (Frazer 1964; Lever 1977). Changes in policy over the past 30 years (e.g. the Wildlife and Countryside act, 1981) have attempted to restrict import and made the release of non-native species in British countryside illegal. As a result, more recent introductions are more likely to arise from already established populations (or captive-bred animals), rather than directly sourced from the native range. If so, this may significantly enhance the ability of the species to persist and expand as the oldest

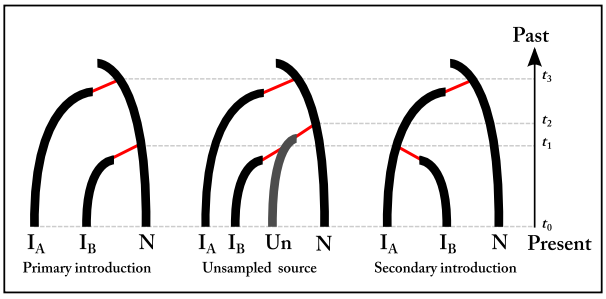

Fig. 1 Graphical representation of *Podarcis muralis* introduction scenarios in England tested by the ABC approach. The *'primary introduction model'* where the introduced population I_B originated independently from the native (N) range, the *'unsampled source model'* where the introduced population I_B originated from an unsampled (Un) source originating from the native range (e.g. a captive source population), and the *'secondary introduction model'* where the introduced population I_B originated from an established introduced population I_A. The thin (red) line indicates a reduction in the effective population size due a bottleneck event following the introduction, and t0, t1, t2 and t3 represent time of introduction event (in generations). Figure modified from Estoup & Guillemaud (2010).

populations have adapted to the cooler climate in England (While *et al.* 2015).

The aim of this study was to establish the colonization pathway(s) of *P. muralis* in England and ultimately to explain their current distribution. We used mitochondrial sequences and nuclear microsatellite markers in a phylogeographic approach to identify potential source regions in the native range. We subsequently used a Bayesian clustering analysis to identify the most likely number of clusters/origins of the non-native populations. We employed approximate Bayesian computation (ABC) methods to test, for each population, three models for their colonization history: (i) *'primary introduction model'* (independent introductions from the native range), (ii) *'unsampled source model'* (introduction from an unsampled source, such as a captive colony) and (iii) *'secondary introduction model'* (secondary introductions from the non-native range). Finally, we constructed networks based on genetic similarity to further investigate and visualize the relationship among non-native populations and the extent of admixture.

Materials and methods

Sample collection and laboratory analyses

We sampled 1328 individuals from 23 non-native populations in England and 34 native populations from France and Italy between 2008 and 2013 (Fig. 1, Table 1). The native source regions were chosen based on previously identified geographic regions for

Table 1 Sampling locations in introduced and native range. Number of samples (N_S), number of identified mtDNA haplotypes (N_H), their assigned lineage and for introduced populations membership in a defined cluster (K)

Sampled locations	Abbreviation	Sampling date	Coordinates (Latitude – Longitude)		N_S*	N_H	Lineage†	Genetic Cluster (K)‡
Introduced Range§								
Abbotsbury	AB	2011	50.67	−2.60	25	2	VEN	K4, K8
Birdbrook	BB	2011	51.46	0.04	13	2	VEN, ROM	K6, K7
Boscombe	BS	2009	50.72	−1.84	25	3	TUS, VEN	K8, K9
Bristol	BR	2009	51.43	−2.60	5	2	TUS, VEN	K4
Bury	BU	2009	50.91	−0.56	20	2	EFR	K3
Cheyne Weare	CW	2009	50.63	−2.05	25	3	EFR, WFR	K3
Corfe Castle	CC	2008	50.53	−2.44	25	3	VEN	K8
Dancing Ledge	DL	2010	50.60	−1.99	25	2	VEN	K6
Eastbourne	EB	2011	50.77	0.29	5	2	VEN, ROM	K5, K7
East Portland	EP	2010	50.53	−2.44	25	3	EFR, WFR	K3
Folkestone	FS	2009	51.08	1.17	21	2	VEN	K7
Holmsley	HO	2009	50.79	−1.70	25	5	VEN, TUS, WFR	K4, K8, K9
Newton Ferrers	NF	2011	50.32	−4.04	25	1	VEN	K2
Poole	PO	2009	50.72	−1.98	25	3	VEN	K6, K8
Seacombe	SC	2010	50.62	−1.96	18	2	VEN	K6
Shoreham	SH	2009	50.83	−0.26	25	1	VEN	K5
Shorewell	SW	2011	50.64	−1.35	25	3	VEN, TUS	K9
Ventnor Botanical Garden	VB	2009	50.59	−1.25	25	3	VEN, TUS	K5, K9
Ventnor Town	VT	2009	50.59	−1.21	25	1	TUS	K9
Wembdon	WB	2011	51.13	−3.02	25	1	WFR	K1
Wellington	WE	2009	50.98	−3.22	25	2	WFR	K3
Winspit	WS	2009	50.59	−2.01	25	4	VEN	K8
West Worthing	WW	2009	50.82	−0.36	25	1	VEN	K5, K6
Native range (Italy)								
Cento	CE	2013	44.73	11.29	25 (12)	6	ROM, VEN	
Bassano Di Grappa (Campesse)	BG	2012	45.80	11.71	25 (8)	2	SAL	
Badia Polesine	BP	2012	45.10	11.49	25 (8)	5	SAL	
Barbarano Vicentino	BV	2012	45.41	11.54	25 (8)	2	SAL	
Mizzole	MZ	2012	45.48	11.06	25 (8)	2	SAL	
Calci	CA	2012	43.72	10.52	25 (12)	3	TUS	
Chianni	CN	2013	43.48	10.64	25 (12)	5	TUS	
Crespina	CR	2012	43.57	10.56	24 (12)	4	TUS	
Greve in Chianti	GC	2013	43.59	11.31	25 (12)	5	TUS	
Montemassi	MM	2013	42.99	11.06	25 (12)	5	TUS	
Prato	PR	2013	43.90	11.11	25 (12)	6	TUS	
Travale	TR	2013	43.17	11.01	25 (12)	4	TUS	
Colle di Val'Elsa	VE	2013	43.42	11.11	25 (12)	4	TUS	
Buti	BT	2012	43.73	10.59	25 (12)	7	TUS, SAL	
Viareggio	VI	2012	43.84	10.26	25 (12)	5	TUS, SAL	
Vignola	VG	2013	44.48	11.01	22 (12)	5	TUS, VEN	
Castellarano	CT	2013	44.51	10.73	25 (12)	3	VEN	
Motta Di Livenza	ML	2012	45.78	12.61	22 (8)	3	VEN	
Nonantola	NO	2013	44.68	11.04	25 (12)	8	VEN	
Olina	OL	2013	44.31	10.78	16 (12)	6	VEN	
Pian Di Venola	PV	2012	44.33	11.19	25 (12)	6	VEN	
Native range (France)								
Bastide	BA	2010	42.94	1.06	25 (5)	2¶	WFR	
Dinan	DN	2013	48.45	−2.05	25 (5)	2¶	WFR	
Fonteirs Cabardes	FC	2012	43.37	2.25	25 (5)	3¶	WFR	
Saint Gervais	GE	2012	46.90	−2.00	25 (5)	1¶	WFR	
Josselin	JO	2013	47.95	−2.55	25 (5)	1¶	WFR	
Saint Lizier	LI	2012	43.00	1.14	20 (5)	2¶	WFR	

Table 1 *Continued*

Sampled locations	Abbreviation	Sampling date	Coordinates (Latitude – Longitude)		N_S*	N_H	Lineage†	Genetic Cluster (K)‡
Saint Michel	MI	2012	46.35	−1.25	25 (5)	1¶	WFR	
Nebias	NE	2012	42.90	2.12	25 (5)	3¶	WFR	
Pontchateau	PC	2013	47.44	−2.09	25 (5)	1¶	WFR	
Puybelliard	PU	2012	46.71	−1.03	22 (5)	1¶	WFR	
Pouzauges	PZ	2012	46.78	−0.84	25 (5)	1¶	WFR	
Saint Girons	SG	2010	42.98	1.15	25 (5)	2¶	WFR	
Vitre	VR	2013	48.12	−1.21	20 (5)	1¶	WFR	

*All individuals from introduced populations were sequenced. For native populations, a sample of individuals (number in parenthesis) per population was sequenced.
†Lineage abbreviations correspond to Venetian (VEN), Tuscan (TUS), Romagna (ROM), Southern Alps (SAL), Western France (WFR) and Eastern France (EFR).
‡Membership in a genetic cluster as defined by the STRUCTURE analysis (see Table S5, Supporting information for proportions of membership, Q).
§Data from Michaelides *et al.* (2013).
¶Sequences analysed in Michaelides *et al.* (2015).

mitochondrial clades (with the exception of the Eastern France clade that we did not sample; Schulte *et al.* 2012a,b; Michaelides *et al.* 2013; Salvi *et al.* 2013). Lizards were caught by hand or noosing, and a small (ca 5 mm) part of the tail was removed by inducing tail release with a pair of tweezers or, when the tail was regrown, using surgical scissors to provide tissue for genetic analysis. All lizards were released at the site of capture following sampling. We extracted genomic DNA from ethanol (70–90%)-preserved tissue with the DNeasy® 96 plate kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions (with overnight lysis).

We genotyped all individuals at 16 polymorphic microsatellite loci: seven described by Richard *et al.* (2012) and nine developed by Heathcote *et al.* (2014) in five multiplexed PCRs (see Table S1, Supporting information) in a total volume of 11 μL reaction mix containing 1 μL of genomic DNA, 5 μL of Qiagen MasterMix, 0.2 μL of each primer (forward and reverse in equal concentrations) and 3.8 μL (for multiplexes 1, 2, 3 and 5) or 3.6 μL (for multiplex 4) of PCR-grade dH_2O. PCR conditions were as follows: 15 min of initialization step at 95 °C, 26 cycles of 30 s at 94 °C, 90 s at 57 °C (for multiplexes 1, 2 and 3) or 55 °C (for multiplexes 4 and 5) and 1 min at 72 °C and a final extension step of 20 min at 60 °C. The 5′-end of each forward primer was labelled with a fluorescent dye either 6-FAM, HEX or NED. PCR products were run with an internal ladder (red ROX-500), on an ABI 3130 Genetic Analyser (Applied Biosystems Inc., Warrington, UK). We scored alleles in GENEIOUS 6.1.7, and any ambiguous peaks were repeated to confirm genotype.

For a subset of native samples (5–12 individuals per population), we sequenced a region of mitochondrion DNA (mtDNA) cytochrome b gene (*cyt-b*) by polymerase chain reaction (PCR) using the primer pair LGlulk [5′-AACCGCCTGTTGTCTTCAACTA-3′] and Hpod [3′-GGTGGAATGGGATTTTGTCTG-5′] (Podnar *et al.* 2007; Schulte *et al.* 2012b; Michaelides *et al.* 2013). All sampled individuals in non-native populations have been previously sequenced (Michaelides *et al.* 2013). Amplifications were carried out in a total volume of 15 μL consisting of 7.5 μL of MyTaq HS Mix (Bioline), 0.45 μL (8 pm) of each primer (Eurofins), 4.6 μL PCR-grade H_2O and 2 μL template DNA. 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 BIGDye Terminator v3.1 Ready Reaction kit (Applied Biosystems) in both directions. Products were precipitated in isopropanol and analysed on an ABI 3130 automated capillary sequencer (Applied Biosystems). Mitochondrial DNA sequences from both directions were corrected by eye and aligned to obtain a consensus sequence. Accepted sequences were then aligned using MAFFT (Katoh *et al.* 2002) implemented in GENEIOUS 6.1.7 (Drummond *et al.* 2011) and trimmed into a uniform length of 656 base pairs (bp). We translated the sequenced *cyt-b* region to amino acid sequences, to verify that no premature stop codons disrupted the reading frame. Unique sequences were submitted to GenBank under the accession numbers KP972470-KP972539.

Mitochondrial DNA analyses

We combined unique haplotypes from Italy (this study) with 12 haplotypes from England (Michaelides *et al.* 2013), and 129 sequences (of varying lengths) obtained from GenBank (Podnar *et al.* 2007; Schulte *et al.* 2008, 2012b; Giovannotti *et al.* 2010; Bellati *et al.* 2011; Gassert *et al.* 2013; Salvi *et al.* 2013; Michaelides *et al.* 2015) to build a phylogenetic tree and assign each population to a mitochondrial lineage (see details of geographic localities of all sequences used in Table S2, Supporting information). We implemented Bayesian inference (BI) analyses in MRBAYES (Huelsenbeck & Ronquist 2001) under the GTR + G + I nucleotide substitution model as selected by the best fit model applying the Akaike Information Criterion (AIC) in MEGA 5.2 (Tamura *et al.* 2011). Three sequences belonging to *Podarcis siculus* (AY770869) (Podnar *et al.* 2005), *P. liolepis* (JQ403296) (Schulte *et al.* 2012a) and *P. erchardi* (FJ867395) (Giovannotti *et al.* 2010) were used as outgroups. The BI analysis was run with four chains of 2 000 000 generations and sampling every 100 trees. We discarded (burn-in length) the first 10% 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.

To investigate evolutionary relationships between native and introduced haplotypes, we constructed a parsimonious phylogenetic network using a median-joining algorithm in NETWORK V.4.6.1.1 (Bandelt *et al.* 1999). We combined unique sequences from this study with sequences from non-native populations (Michaelides *et al.* 2013) and native French sequences (Michaelides *et al.* 2015). We also used this analysis to find identical UK haplotypes within the sampled native haplotypes.

Microsatellite analyses

We first tested for the presence of null alleles, effects of stuttering and large allele dropout using MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). We found no evidence of stuttering or large allele dropout. While some loci showed evidence of null alleles, these were not present across all populations; therefore, we retained all 16 loci for further analyses. To infer the population structure of the non-native populations, we implemented a Bayesian inference clustering method in STRUCTURE v.2.3.4 (Pritchard *et al.* 2000) using the admixture model (Falush *et al.* 2003) with correlated allele frequencies. We ran simulations with a burn-in of 100 000 iterations and a run length of 10^6 iterations from $K = 1$ through 23. Runs for each K were replicated five times and the best K was determined according to the method described by Evanno *et al.* (2005) in the online software STRUCTURE HARVESTER v.0.6.93 (Earl & vonHoldt 2011). We also ran the corresponding analysis including both native and non-native samples first under the same prior parameters and second using sampling locations (England, Italy and France) as prior information.

Approximate Bayesian computation (ABC). We estimated the relative likelihood of alternative scenarios (Fig. 1) that could explain the colonization routes of wall lizards in England using approximate Bayesian computation (ABC, Beaumont *et al.* 2002) in the program DIYABC v.2.0.4 (Cornuet *et al.* 2008, 2010, 2014). Because of the large number of populations, it is not feasible to separate the many scenarios that invoke specific native or introduced locations as sources. We therefore followed a sequential approach with a timeline based on the approximate date of introduction for each non-native population (see Michaelides *et al.* 2013). Using the oldest extant population (Ventnor, VT) as the starting point, we tested for each population whether it originated from anywhere within the native range (primary introduction model), from an unsampled population (unsampled source model) or from at least one previously established non-native population (secondary introduction model). To do this, we pooled native populations of the specific lineage together to create a native (N) pool of genotypes that could be evaluated against the total pool of genotypes of previously introduced, non-native, populations.

The parameters defining each scenario (i.e. effective population sizes (N_E), effective size of founders (N_F), time of introduction event (T_I) and duration of bottleneck events (B_D)) were considered random variables drawn from prior distributions (see Table S3, Supporting information). The mutation model for microsatellite loci was assumed to be a generalized stepwise-mutation (GSM) model (Estoup *et al.* 2002) and default values were kept (Cornuet *et al.* 2008, 2010, 2014). The coalescent-based algorithm simulates data sets for a number of predefined scenarios and compares the summary statistics of these with the summary statistics of the observed data. Summary statistics used in ABC were one-sample summary statistics including mean genetic diversity and mean size variance, and two-sample summary statistics including mean genetic diversity, mean size variance, pairwise F_{ST} values, shared allele distance and $d\mu^2$ distance. We first performed pre-evaluation of scenarios and prior distributions (option implemented in DIYABC v.2.04) to check that at least one combination of scenarios and priors can produce simulated data sets that are close enough to the observed data set. We then simulated 3×10^6 data sets and estimated the posterior probabilities of competing scenarios using a polychotomous logistic regression on 1% of simulated data sets

closest to the observed data set. For this analysis, summary statistics were transformed by linear discriminant analysis (LDA) (Estoup *et al.* 2012). In cases where confidence intervals were nonoverlapping between scenarios, we considered the one with the highest posterior probability to be well supported. To further assess confidence in selecting the most probable scenario, we analysed 500 pseudo-observed data sets using parameter values drawn from prior distributions (Table S3, Supporting information) and LDA-transformed summary statistics to calculate type I error (the probability of excluding the selected scenario when it is actually the true scenario) and type II error (the probability of selecting the scenario when it is not the true scenario).

Network analysis. To better understand the relationship among non-native populations and the extent of admixture through multiple introductions, we performed two network analyses: first, using proportions of membership (Q) in a defined cluster as a metric of relationship, and second, using genetic differentiation based on F_{ST} values. In these networks, each node represents a non-native population and the edges represent a relationship (genetic similarity). We excluded two populations (Bristol, BR; and Eastbourne, EB) with small sample size (five individuals). Using the admixture scores output from STRUCTURE, we constructed networks based on the average pairwise proportion of membership (Q) in a cluster. We considered two different threshold Q-values of 0.1 and 0.2, respectively, to be sufficient for a population to warrant membership in a cluster (see Vaha & Primmer 2006). We then averaged Q between two populations within a cluster and summed average values across clusters (where populations shared more than one cluster). We computed pairwise genetic differentiation (as F_{ST}) among non-native populations from microsatellite data in Arlequin 3.5.1.3 (Excoffier & Lischer 2010). To construct the network, we first included pairwise values below 0.15, because an F_{ST} value above this threshold is considered an indication of significant genetic differentiation among populations (Balloux & Lugon-Moulin 2002; Frankham *et al.* 2002). However, high polymorphism in the microsatellite loci and chance events might reduce genetic differentiation and overestimate real relationships (Wright 1978; Balloux & Lugon-Moulin 2002). We therefore used, in addition, a stricter threshold F_{ST} of 0.10. All networks were constructed in R (R Development Core Team 2011) using the packages 'igraph' (Csardi & Nepusz 2006) and 'popgraph' (Dyer 2014).

Results

We identified 70 new unique haplotypes in Italy that together with previously identified haplotypes from France (six haplotypes; Michaelides *et al.* 2015) and the UK (12 haplotypes; Michaelides *et al.* 2013) form six well-supported clades (Venetian, Tuscan, Romagna, Western France, Eastern France and Southern Alps clades; Schulte *et al.* 2011; see Table 1 and Fig. S1, Supporting information). The geographic distribution of these lineages in our sample is shown in Fig. 2. Four populations in the native range (in Italy) were found to harbour haplotypes from two different lineages (Fig. 2). The median-joining network grouped all sequences into six haplogroups (Fig. 3). The most common non-native haplotype (UKH4) was identical to the most common native Venetian haplotype (VEN1). Both Tuscan haplotypes in the introduced range were represented in native samples, with one (UKH11) being identical to the most common native Tuscan haplotype (TUS2). The most common non-native French haplotype (UKH6) was identical to the most common native Western France haplotype (WFR-H5).

Genetic structure

The Bayesian clustering analysis in STRUCTURE (see Fig. S2, Supporting information) revealed that $K = 9$ clusters best capture the genetic structure of the non-native populations in England. Fifteen populations belonged to a single cluster ($Q > 0.85$ and $Q < 0.1$ for any other cluster; see Table S5, Supporting information). The remaining eight populations showed evidence of admixture, with a considerable ($Q > 0.1$) proportion of membership in two or three clusters (Table S5). A STRUCTURE analysis combining all populations showed identical results (both with and without sampling locations as prior). French populations (both native and non-native) were grouped into two clusters (western and southwestern France), whereas the structure of Italian populations showed substantially higher differentiation between native and non-native populations (see Fig. S3, Supporting information).

Colonization scenario testing using ABC

We tested each introduced population to determine whether it was established from a native population (primary introduction scenario), from an unsampled source region or from a previously introduced population (secondary introduction scenario). Pre-evaluation of scenarios and prior distributions showed that the summary statistics from the observed data produced eigenvectors that were within the margins of the sets of simulated data sets (data not shown). We found high support (posterior probability (P), $P > 0.9$) for the secondary introduction model for ten populations (40%; Table 2). One population (Folkestone, FS) was probably a secondary introduction ($P = 0.44$). From the remaining

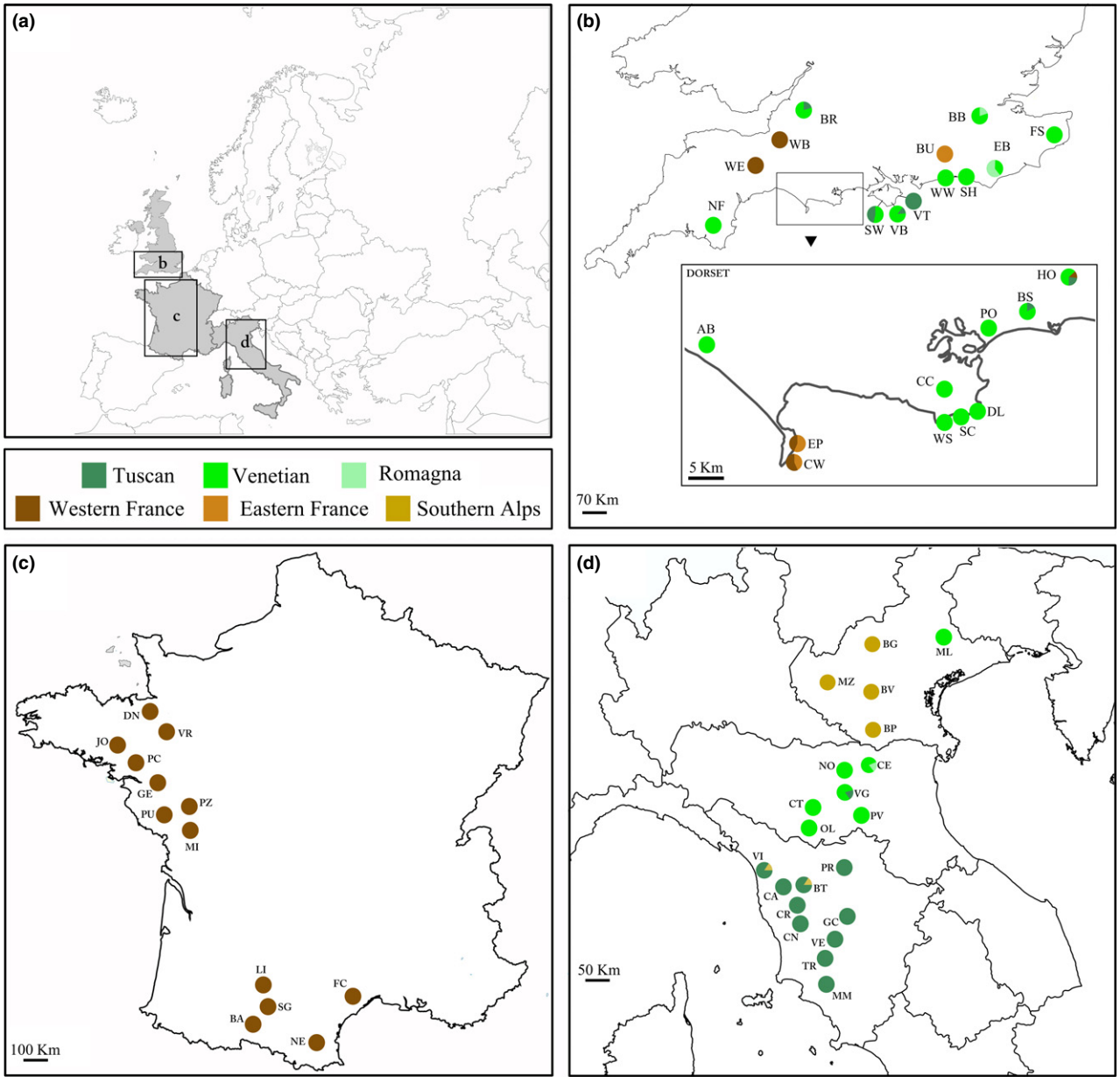

Fig. 2 Sampling locations and mtDNA lineage distribution; (a) location of the three sampling regions; (b) distribution of introduced populations in England (modified from Michaelides *et al.* 2013), (c) native populations in France (modified from Michaelides *et al.* 2015), (d) native populations in Italy. Population pie chart is coloured according to percentage of mtDNA lineage origin (see Table 1).

twelve populations, five could be confirmed as primary introductions from the native range, whereas for the remaining seven populations we were unable to separate with high confidence an introduction from the sampled native range or from an unsampled source. Two of these populations (BR, EB) have small sample size. For two of the French-origin populations (EP, CW), ABC suggested primary introduction for East Portland (EP, $P = 0.38$) and secondary introduction for CW ($P = 0.35$). The weak support is likely because of the lack of samples from the eastern part of the range of this clade (both non-native populations harbour haplotypes from both Western France and Eastern France clades). Confidence in scenario choice (type I and type II errors) for all populations is shown in Table S4.

Network analyses

We investigated further the relationship among non-native populations by constructing networks based on

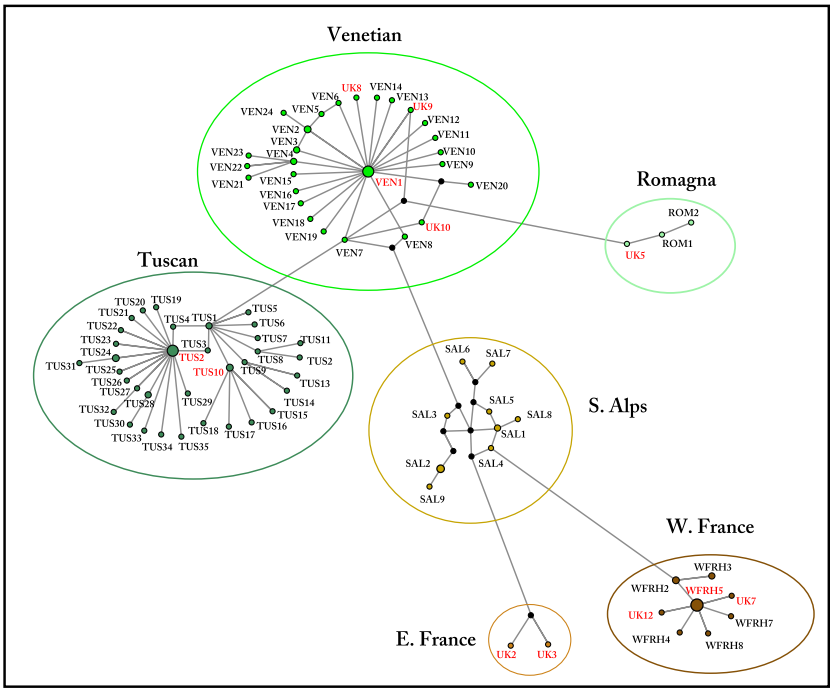

Fig. 3 Median-joining network of mtDNA sequences. Combined sequences analysed in this study with UK sequences (Michaelides *et al.* 2013) and French sequences (Michaelides *et al.* 2015). Six haplogroups have been identified corresponding to six lineages (Tuscan [TUS], Venetian [VEN], Romagna [ROM], Southern Alps [SAL], Western France [WFR] and Eastern France). Black dots represent median vectors required to connect sequences with maximum parsimony. The diameter of each circle (haplotype) corresponds to the number of populations sharing that haplotype. Introduced haplotypes (UKH1-UKH12) are indicated in red (UKH1 is identical to T10, UKH4 to V1, UKH6 to FRH5, and UKH11 to T2).

genetic similarity. The threshold for membership to a cluster of Q = 0.2 best corresponded to the results from STRUCTURE and shows two populations without connections, two small networks of two and four populations and the remaining 13 populations formed a structured network (Fig. 4; see Fig. S4a for results for Q = 0.1). The F_{ST}-based networks resulted in very similar results (Figs S4b and S4c). The likely origin and introduction history of each of the populations based on these analyses and other sources of information is described in more detail in Table S6.

Discussion

The first step to understand biological invasions is to establish the pathways of colonization – where did the non-native species come from and how did they get there (Estoup & Guillemaud 2010)? Our analyses reveal that the common wall lizard in England originates from at least nine introduction events from the native range. Secondary introductions from a previously established non-native population were well supported for eleven (47%) populations, all of which are restricted to the regions of Dorset and the Isle of Wight. There was also evidence for multiple introductions of animals from different geographic origin into the same non-native location, which create opportunities for hybridization.

The simplest scenario for the colonization of a particular area by an alien species is that all populations in the non-native range derive from a single source population in the native range (as for example *Anolis chlorocyanus* in Kolbe *et al.* 2007). However, multiple native-range sources is a common characteristic of biological invasions (Dlugosch & Parker 2008), including for invasive lizards (Kolbe *et al.* 2004, 2007; Chapple *et al.* 2012; Schulte *et al.* 2012b). The presence of haplotypes in the non-native populations from five geographically separated lineages shows that there are multiple native-range sources of wall lizards in England. The majority of introductions are from Italy, and we can establish with some confidence that the source regions are several different populations in Tuscany and in the vicinity of Bologna. Despite that these two regions are inhabited by different lineages, which appear to hybridize in contact zones, they share the primarily green-backed morphology and exaggeration of male secondary sexual characters that is typical of the subspecies *P. m. nigriventris* (Böhme 1986). As other parts of northern Italy are inhabited by the more common brown phenotype, this is consistent with preferential trading of animals that are considered charismatic (Kolbe *et al.* 2012). However, there were also several independent introductions of animals from France where the green form does not occur naturally.

Anecdotal evidence suggests that the earliest established extant populations in England involved animals that were either brought back by herpetologists or bought in pet shops (Table S6). Our data suggest that the commercial pet trade at that time did not make use of the existing population on the Isle of Wight (which is

Table 2 Posterior probabilities of the selected scenario for each non-native population tested by ABC. The selected scenario was the one with the significantly highest posterior probability value and with the 95% confidence interval (95% CI) not overlapping with the 95% CI of any other compared scenario. When the 95% CI overlaps, all values are reported.

Introduced population*	Posterior probability of selected scenario [confidence intervals, CI]		
	Primary introduction	Unsampled source region	Secondary introduction
VT	Oldest population introduced in England, considered a primary introduction		
BU	0.5935 [0.5830, 0.6039]		
BB	0.9319 [0.9124, 0.9514]		
SH	0.7822 [0.7642, 0.8003]		
NF		0.6440 [0.5173, 0.7706]	
WE	0.6029 [0.5930, 0.6127]		
SW			1.0000 [1.0000, 1.0000]
HO			0.9998 [0.9996, 1.0000]
WS			0.9106 [0.8750, 0.9463]
SC			0.9367 [0.8986, 0.9749]
WB	0.5591 [0.2183, 0.9000]	0.4409 [0.1000, 0.7817]	
DL			0.9951 [0.9929, 0.9973]
PO			0.9960 [0.9939, 0.9981]
FS			0.4444 [0.4014, 0.4875]
BS			0.9999 [0.9997, 1.0000]
EP	0.3817 [0.3651–0.3984]		
CW	0.3333[0.3030–0.3635]	0.3123[0.2835–0.3412]	0.3544 [0.3047–0.4040]
AB			0.9999 [0.9999, 1.0000]
VB			1.0000 [0.9999, 1.0000]
WW	0.5010 [0.4931, 0.5089]	0.4969 [0.4890, 0.5048]	
BR†	0.4160 [0.0462, 0.7859]	0.5561 [0.1925, 0.9198]	
CC			0.9963 [0.9882, 1.0000]
EB†	0.4063 [0.3078, 0.5048]	0.4980 [0.4011, 0.5949]	

*Populations are ordered according to approximate time of introduction.
†Limited sample size (=5).

of Tuscan *P. m. nigriventris* origin), nor the previously extensive import from Jersey (which was officially banned in the 1940s; Michaelides *et al.* 2015), but instead imported animals direct from the Bologna–Modena region. The patterns in England can be compared to the origin of wall lizards in Central Europe, which also includes several different clades (Schulte *et al.* 2012b). For example, the source area of the largest known invasive population in the Passau region in Germany has also been assigned to the Bologna–Modena region (Schulte *et al.* 2013). However, in other non-native populations in Central Europe, the Eastern France and Southern Alps clades are most common, and the presence of Venetian haplotypes appears to be partly because animals derive from a hybrid zone with the Southern Alps clade (Schulte *et al.* 2012b) which suggests a different origin to English populations. Although the colonization routes have not been established for German non-native populations of wall lizards, these data are consistent with many independent introductions in both England and Germany, the origin of which depends on country-specific accessibility of animals.

The shared haplotypes and genetic structure of non-native populations in England suggest that previously established populations have served as stepping stones for further introductions. Our ABC analyses provided strong support that eleven of the 23 populations originate from wild non-native sources in England. These form two clusters, one on the Isle of Wight and one on the south coast of Dorset, where eight of ten populations are secondary introductions. Recent studies of both animals and plants have reported secondary introductions from established non-native populations, but the majority of these probably represent unassisted range expansion or nondeliberate human introductions (*Anolis sagrei*, Kolbe *et al.* 2004; *Diabrotica virgifera virgifera*, Miller *et al.* 2005; *Harmonia axyridis*, Lombaert *et al.* 2010; *Solenopsis invicta*, Ascunce *et al.* 2011; *Silene latifolia*, Keller *et al.* 2012). We can exclude natural range expansion as an explanation on the basis of their geographic distribution, which has major gaps despite suitable habitat, and the lack of evidence of dispersal even between closely situated sites (Langham 2014; T Uller & GM While pers obs; While *et al.* 2015). Nondeliberate introductions are also unlikely, given that the majority

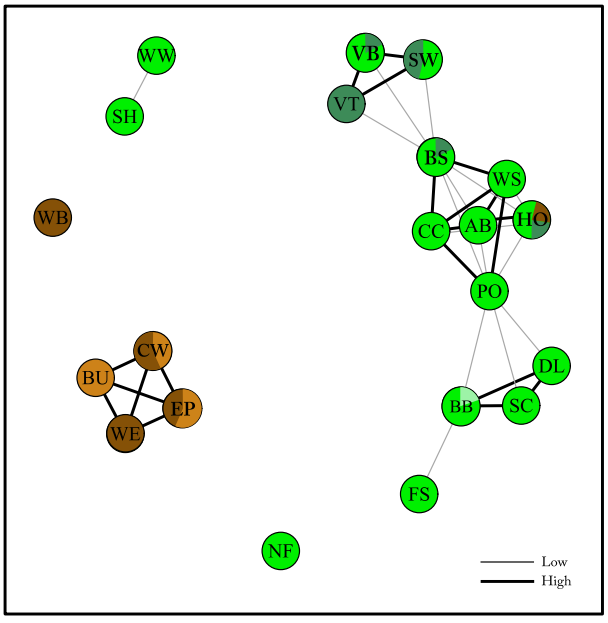

Fig. 4 Network analysis of introduced populations in England. The network is constructed based on average pairwise proportion of membership (Q) in a cluster (*K*) as defined by STRUCTURE. Populations are colour-coded according to mtDNA lineage. The thickness of the line connecting two nodes (populations) corresponds to the strength of the pairwise similarity (bottom right corner of each panel).

of secondary introductions are in disused quarries or other suitable habitat rather than in villages or gardens, which would be the pattern if the animals originated from escaped pets or were accidentally translocated. In contrast, the locations of several primary introductions are entirely consistent with accidental introductions (Table S6). While we cannot rule out the possibility that the populations supported as secondary introductions in our study have a common source (e.g. a captive population) rather than serving as stepping stones *per se*, the fact that we see structure in the network linking these populations suggests that at least some have served as true stepping stones. In contrast, the cluster of populations further east on the English south coast appear to have separate origins from populations on the central south coast and on the Isle of Wight, and several are likely to be independent primary introductions from the native range.

The local nature of secondary introductions along the Dorset coast (primarily in disused quarries) is indicative of an isolated but deliberate attempt to establish the species in the region. Similarly, it is noticeable that the oldest, perhaps largest, and well-known population on Isle of Wight (Ventnor, VT) has only been used as a source for two local introductions. These results emphasize that stepping-stone populations may have very local effects even when human-mediated processes are the primary source of spread. On the contrary, stepping-stone populations may have wider effects in Germany, where 15 non-native populations across the country share identical haplotypes of the Venetian clade indicating human-mediated secondary introductions (Schulte *et al.* 2011, 2012c). Somewhat surprisingly, the data in Table 2 do not support our expectation that secondary introductions should become more common over time, as a result of constraints on importation due to more strict legislation and increased public ethical and legislative issues. Instead, the data suggest active translocation of animals of two or three introduced populations within a limited geographic region from the mid-1980s, possibly mixed with animals from other native sources, with additional independent primary introductions continuing to take place elsewhere well into the 21st century. Despite being geographically isolated, the presence of secondary introductions may have significant implications for further human-mediated expansion. Animals introduced from the native range face serious climatic challenges, and introductions are likely to fail. However, recent evidence from non-native populations of both Italian and French origins show they have adapted to the cooler climate in England (While *et al.* 2015). This suggests that the opportunity for natural range expansion may increase over time and that preventing translocations is important to avoid expansion of the species in England.

While our analyses provide strong evidence for multiple origins of wall lizards in England as a whole, the evidence for admixture within populations is more ambiguous. To some extent, this ambiguity results from the complex phylogeography of the species in its native range. A fine geographic structure in mtDNA haplotypic variation in the native range may facilitate the detection of multiple sources (e.g. *A. sagrei* in Kolbe *et al.* 2004). In our native-range sampling, we identified four populations in Italy that contained haplotypes from two lineages, suggesting potential hybrid regions. This makes the identification of the source population and the number of introductions more complicated as the genetic structure of non-native populations could be attributed to either a single introduction from a hybrid region or two separate introductions. The likely presence of bottlenecks further complicates the situation. Nevertheless, we were able to separate these scenarios with some confidence in some cases. For example, the population in London (Birdbrook, BB) harbours haplotypes from two different Italian lineages. The ABC analysis suggested that the population originated independently from the native range, which is consistent with data showing one population (Cento, CE) that harbours the same combination of haplotypes (from two

lineages) as the non-native population. On the contrary, our analyses do support that two populations on the Isle of Wight (Ventnor Botanical Garden, VB; and Shorewell, SW) are secondary introductions from the oldest established non-native population on the island (VT) and at least one other population from England.

In conclusion, the colonization of *Podarcis muralis* in England involves at least nine introduction events from multiple native sources. This probably reflects that there have been a number of private and commercial import channels up until at least the 1990s. Several populations appear to have multiple origins, although some of these may be introductions of animals from hybrid zones. At least 47% of the introduced populations were established using animals already present in England. However, secondary introductions are geographically restricted, suggesting that non-native populations have not been widely exploited for the pet trade within the UK.

Acknowledgements

We are indebted to Steve Langham for his efforts and help in locating introduced wall lizard populations. We are also grateful to Charles Snell, Martin Noble, Fred and Pat Howarth, Shona McDonough, the Lever family, Tony Pashley, Mark Anderson, Ian Boyd, Nick Squirrel, Tim Bernhard, Tanya French, and Anthony Mitchell for outstanding help with providing access to private gardens and lands, giving us pointers that helped establish the geographic limits of populations, and retrieve details on the introduction history. Roberto Sacchi and Marco Zuffi provided invaluable logistic support in Italy and Fabien Aubret assisted in France. Lindall Kidd, Hannah MacGregor, Rob Heathcote and Ben Daly helped in the field. Finally, we thank three anonymous reviewers who improved the manuscript by their comments. Funding for this project was obtained from the British Ecological Society, the National Geographic Society and the Royal Society (all to TU). SNM is supported by the AG Leventis Foundation and the Biotechnology & Biological Sciences Research Council (BBSRC). GMW was funded by a FP7 Marie Curie Fellowship. TU is supported by the Royal Society of London and the Knut and Alice Wallenberg Foundations.

References

Allan GM, Prelypchan CJ, Gregory PT (2006) Population profile of an introduced species, the common wall lizard (*Podarcis muralis*), on Vancouver Island, Canada. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **84**, 51–57.

Ascunce MS, Yang CC, Oakey J *et al.* (2011) Global invasion history of the fire ant *Solenopsis invicta*. *Science*, **331**, 1066–1068.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155–165.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Beaumont MA, Zhang WY, Balding DJ (2002) Approximate Bayesian computation in population genetics. *Genetics*, **162**, 2025–2035.

Bellati A, Pellitteri-Rosa D, Sacchi R *et al.* (2011) Molecular survey of morphological subspecies reveals new mitochondrial lineages in *Podarcis muralis* (Squamata: Lacertidae) from the Tuscan Archipelago (Italy). *Journal of Zoological Systematics and Evolutionary Research*, **49**, 240–250.

Böhme W (1986) Handbuch der Amphibien und Reptilien Europas. Bandll/2, Echsen (Sauria) III (Lacertidae lll; Podarcis) Aula-Verlag, Wiesbaden.

Burke R, Deichsel G (2008) Lacertid lizard introductions into North America: history and future. In: *Urban Herpetology* (eds Mitchell JC, Brown REJ, Bartholomew B), pp. 347–353. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah.

Chapple DG, Miller KA, Kraus F, Thompson MB (2012) Divergent introduction histories among invasive populations of the delicate skink (*Lampropholis delicata*): has the importance of genetic admixture in the success of biological invasions been overemphasized? *Diversity and Distributions*, **19**, 134–146.

Cornuet JM, Santos F, Beaumont MA *et al.* (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *BMC Bioinformatics*, **24**, 2713–2719.

Cornuet J-M, Ravigné V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics*, **11**, 401.

Cornuet JM, Pudlo P, Veyssier J *et al.* (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *BMC Bioinformatics*, **30**, 1187–1189.

Csardi G, Nepusz T (2006) The igraph software package for complex network research. *InterJournal Complex Systems*, **1695**, 1–9.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Drummond AJ, Ashton B, Buxton S *et al.* (2011) Geneious v6.1.7, p. Available from: http://www.geneious.com/.

Dyer RJ (2014) Popgraph: This is an R package that constructs and manipulates population graphs, R Package version 1.4. Available from: http://CRAN.R-project.org/package=popgraph.

Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, **19**, 4113–4130.

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology*, **11**, 1591–1604.

Estoup A, Lombaert E, Marin JM *et al.* (2012) Estimation of demo-genetic model probabilities with Approximate Bayesian Computation using linear discriminant analysis on summary statistics. *Molecular Ecology Resources*, **12**, 846–855.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Floerl O, Inglis GJ, Dey K, Smith A (2009) The importance of transport hubs in stepping-stone invasions. *Journal of Applied Ecology*, **46**, 37–45.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.

Frazer JFD (1964) Introduced species of amphibians and reptiles in mainland Britain. *British Journal of Herpetology*, **3**, 145–150.

Gassert F, Schulte U, Husemann M *et al.* (2013) From southern refugia to the northern range margin: genetic population structure of the common wall lizard, *Podarcis muralis*. *Journal of Biogeography*, **40**, 1475–1489.

Giovannotti M, Nisi-Cerioni P, Caputo V (2010) Mitochondrial DNA sequence analysis reveals multiple Pleistocene glacial refugia for *Podarcis muralis* (Laurenti, 1768) in the Italian Peninsula. *Italian Journal of Zoology*, **77**, 277–288.

Gleed-Owen CP (2004) Green lizards and wall lizards on Bournemouth cliffs. *Herpetological Bulletin*, **88**, 3–7.

Heathcote RJP, Dawson DA, Uller T (2014) Characterisation of nine European wall lizard (*Podarcis muralis*) microsatellite loci of utility across sub-species. *Conservation Genetics Resources*, **7**, 85–87.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: bayesian inference of phylogenetic trees. *BMC Bioinformatics*, **17**, 754–755.

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, **46**, 10–18.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecology Letters*, **11**, 852–866.

Keller SR, Gilbert KJ, Fields PD, Taylor DR (2012) Bayesian inference of a complex invasion history revealed by nuclear and chloroplast genetic diversity in the colonizing plant, *Silene latifolia*. *Molecular Ecology*, **21**, 4721–4734.

Kolbe JJ, Glor RE, Rodríguez Schettino L *et al.* (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Kolbe JJ, Glor RE, Schettino LR *et al.* (2007) Multiple sources, admixture, and genetic variation in introduced Anolis lizard populations. *Conservation Biology*, **21**, 1612–1625.

Kolbe JJ, Lavin BR, Burke RL *et al.* (2012) The desire for variety: Italian wall lizard (*Podarcis siculus*) populations introduced to the United States via the pet trade are derived from multiple native-range sources. *Biological Invasions*, **15**, 775–783.

Kraus F (2009) *Alien Reptiles and Amphibians: A Scientific Compendium and Analysis*. Springer Publishing Company, New York City, New York.

Krysko KL, Burgess JP, Rochford MR *et al.* (2011) Verified non-indigenous amphibians and reptiles in Florida from 1863 through 2010: outlining the invasion process and identifying invasion pathways and stages. *Zootaxa*, **3028**, 1–64.

Langham S (2014) The Wall Lizard Project - Surrey Amphibian and Reptile Group. http://www.surrey-arg.org.uk/SARG/02000-Activities/SurveyAndMonitoring/WallLizard/PMSitePicker.asp.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386–391.

Lever C (1977) *The Naturalized Animals of the British Isles*. Hutchinson, London.

Lombaert E, Guillemaud T, Cornuet J-M *et al.* (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. *PLoS ONE*, **5**, e9743.

Long JS (1981) *Introduced Birds of the World: The Worldwide History, Distribution and Influence of Birds Introduced to new Environments*. David & Charles, London.

Mack RN, Simberloff D, Lonsdale WM *et al.* (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Michaelides S, While G, Bell C, Uller T (2013) Human introductions create opportunities for intra-specific hybridization in an alien lizard. *Biological Invasions*, **15**, 1101–1112.

Michaelides S, Cornish N, Griffiths R *et al.* (2015) Phylogeography and conservation genetics of the common wall lizard, *Podarcis muralis*, on islands at its northern range. *PLoS ONE*, **10**, e0117113.

Miller N, Estoup A, Toepfer S *et al.* (2005) Multiple transatlantic introductions of the western corn rootworm. *Science*, **310**, 992.

Podnar M, Mayer W, Tvrtkovic N (2005) Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology*, **14**, 575–588.

Podnar M, Haring E, Pinsker W, Mayer W, Ballantine WJ (2007) Unusual origin of a nuclear pseudogene in the Italian wall lizard: intergenomic and interspecific transfer of a large section of the mitochondrial genome in the genus *Podarcis* (Lacertidae). *Journal of Molecular Evolution*, **64**, 308–320.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Richard M, Stevens VM, Hénanff ML, Coulon A (2012) Fourteen new polymorphic microsatellite loci for the wall lizard *Podarcis muralis* (Sauria: Lacertidae). *Molecular Ecology Resources*, **00**, 1–5.

Ruiz GM, Carlton J (2003) *Invasive Species: Vectors And Management Strategies*. Island Press, Washington, District of Columbia.

Salvi D, Harris DJ, Kaliontzopoulou A, Carretero MA, Pinho C (2013) Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evolutionary biology*, **13**, 147.

Sax DF, Stachowicz JJ, Brown JH *et al.* (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution*, **22**, 465–471.

Schulte U (2008) *Die Mauereidechse*. Laurenti Verlag, Bielefeld.

Schulte U, Thiesmeier B, Wayer W, Schweiger S (2008) Allochthone Vorkommen der Mauereidechse (*Podarcis muralis*) in Deutschland. *Zeitschrift für Feldherpetologie*, **15**, 139–156.

Schulte U, Bidinger K, Deichsel G *et al.* (2011) Verbreitung, geografische Herkunft und naturschutzrechtliche Aspekte allochthoner Vorkommen der Mauereidechse (*Podarcis muralis*) in Deutschland. *Zeitschrift für Feldherpetologie*, **18**, 161–180.

Schulte U, Gassert F, Geniez P, Veith M, Hochkirch A (2012a) Origin and genetic diversity of an introduced wall lizard population and its cryptic congener. *Amphibia-Reptilia*, **33**, 129–140.

Schulte U, Hochkirch A, Loetters S *et al.* (2012b) Cryptic niche conservatism among evolutionary lineages of an invasive lizard. *Global Ecology and Biogeography*, **21**, 198–211.

Schulte U, Veith M, Hochkirch A (2012c) Rapid genetic assimilation of native wall lizard populations (*Podarcis muralis*) through extensive hybridization with introduced lineages. *Molecular Ecology*, **21**, 4313–4326.

Schulte U, Veith M, Mingo V, Modica C, Hochkirch A (2013) Strong genetic differentiation due to multiple founder events during a recent range expansion of an introduced wall lizard population. *Biological Invasions*, **15**, 2639–2649.

Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. *Proceedings of the National Academy of Sciences USA*, **98**, 1095–1100.

Tamura K, Peterson D, Peterson N *et al.* (2011) MEGA5: molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Vaha JP, Primmer CR (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology*, **15**, 63–72.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

While GM, Williamson J, Prescott G *et al.* (2015) Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings of the Royal Society London Series B: Biological Sciences*, **282**, 20142638.

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution*, **24**, 136–144.

Wright S (1978) *Evolution and the Genetics of Population, Variability Within and Among Natural Populations*. The University of Chicago Press, Chicago, Illinois.

S.N.M., G.M.W. and T.U. conceived of and managed the project, collected data and wrote the paper. S.N.M. analyzed the data. N.Z. collected data. All authors commented on the paper.

Data accessibility

Sampling locations: Table 1. mtDNA sequences from this study: GenBank accession numbers KP972470-KP972539. Microsatellite genotypes (16 loci) and mtDNA cyt-b gene alignment of 211 sequences used in phylogenetic analysis: doi:10.5061/dryad.6k2qm

Supporting information

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

Fig. S1 Bayesian inference consensus tree derived from mitochondrial *cyt-b* sequences.

Fig. S2 Bayesian Clustering Analysis in STRUCTURE.

Fig. S3 Bayesian Clustering Analysis in STRUCTURE including both native and introduced populations.

Fig. S4 Networks based on genetic similarity.

Table S1 Microsatellite marker multiplexes used to genotype all individuals in this study.

Table S2 List of all cytochrome b (*cyt-b*) sequence data used in the phylogenetic analysis.

Table S3 Prior distributions of the historical, demographic and mutation parameters used in ABC analyses.

Table S4 Confidence in scenario choice by estimating type I and II errors.

Table S5 Proportion of membership (Q) in each cluster.

Table S6 Introduction history of *Podarcis muralis* populations in England.