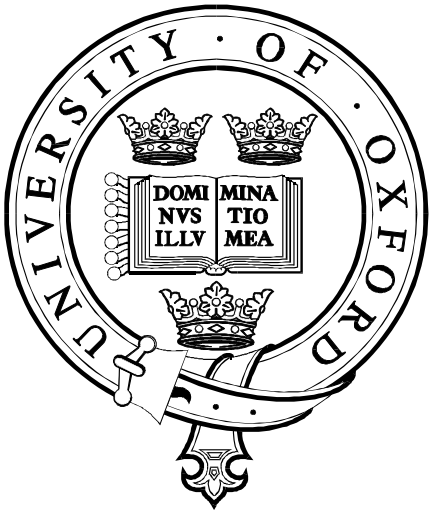
GENETICS OF COLONISATION IN THE COMMON WALL LIZARD



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CHAPTER 2| PHYLOGEOGRAPHY AND CONSERVATION GENETICS OF THE COMMON WALL LIZARD, *PODARCIS MURALIS* ON ISLANDS AT ITS NORTHERN RANGE

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CHAPTER 3| HUMAN INTRODUCTIONS CREATE OPPORTUNITIES FOR INTRA-SPECIFIC HYBRIDIZATION IN AN ALIEN LIZARD

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CHAPTER 4| WIDESPREAD PRIMARY, BUT GEOGRAPHICALLY RESTRICTED SECONDARY, HUMAN INTRODUCTIONS OF WALL LIZARDS, *PODARCIS MURALIS*

SNM collected data, conducted lab work and all statistical analyses and wrote the manuscript in collaboration with **GMW** and **TU. NZ** collected data and conducted lab work, **TU** collected data and supervised **SNM** and **GMW** collected data. All authors commented on all versions of the manuscript.

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CHAPTER 5| LOSS OF GENETIC DIVERSITY AND INCREASED EMBRYONIC MORTALITY IN NON-NATIVE LIZARD POPULATIONS

SNM collected data, conducted lab work and all statistical analyses and wrote the manuscript in collaboration with **GMW** and **TU. NZ** collected data and conducted lab work, **TU** collected data and supervised **SNM, GMW** collected data, **MALZ, RS, BC** and **FA** provided data from native populations.

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THESIS ABSTRACT

In this thesis I set out to further our understanding of the causes and consequences of genetic variation after colonisation events. Specifically, I focused on how historical processes shape genetic diversity and to what extent we can link colonisation history, genetic diversity, individual fitness and population viability. To achieve this, I used a combination of molecular markers, analytical tools and the common wall lizard, *Podarcis muralis* as a study system. I first infer the origin and genetic architecture of isolated population on islands at the range margin, in relation to mainland populations, to determine whether their current distribution and genetic structure are a result of a historical colonisation event or a more recent introduction. I then unravel the details of human-mediated introductions of *P. muralis* in England to further test which factors affect their genetic structure. I ask about the contribution of multiple introductions and admixture, the importance of number of founders and the year since their introduction and whether bottleneck events during primary and /or secondary introduction predict the level of genetic diversity in the non-native range. Throughout this study I obtain information on population genetic structure and composition from both native and non-native ranges. This is essential since the (complex) phylogeographic structure of *P. muralis* in the native range determines the distribution and structure of genetic diversity from which colonists are drawn and the details of colonisation will then reflect in the genetics of non-native populations. Lastly, I assess the consequences of colonisation on reproductive fitness and test for heterozygosity fitness correlations at the individual and population level. Overall, this thesis demonstrates why reconstructing the colonisation history is important when aiming to understand the causes and consequences of genetic variation during colonisation. This information is critical when assessing the relationship between genetic diversity and establishment success. Whether non-native populations have retained sufficient evolutionary potential to adapt to their new climate their long-term viability will be dictated by availability of suitable habitat rather than by internal population factors.

CHAPTER 1

BACKGROUND

Among the most fundamental challenges in ecology and evolution are to understand the causes and consequences of species distribution and variation. These processes can be studied at different temporal and spatial scales, and at different levels of biological organisation, from genes to communities. There is also a practical dimension to this work; conservation of species and populations require knowledge of what drives changes in species distribution and makes populations more vulnerable to extinction. Population genetics play a central role in this endeavour. For example, lack of genetic variation should result in reduced ability to adapt and increased risk of extinction (Frankham 2005a). Low genetic variation is also symptomatic of small population size, where inbreeding may be high and hence drive populations to extinction through inbreeding depression (Keller & Waller 2002). Consequently, conservation biology is increasingly dedicated to the genetics of small and vulnerable populations (Frankham *et al.* 2002). However, despite extensive experimental and theoretical research to assess the roles of demography and genetics for population viability, there is still lack of explicit hypothesis testing in natural populations. This is partly because of the logistical challenges of replication and experimentation in wild populations. *Colonising species* (see GLOSSARY), referring to species that are able to overcome geographical barriers (both naturally or human-aided), allow us to study ecological and evolutionary processes occurring in real-time and can be seen as 'natural experiments' (Sakai *et al.* 2001; Lee 2002; Sax *et al.* 2007). The theme of this thesis is to investigate the causes and consequences of genetic variation after colonisation events, using the common wall lizard (*Podarcis muralis*) as a study system.

COLONISATION EVENTS: NATURAL RANGE EXPANSIONS *VS.* ANTHROPOGENIC INTRODUCTIONS

The geographic distribution of a species can be highly dynamic; it is modified over time as populations expand or contract in response to biotic and abiotic conditions (Sexton *et al.* 2009). The rate and distance of individual dispersal is a key life-history trait of fundamental importance to range formations and (re)colonisation events. For example, long-distance migrants were the first to recolonise post-glacial Europe during the Pleistocene (Hewitt 1999) and colonisation ability is reflected in species compositions of islands (MacArthur & Wilson 1967). Such events occurred over long time periods; however, range expansions (shifts) have also been documented in parallel with climate change in many taxonomic groups and geographical locations during the 20th century (Parmesan 2006). Modifications in species distribution, population size and connectivity are reflected in the amount and structuring of genetic diversity within contemporary populations, largely due to the effects of natural selection, genetic drift and gene flow.

In contrast to natural range expansion, which is usually a slow process, human activities are increasingly modifying the distribution and abundance of species (Mack *et al.* 2000). For human-mediated introductions, the intrinsic dispersal ability of species may not be very important. Instead, behavioural traits may play a greater role in range expansion by increasing the propensity for a species to be transported. For example, species that expand their range through human activities tend to be found in close proximity to human-occupied environments (e.g., urban habitats) and may exhibit behaviours that make them actively hide and/or seek shelter (e.g., the delicate skink, *Lampropholis delicata*, Chapple *et al.* 2011), thus increasing their likelihood of being transported to new locations (Chapple *et al.* 2012). Such rapid colonisation

events have dramatic consequences for the amount and structuring of the genetic diversity within the newly founded population.

The colonisation process is often associated with a small number of individuals that depart from a location (transportation stage), arrive in a new area (introduction stage), establish and spread. Since genetic variability is the basis of evolutionary change (Lewontin 1974), the amount and structuring of genetic variation that is transferred by colonising individuals from one location to another could influence the likelihood of successful establishment, future spread and the long-term viability of populations. It is therefore a central focus of research to document the causes and consequences of genetic variation during colonisation events (Figure 1 and 2).

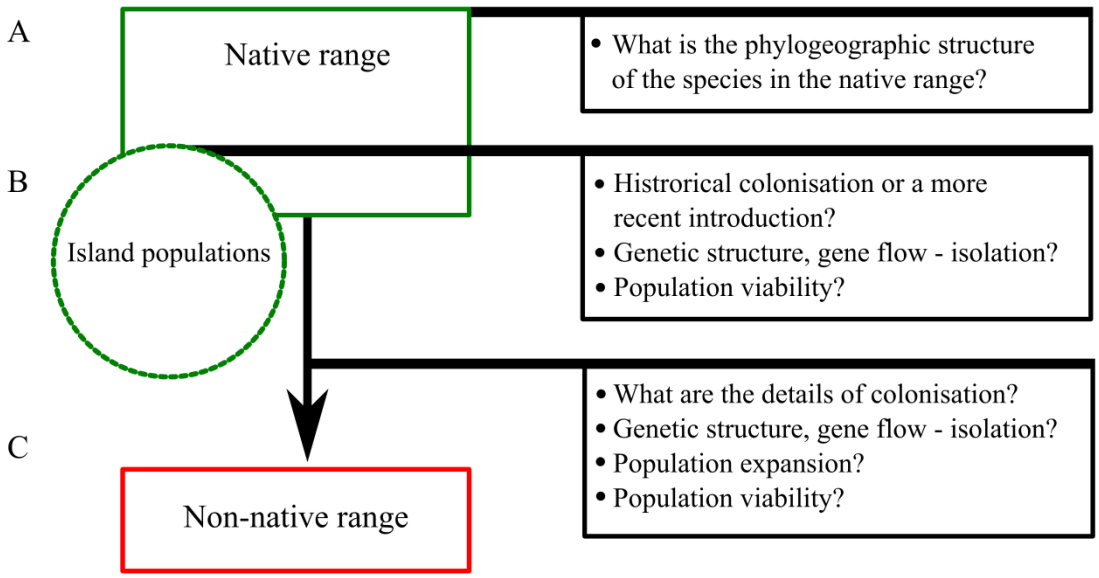

Figure 1 | Schematic illustration of the key questions addressed within this thesis. A) In relation to the native range of a species, B) Colonisation events on islands at the range margin and C) Human-mediated introductions outside the native range.

THE GENETICS OF COLONISING SPECIES

Over the last 50 years since the publication of *The Genetics of Colonizing Species (1965)*, edited by H.G. Baker and G.L. Stebbins, research has been focused on integrating ecology, genetics and evolution in order to understand the causes and consequences of colonisation (Barrett 2015; Bock *et al.* 2015; Dlugosch *et al.* 2015). Traditionally, genetic variation was measured at the protein level, but over the last two decades neutral DNA markers, such as, nuclear microsatellites and maternally-inherited mitochondrial (mtDNA) or chloroplast DNA (cpDNA), have been commonly used to quantify the levels and patterns of genetic diversity and phylogeographic structure (Frankham *et al.* 2002). The molecular toolbox is expanding (e.g. full genomes are increasingly available) and the field of *invasion genetics* is developing rapidly. One major challenge is to unravel the (sometimes complex) colonisation history and identify the factors determining the amount and structure of genetic diversity in contemporary populations following a colonisation event (Figure 2).

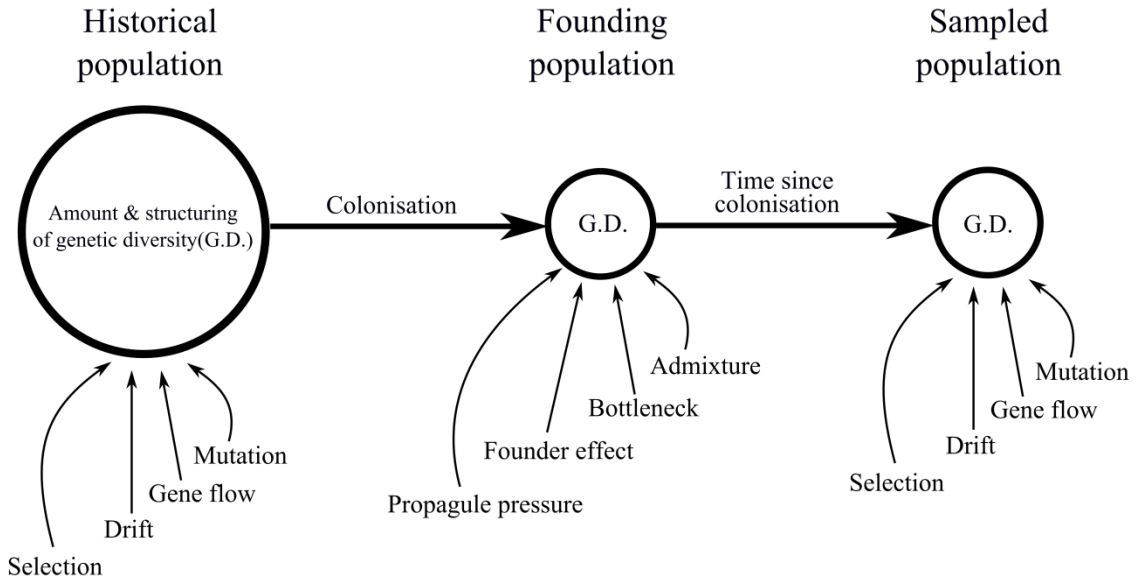

Figure 2 | Path diagram illustrating the colonisation process. Vertical arrows show the factors shaping the amount and structuring of genetic diversity (G.D.) of populations.

Contemporary levels of genetic diversity in recently colonising species reflect a number of different processes (Figure 2). Firstly, the evolutionary history of a species in its native range will determine the structure of genetic diversity from which colonists are drawn (Keller & Taylor 2008). Secondly, natural selection, genetic drift and gene flow will have a strong influence on the genetic architecture of colonising populations and consequently, the rate and extent of divergence from the source populations. Finally, fluctuations in population size through time and the spatial distribution of neighbouring populations affect the loss (via stochastic drift) or replenishment (via gene flow) of genetic variation (Eckert *et al.* 2008). Hence, analysis of the evolutionary history of populations across the geographical range of a species can provide information about the distribution of diversity available prior to colonisation (Eckert *et al.* 2008; Keller & Taylor 2008). History, selection and chance events will then affect genetic diversity during the colonisation process (Keller & Taylor 2008).

Over the course of a colonisation event, species may experience dramatic changes in population structure and genetic diversity due to *founder* and *bottleneck events* (Sakai *et al.* 2001; Dlugosch & Parker 2008). *Propagule pressure* and the genotypes of founder individuals will be subject to genetic drift and theory predicts a depletion of genetic variation in founding populations (Nei *et al.* 1975). Since many colonisation events involve a small number of propagules, a severe reduction in genetic diversity is likely to affect their establishment success and reduce the adaptive potential of the founding population. Yet, many widespread, *invasive populations* have successfully established from relatively few propagules and these cases represent the *genetic paradox of invasion* (Frankham 2005b).

GLOSSARY

- **Admixture:** Mixing of genotypes from geographically and genetically distinct populations.
- **Bottleneck event:** Decrease in genetic diversity resulting from a significant reduction in population size for at least one generation.
- **Colonisations:** Natural extra-range dispersal (e.g. the colonisation of islands) and human-aided introductions into new environments.
- **Exotic:** Non-native plants or animals introduced intentionally or accidently to an area through human activity. Synonyms; *alien*, non-native, *non-indigenous*, *introduced.*
- **Founder effect:** The occurrence in which founders of a new population carry only a fraction of the total genetic variation in the source population because of sampling error resulting from their small numbers.
- **Genetic paradox:** A dilemma in invasion biology: how do newly founded populations overcome low genetic diversity and expected low evolutionary potential, typically associated with extinction risk, to become established outside of their native range? (Roman & Darling 2007b)
- **Hybrid vigour (heterosis)**: Phenotypic superiority of a hybrid over its parents, presumably due to increased levels of heterozygosity.
- **Hybridisation:** Crossing between two or more distinct lineages, typically subspecies or species.
- **Inbreeding depression:** When inbreeding has a significant cost to fitness, for example by decreasing survival.
- **Inbreeding:** Inbreeding occurs when closely related individuals mate with each other.
- **Invasion genetics:** The study of the historical, ecological and demographic processes responsible for the patterns of genetic diversity in populations and their influence on invasion success and contemporary evolution during biological invasion (Barrett 2015).
- **Invasive species**: A non-native species that harms the native biota, spreads from the point of introductions and becomes abundant
- **Multiple introductions:** Repeated introduction events of species into the same non-native location. The introductions could be from the same native location or different locations (promoting admixture).
- **Mutation load:** reduction in fitness due to the presence of deleterious mutations segregating at mutation-selection balance (Agrawal & Whitlock 2012).
- **Propagule pressure:** The sum of all release events of the number of individuals introduced at a given location (Simberloff 2009).

The genetic paradox of invasion is now, at least partly, resolved since there is compelling evidence to suggest that the effects of bottlenecks are often counteracted by *multiple introductions* and *admixture*, which could increase genetic variation (Roman & Darling 2007; Dlugosch & Parker 2008; Uller & Leimu 2011; Rius & Darling 2014). Multiple genotypes within an introduced population, either as several independent or repeated introductions, can erase the genetic signature of a founder effect via gene flow. For example, Kolbe *et al*. (2004) identified eight independent introductions of the brown anole in Florida, from locations across their native range.

Mixing of genetic material from distinct native sources resulted in introduced populations that harbour substantially more genetic variation than the source populations, and such cases have been found in many human-aided introductions (e.g. Kolbe *et al.* 2007; Lavergne & Molofsky 2007; Facon *et al.* 2008). In addition to increased genetic diversity (and phenotypic viability or *hybrid vigour*), admixture has been found to benefit newly established populations through masking or purging deleterious mutations and as a result reducing the likelihood of *inbreeding depression* (Ellstrand & Schierenbeck 2000; Keller & Waller 2002). Therefore, admixture can enhance the opportunity for evolution within introduced populations and may facilitate rapid divergence between native and introduced populations (Lavergne and Molofsky 2007; Keller and Taylor 2010). However, the importance of admixture for the fitness of individuals and the successful establishment of non-native populations remain poorly understood (Rius & Darling 2014).

CONSEQUENCES OF COLONISATION ON POPULATION VIABILITY

Colonisations are commonly associated with periods of small population size. Lessons from island biogeography and conservation genetics demonstrate that prolonged periods of isolation and low population size result in increased among-population differentiation and may also cause severe inbreeding. Inbreeding increases homozygosity and could lead to the expression of deleterious recessive mutations (*mutation load)* which reduces the fitness of individuals (i.e., inbreeding depression) and the viability of populations (Keller & Waller 2002). For example, established *exotic* bird species in New Zealand experienced severe bottlenecks that reduced genetic diversity and increased hatching failure (Merilä *et al.* 1996; Briskie & Mackintosh 2004; Heber & Briskie 2010). Such signatures of negative genetic effects

have been assessed by looking for correlations between individual heterozygosity (typically measured using neutral markers which provide estimates of average heterozygosity across the genome, Szulkin *et al.* 2010) and fitness-related traits, such as fecundity or survival (Heterozygosity-Fitness Correlations, HFCs; Chapman *et al.* 2009; Szulkin *et al.* 2010). Despite overall weak effect sizes (Chapman *et al.* 2009) such associations can be informative about the fitness consequences of demographic and stochastic events during colonisation that are likely to affect population viability in the long-term.

STUDYING COLONISATION EVENTS: KNOWNS AND UNKNOWNS

Over the last few decades, the application of molecular marker techniques and advanced analytical methods has resulted in an increasing number of studies on the genetics of colonising species (with emphasis on invasive species). We now know that founder and bottleneck events can have negative or no effect on colonisation success and that increased genetic variability is likely to be due to multiple introductions and intraspecific admixture (Dlugosch & Parker 2008; Uller & Leimu 2011; Rius & Darling 2014; Dlugosch *et al.* 2015). However, inferring processes underlying the genetic architecture of introduced populations and their successful establishment remains challenging, largely because of a lack of information about colonisation history (Estoup & Guillemaud 2010). This lack of historical context could lead to the misidentification of the source(s) of introduced populations, which is expected to be more likely when there is weak phylogeographic structure in the native range or if sampling has been incomplete or inappropriate (Taylor & Keller 2007; Muirhead *et al.* 2008; Estoup & Guillemaud 2010). Furthermore, genetic drift (or selection) could enhance rapid genetic divergence between native and introduced

populations making the source population more difficult to identify. To understand the relationship between genetic diversity and establishment success, as well as the short- and long-term population viability, it is critical to obtain information on population genetic structure and composition from both native and non-native ranges and with a sufficient geographic coverage to track most of the genetic diversity potentially sampled during the invasion process (Dlugosch & Parker 2008; Muirhead *et al.* 2008; Estoup & Guillemaud 2010). By comparing genetic variability in native and invasive populations, it is also possible to deduce the demographic and evolutionary changes (including genetic drift and selection) that have shaped the introduced population.

Disentangling bottleneck and admixture events and their effects on fitness will enable a deeper understanding of the evolutionary constraints and opportunities experienced by founding populations (Dlugosch *et al.* 2015). With the exception of a few studies on plants (Taylor & Keller 2007; Keller & Taylor 2008; Keller & Taylor 2010; Taylor *et al.* 2010; Keller *et al.* 2012; Keller *et al.* 2014) and invertebrates (Facon *et al.* 2008; Lombaert *et al.* 2010; Facon *et al.* 2011a; Facon *et al.* 2011b; Lombaert *et al.* 2014), we still lack a comprehensive study of the ecological and evolutionary processes occurring in real-time in a vertebrate species. The common wall lizard (see Box 1) has several features that make it a very useful system for studying the genetics of colonising species. The species has a widespread distribution, has undergone a number of range expansion and contractions in the past, and the isolated island populations and human-aided introductions outside the native range provide a platform to address fundamental questions regarding the genetics of colonisation.

BOX 1 | THE STUDY SYSTEM, *PODARCIS MURALIS* (LAURENTI 1768)

The common wall lizard (*P. muralis*) is a small lizard that grows up to 75 mm snout to vent length and 20cm in total length. It is a sexually dimorphic species, where males exhibit more pronounced secondary sexual characters. Females lay one to three clutches a year of 2-10 eggs that typically hatch in 6-11 weeks (development is temperature dependent). The offspring reach sexual maturity after 1-2 years and as adults can live up to six years in the wild [1, 2].

Classification

Kingdom:	Animalia
Phylum:	Chordata
Class:	Reptilia
Order:	Squamata
Family:	Lacertidae
Genus:	*Podarcis*
Species:	***P. muralis***

Typically saxicolous and strongly associated with modified or artificial habitats (e.g., brick and stone walls), the common wall lizard is widely distributed in Europe (Fig. 1) and is found from sea level up to 2,500m altitude. Several genetically and geographically distinct clades have been identified and at least five sub-species are currently recognized, largely based on morphological variation in dorsal coloration and patterning (Fig. 2, [1,2,3,4,5]).

Fig. 1 | Native range of *P. muralis*.

Outside the native range, the species has been introduced to North America and to many locations in Germany and the UK [5,6,7,8]. In the UK alone, about 50 introductions are known with more than 20 extant populations that are the result of escapees or deliberate release of captive animals and/or their offspring. Data on introductions, the number of founders and their origin exist for many of these populations, partly through herpetological reports and partly through information provided by the landowners.

The wall lizard system represents an excellent model to address important questions in invasion biology. The number of replicated introductions (>20 non-native populations), historical and observational data, extensive sampling in the native range and a combination of molecular markers and advanced analytical approaches provide an ideal framework to shed light on the evolutionary dynamics of human-assisted colonisation.

Fig. 2 | Morphological variation in *P. muralis*.

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THESIS OVERVIEW

In this thesis, I investigate the causes and consequences of genetic variation after colonisation events. In the following four chapters (Table 1), my aim is to increase our understanding of the origin of wall lizards in Britain and on islands in the British Channel, how historical processes shape genetic diversity, and to what extent we can link introduction history, genetic diversity, individual fitness and population viability in non-native populations.

Chapter 2 first deals with marginal populations of *P. muralis* in the native range and specifically populations on Jersey and the Channel Islands, which were part of the mainland until approximately 7000 years ago. Island populations are interesting because they experience more rapid cycles of extinction, recolonization (with associated founder effects), severe population bottlenecks and asymmetric gene flow. The situation on Jersey is of particular interest because the species' distribution on the island raises the possibility that at least some populations may have been introduced. I assess the genealogical relationship between island and mainland populations (using a mitochondrial DNA marker (mtDNA)), and their genetic architecture (inferred from microsatellite data), and discuss the current distribution of the species at the range limit and the implication of the results for the conservation status of the species.

Chapter 3 moves beyond the native range and aims to elucidate the geographical origin(s) of human-mediated introductions of *P. muralis* in the UK. I use a phylogenetic approach to match haplotypes (mtDNA) found in non-native populations with available haplotypes from across the native distribution of the species, thereby identifying their genetic and geographic origins.

Chapter 4 builds on the work from chapter 3 and aims to reconstruct the routes of human-mediated introductions. With extensive sampling in the native range, a combination of molecular markers (mtDNA and microsatellite loci) and advanced computational methods, corroborated with historical data, my aim is to infer the complex colonisation history of non-native populations in the UK. Of particular interest is the extent to which introductions are primary or secondary, the geographic scale of secondary introductions, and whether or not introduced populations bring together genetic lineages that are allopatric in the native range.

Chapter 5 makes use of the colonisation history established in Chapter 4 to investigate the level of genetic variation in non-native populations in relation to the native region and its relationship to individual fitness and population viability. I first document the loss of genetic variation in the non-native range. Second, I assess which factors best predict the level of genetic diversity and finally, using data on reproductive fitness (fecundity, infertility and embryonic mortality), I test for heterozygosity-fitness correlations at the population and individual level.

Table 1 | Thesis outline

	Challenge	Material & Methods
Chapter 2	Genetic architecture of island populations at the range limit of *P. muralis*	• 484 individuals 21 populations (four locations on Jersey island, islets of Chausey Archipelago and 16 mainland populations) • mtDNA cyt-b gene (656bp) • Microsatellite markers (10 loci)
Chapter 3	Human-mediated introductions outside the native range: tracing the origin of *P. muralis* in England	• 507 individuals from 23 non-native populations • mtDNA cyt-b gene (656bp) • 175 published sequences from native distribution
Chapter 4	Colonisation history of non-native populations of *P. muralis* in England	• 1328 individuals from 23 non-native populations and 34 native populations • mtDNA cyt-b gene (656bp) • Microsatellite markers (16 loci)
Chapter 5	Consequences of colonisation history and demographic events on genetic structure and its relationship to inbreeding depression of *P. muralis*	• 1546 individuals from 21 non-native and 45 native populations • mtDNA cyt-b gene (656bp) • Microsatellite markers (13 loci) • Data on fecundity, fertility and embryonic mortality from 13 introduced and 11 native populations

CHAPTER 2

PLOS ONE

RESEARCH ARTICLE

Phylogeography and Conservation Genetics of the Common Wall Lizard, *Podarcis muralis*, on Islands at Its Northern Range

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ABSTRACT

Populations at range limits are often characterised by lower genetic diversity, increased genetic isolation and differentiation relative to populations at the core of geographical ranges. Furthermore, it is increasingly recognised that populations situated at range limits might be the result of human introductions rather than natural dispersal. It is therefore important to document the origin and genetic diversity of marginal populations to establish conservation priorities. In this study, we investigate the phylogeography and genetic structure of peripheral populations of the common European wall lizard, *Podarcis muralis,* on Jersey (Channel Islands, UK) and in the Chausey archipelago. We sequenced a fragment of the mitochondrial cytochrome b gene in 200 individuals of *P. muralis* to infer the phylogeography of the island populations using Bayesian approaches. We also genotyped 484 individuals from 21 populations at 10 polymorphic microsatellite loci to evaluate the genetic structure and diversity of island and mainland (Western France) populations. We detected four unique haplotypes in the island populations that formed a sub-clade within the Western France clade. There was a significant reduction in genetic diversity (H_O, H_E and A_R) of the island populations in relation to the mainland. The small fragmented island populations at the northern range margin of the common wall lizard distribution are most likely native, with genetic differentiation reflecting isolation following sea level increase approximately 7000 BP. Genetic diversity is lower on islands than in marginal populations on the mainland, potentially as a result of early founder effects or long-term isolation. The combination of restriction to specific localities and an inability to expand their range into adjacent suitable locations might make the island populations more vulnerable to extinction.

INTRODUCTION

There is a growing interest in the patterns and processes associated with geographical variation in population genetic structure across species' ranges since these often shift, expand and contract over time (Brown *et al.* 1996; Davis & Shaw 2001; Eckert *et al.* 2008; Sexton *et al.* 2009). Historical and contemporary changes to population size and gene flow influence genetic diversity and population differentiation (Vucetich & Waite 2003; Eckert *et al.* 2008). These changes are particularly important in populations at geographical range limits, since these populations experience more rapid cycles of extinction, recolonization (with the associated founder events), severe population bottlenecks and asymmetric gene flow (Eckert *et al.* 2008). As a consequence, marginal populations tend to show greater than expected isolation by distance and have lower genetic diversity than populations located within the species' range (Eckert *et al.* 2008). They are therefore often of particular conservation interest (Lesica & Allendorf 1995; Hampe & Petit 2005).

To complicate matters, it is increasingly recognized that isolated populations at the edge of species' distributions might not have dispersed, or become isolated, naturally but instead might have been assisted by humans. This has the potential to result in genetic admixture when animals are introduced from multiple source populations. As a consequence of human-mediated dispersal and resulting admixture, marginal populations might actually show higher genetic diversity than geographically more central populations (Roman & Darling 2007; Uller & Leimu 2011). Therefore, it is important to establish the origin of marginal populations to be able to assign conservation priorities. This is well exemplified by the changing status of the pool

frog (*Pelophylax lessonae*) in Britain. Initially considered to be present solely as a result of human introductions the native status of pool frogs was confirmed just in time to witness its extinction (Beebee *et al.* 2005). The species is now the focus of an active reintroduction program (Buckley & Foster 2005).

The common wall lizard (*Podarcis muralis*) exhibits a wide distribution across central and southern Europe. It also occurs in peripheral populations in Northern Europe where its status as a native species is debated. For example, while populations of wall lizards are known to be non-native in England (Michaelides *et al.* 2013) and parts of Germany (Schulte *et al.* 2008), some isolated populations at the northern range limit in France, the Netherlands, and in Eastern Europe are of uncertain origin (Schulte 2008). Of particular interest are populations on islands in the Golfe Normand-Breton, which were previously part of the French continental landmass and have been separated following climate and sea level changes about 7,000 BP (Jones 1993; Livory 1997). Jersey, the largest of Channel Islands (11,630ha) (Berry 2009) and the Chausey archipelago (a group of islands, totalling 59ha) are now 25.5 and 17 km west of Normandy Coast, respectively (Walters & Ineich 2006; Berry 2009). The presence and distribution of wall lizards on Jersey has been described by a number of authors (Ansted & Latham 1865; Sinel 1908; Le Sueur 1976) and it has been widely assumed that *P. muralis* is native to these islands. However, the species distribution on Jersey is noticeably patchy and restricted to old walls and ramparts on the north-eastern and eastern coast of the island (Avery & Perkins 1989), which suggests that they could have been introduced following the construction of the forts. Indeed, a population on the south east coastline of Jersey, cut off from the rest of the Island at

high tide, is known to be a more recent introduction, although the origin of those animals is unknown (Cornish 2011).

The origin and genetic diversity of populations of *P. muralis* on the Channel Islands is of much interest as they are currently considered threatened and enjoy full protection status, despite that its present distribution is indicative of more recent introductions. Natural colonization of islands could have occurred from southern refugia, following climatic warming at the end of the Pleistocene and before the rising sea level, followed by separation from the mainland. Alternatively, colonization could have occurred subsequent to island isolation via rafting or the quarrying of granite. The aim of this study was to infer the origin of *P. muralis* populations on Jersey and Chausey Island and investigate the population genetic structure and diversity in relation to mainland populations. Based on our results we discuss conservation implications for these peripheral populations.

MATERIALS AND METHODS

Study species

The European wall lizard, *Podarcis muralis* (Laurenti, 1768) has a wide distribution in central and southern Europe (Gruschwitz & Böhme 1986) and shows a strong phylogeographic structure with several genetically and geographically distinct clades (Giovannotti *et al.* 2010; Schulte *et al.* 2012). This genetic structure is likely to have originated during isolation in southern glacial refugia in Italy on the Apennine Peninsula (Giovannotti *et al.* 2010), the Balkans and on the Iberian Peninsula (Gruschwitz & Böhme 1986; Schulte *et al.* 2012). The postglacial recolonization of western Europe expands to the northwest along the French coast of the English

Channel, across southern Belgium and southernmost Netherlands towards south-western Germany (Gruschwitz & Böhme 1986).

Sampling, sequencing and genotyping

We sampled 484 individuals from 21 populations between 2008 and 2013 (see Table 1 and Figure 1 in results section). We sampled lizards from all four locations on Jersey (St. Aubin Fort, Mont Orgueil Castle and Gorey, L'Etacquerel Fort and Fort Leicester, see Table S3 in Supplementary Information), from the Chausey archipelago (where the lizard is more widespread, see Table S3 in Supplementary Information for more information) and from 19 populations in France (see Table S3 in Supplementary Information). We focused on mainland populations at the north-western margin of the species distribution, i.e., close to the Channel Islands, but also included a number of populations in south-western France to compare the observed divergence between island populations with divergence across the entire western France lineage.

Ethics Information

Lizards were captured by noosing, and a small (ca 5mm) 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. The research was approved by the UK Home Office Ethical License PPL30/56 and all work and procedures during fieldwork were carried out under annual licenses and permits from the States of Jersey Government (Department of the Environment) and the French Government (Direction Régionale de l'Environnement, de l'Aménagement et du Logement).

DNA extraction, sequencing and genotyping

We extracted genomic DNA from tail tissue preserved in ethanol (70-90%) with DNeasy 96 plate kit (Qiagen, Valencia, CA) following manufacturer's instructions (with overnight lysis). For the phylogenetic analysis we amplified a 656bp region of mitochondrion cytochrome b gene by polymerase chain reaction (PCR) using the primer pair LGlulk [5′-AACCGCCTGTTGTCTTCAACTA-3′] and Hpod [3′-GGTGGAATGGGATTTTGTCTG-5′] (Deichsel & Schwiger 2004; Podnar *et al.* 2007; Schulte *et al.* 2012; Michaelides *et al.* 2013). Amplifications were carried out in a total volume of 15µl consisting of 7.5µl of MyTaq HS Mix (Bioline), 0.45µl (8pm) of each primer (Eurofins), 4.6µl PCR grade H_2O and 2µl template DNA. PCR conditions were as follows: an initial denaturation step at 94^{o}C for 1 min, followed by 35 cycles at 94^{o}C for 1 min, 53^{o}C for 45sec and 72^{o}C for 1 min and a final extension step at 72^{o}C for 10min. PCR products were purified using the MinElute 96 UF PCR Purification Kit (Qiagen, Valencia, CA).

Sequencing reactions were carried out with BIGDye 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 MAFFT (Katoh *et al.* 2002b) 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 KP118978-KP118990.

To infer the genetic structure and diversity of our populations we genotyped 484 individuals at 10 polymorphic microsatellite loci; four described by Richard *et al.* (2012) and six recently developed by Heathcote *et al*. (2014) (Table S1). Multiplexed PCRs were carried out in a total volume of 11µl reaction mix containing 1µl of genomic DNA, 5µl of Qiagen MasterMix, 0.2µl of each primer (forward and reverse in equal concentrations) and 3.8µl (for multiplex 1 and 2) or 3.6µl (for multiplex 3) of PCR grade dH_2O. PCR conditions were as follows: 15min of initialization step at 95^{o}C, 26 cycles of 30sec at 94 ^{o}C, 90sec at 57 ^{o}C (for multiplex 1 and 2) or 55 ^{o}C (for multiplex 3) and 1min at 72^{o}C and a final extension step of 20min at 60^{o}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.) We scored alleles in GENEIOUS 6.1.7 and any ambiguous peaks were repeated to confirm genotype.

Phylogenetic analyses

We used the phylogenetic tree approach to assign haplotypes to known lineages by combining our sequences with 68 sequences (of varying lengths), obtained from GenBank, across the native distribution of the species (see Table S2 in Supplementary Information (Podnar *et al.* 2007; Schulte *et al.* 2008; Giovannotti *et al.* 2010; Bellati *et al.* 2011; Schulte *et al.* 2012; Gassert *et al.* 2013; Salvi *et al.* 2013)). Three sequences belonging to *P. siculus* (AY185095) (Podnar 2004)*, P. liolepis* (JQ403296) (Schulte *et al.* 2012b) and *P. melisellensis* (AY185097) (Podnar 2004) were used as outgroups in the phylogenetic analysis using Bayesian Inference (BI). We implemented 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). The BI analysis was run with four chains of 1,000,000 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 of our sequences, we constructed a parsimonious phylogenetic network using a median – joining algorithm in Network v.4.6.12 (Bandelt *et al.* 1999). The method uses median vectors as a hypothetical ancestral sequence required to connect existing sequences within the network with maximum parsimony.

Population genetics analyses

We checked the microsatellite data in MICROCHECKER V.2.2.3 (Van Oosterhout *et al.* 2004) for null-alleles, large allele dropouts and scoring errors. Basic genetic diversity indices, observed and expected heterozygosities (H_O, H_E) were calculated with GENALEX 6.5 (Peakall & Smouse 2012) and allelic richness (A_R) with FSTAT v.2.9.3 (Goudet 1995b, 2001). Inbreeding coefficient (F_{IS}) and deviations from Hardy-Weinberg equilibrium were also evaluated at the 0.05 nominal level for multiple tests using sequential Bonferroni corrections in FSTAT v.2.9.3 (Goudet 1995, 2001). We compared H_O, H_E, A_R in island versus mainland populations with a Welch Two Sample t-test and evaluated the correlation between expected heterozygosity and latitude with a Spearman's rank correlation test in R (R Development Core Team 2011).

To infer population structure, we implemented a Bayesian analysis in STRUCTURE v.2.3.4 (Pritchard *et al.* 2000) using the admixture model (Falush *et al.* 2003). The simulations were run with a burn-in of 100,000 iterations and a run length of 10^6 iterations from $K = 1$ through 5. Runs for each K were replicated 10 times and the true 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 tested the level of genetic diversity within populations, among populations and among groups (as defined by the structure clustering analysis) by hierarchical analysis of molecular variance (AMOVA, (Excoffier *et al.* 1992)) in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010). Population differentiation was assessed by calculating the F_{ST} values and visualized with a Principle Coordinate Analysis (PCoA) in GENALEX 6.5 (Peakall & Smouse 2012).

RESULTS

Phylogeography

Analysis of mtDNA sequences of 192 individuals revealed 13 unique haplotypes all nested within the Western France Clade (Figure 2). The most common haplotype on the mainland (France) was WFR-H5, which was also present on Chausey (one individual) but not on Jersey (Figure 1A). The parsimony network showed that WFR-H5 has a central position among French haplotypes and JER-H3 forms the centre of the cluster of Jersey and Chausey haplotypes, which are distinct from the rest of the mainland populations (Figure 1B).

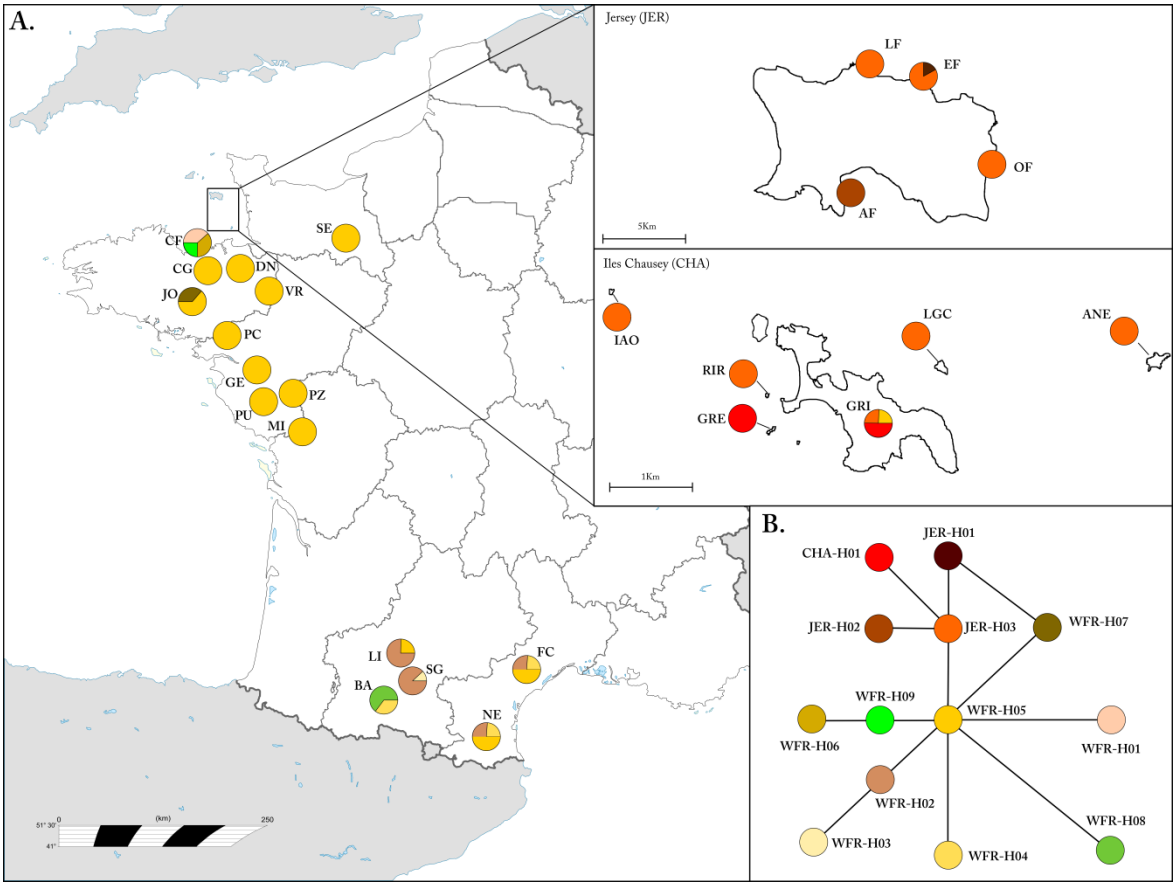

Figure 1 | Distribution of sampled sites and haplotype network. (A) Pie charts indicate the percentage of sampled individuals matched to a specific haplotype (for population abbreviations see Table 1). (B) Parsimonious phylogenetic network reconstructed from 13 unique haplotypes sampled in our populations using a median-joining algorithm.

Table 1 | Results from mtDNA and microsatellite analyses.

Region	Population	Code	Latitude (°N)	Longitude (°E)	N_I*	N_H	Haplotype **	A_R	H_O (s.d)	H_E (s.d)	F_{IS} ***
Jersey	St. Aubin Fort	AF	49.18712	-2.17103	15 (15)	1	JER-H2(15)	2.12	0.237 (0.071)	0.409 (0.063)	**0.452**
	L'Etacquerel Fort	EF	49.238267	-2.06698	17 (17)	2	JER-H1(2), JER-H3(15)	2.14	0.313 (0.077)	0.401 (0.075)	**0.255**
	Fort Leicester	LF	49.240243	-2.08162	14 (14)	1	JER-H3(14)	2.86	0.375 (0.104)	0.532 (0.079)	**0.35**
	Mount Orgueil Castle	OF	49.198904	-2.02013	34 (34)	1	JER-H3(35)	2.71	0.403 (0.1)	0.552 (0.082)	**0.291**
Chausey Archipelago	Iles de Chausey	CH	48.87425	-1.83016	31 (34)	3	JER-H3(30), CHA-H1(3), FR-H05(1)	3.21	0.547 (0.104)	0.613 (0.084)	0.144
France	Cap Frehel	CF	48.66451	-2.32066	12 (11)	3	FR-H1(6), FR-H6(3), FR-H9(2)	2.92	0.508 (0.115)	0.558 (0.101)	0.134
	Chateau du Guildo	CG	48.574464	-2.20691	25 (5)	1	FR-H5(5)	3.05	0.528 (0.092)	0.609 (0.091)	**0.155**
	Dinan	DN	48.454352	-2.04734	25 (5)	1	FR-H5(5)	3.35	0.630 (0.049)	0.646 (0.046)	0.045
	Sees	SE	48.605425	0.172979	24 (5)	1	FR-H5(5)	2.46	0.451 (0.078)	0.480 (0.078)	0.085
	Vitre	VR	48.124012	-1.2144	20 (5)	1	FR-H5(5)	3.52	0.590 (0.079)	0.632 (0.081)	0.092
	Josselin	JO	47.953899	-2.54648	25 (5)	2	FR-H5(3) FR-H7(2)	3.63	0.589 (0.086)	0.634 (0.083)	0.091
	Pontchateau	PC	47.436895	-2.08903	25 (5)	1	FR-H5(5)	3.76	0.513 (0.091)	0.550 (0.093)	0.088
	Puybelliard	PU	46.706436	-1.02946	22 (5)	1	FR-H5(5)	3.61	0.662 (0.079)	0.699 0.081)	0.079
	Pouzagues	PZ	46.78435	-0.83917	25 (5)	1	FR-H5(5)	3.80	0.694 (0.076)	0.718 (0.080)	0.054
	Saint Gervais	GE	46.902738	-1.99874	25 (5)	1	FR-H5(5)	3.66	0.659 (0.085)	0.706 (0.084)	0.088
	Bastide	BA	46.35321	-1.25172	25 (5)	2	FR-H3(2), FR-H8(3)	3.26	0.564 (0.109)	0.635 (0.095)	0.041
	Saint Michel	MI	42.939334	1.055994	25 (5)	1	FR-H5(5)	3.56	0.686 (0.086)	0.699 (0.082)	0.151
	Saint Lizier	LI	43.003259	1.138791	20 (5)	2	FR-H2(4), FR-H5(1)	3.8	0.704 (0.085)	0.708 (0.087)	0.031
	Saint Girons	SG	42.982243	1.146273	25 (5)	2	FR-H2(4), FR-H3(1)	3.66	0.639 (0.108)	0.707 (0.074)	**0.12**
	Nebias	NE	42.896786	2.11586	25 (5)	3	FR-H5(3), FR-H2(1), FR-H4(1)	3.77	0.625 (0.079)	0.718 (0.079)	**0.15**
	Fontiers Cabardes	FC	43.369587	2.248493	25 (5)	3	FR-H4(1), FR-H5(3), FR-H2(1)	3.45	0.610 (0.105)	0.650 (0.098)	0.087

* Number of individuals used in microsatellite analysis and in parenthesis the number of individuals used in mtDNA analysis.
** Number of individuals sharing the same haplotype is shown in parenthesis
*** values in bold indicate significant deviation from Hardy-Weinberg equilibrium after correcting for multiple tests at the nominal level (5%), *p>0.00024*.
NI (number of individuals), NH (number of haplotypes), A_R (allelic richness), H_O (observed heterozygosity), H_E (expected heterozygosity) and F_{IS} (inbreeding coefficient).

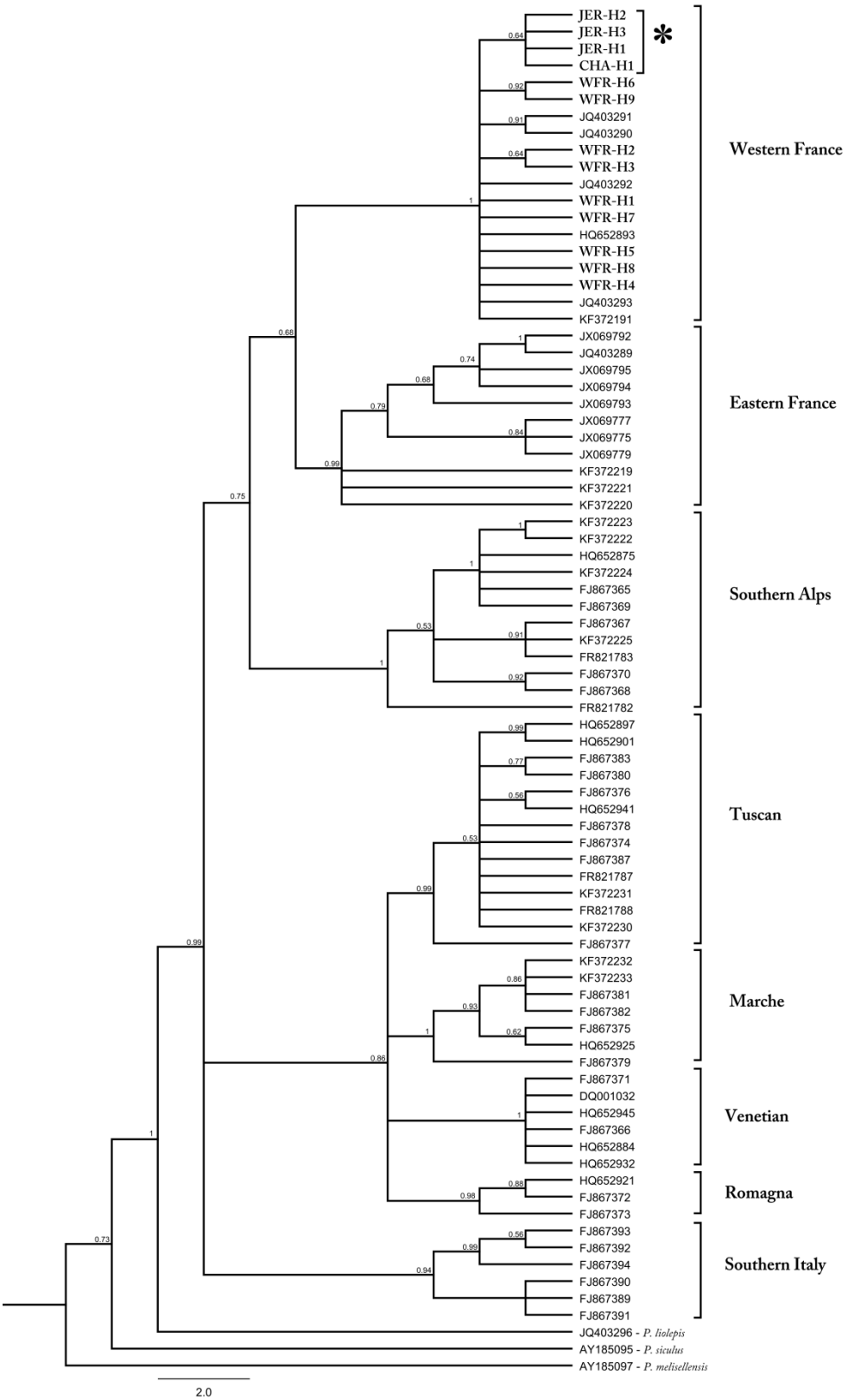

Figure 2 | Bayesian inference consensus tree derived from mitochondrial *cyt-b* sequences. Posterior probabilities (>0.5) are indicated above nodes. Haplotypes analyzed in this study are shown in bold and all were assigned to the Western France Clade. Haplotypes from Jersey and Chausey islands are indicated with an asterisk (*). For information on locality of the sequences see Table S2 in Supplementary Information.

Population genetics

All 484 individuals were genotyped at 10 polymorphic loci, ranging from 10 to 56 alleles with mean number of 20.3 alleles per locus across all populations. Evidence of null alleles was observed in several loci but none were consistent across all populations, therefore we did not exclude them for further analysis (Table S4 in Supplementary Information). Allelic richness, expected and observed heterozygosities (Table 1) were all significantly lower ($p<0.05$) in the island populations of Jersey and Chausey than in mainland France populations (Figure S2 in Supplementary Information). There was a significant negative correlation (r = -0.84, $p<0.05$) between latitude and expected heterozygosity (Figure 3).

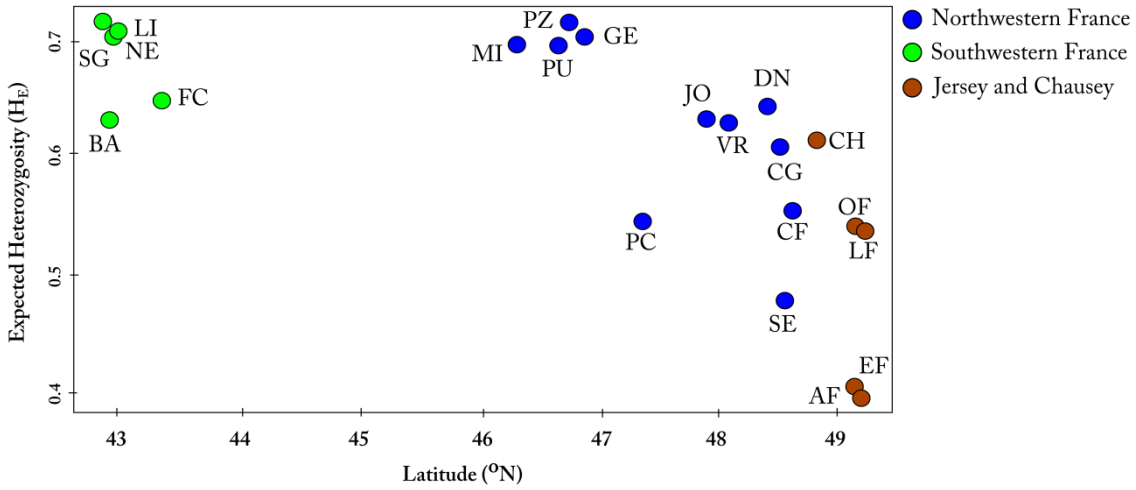

Figure 3 |Correlation between expected heterozygosity (H_E) and latitude. There was a significant negative correlation (r = -0.84, $p<0.05$).

The Bayesian clustering approach implemented in STRUCTURE suggested K = 3 best-fit the genetic data (Figure 4, see also Figure S1 in Supplementary Information). The Principle Coordinate Analysis (PCoA) based on F_{ST} values (see Table S5 in Supplementary Information) between populations confirmed the results from STRUCTURE, identifying three clear groups corresponding to the samples from the Islands, North Western France and South Western France (Figure 5). Analysis of

Molecular Variance (AMOVA) revealed that 28% of the genetic variation was found among the three groups (clusters) and 50% was found within individuals (Table 2).

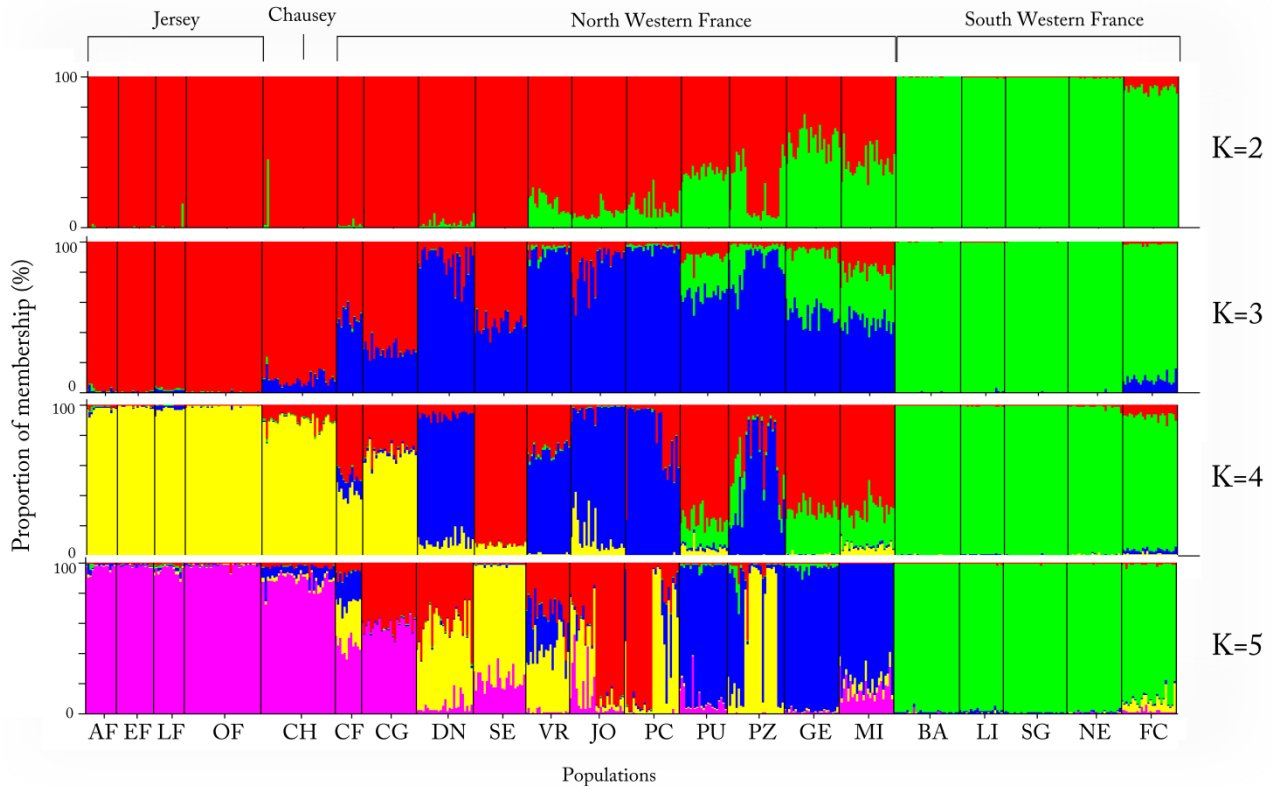

Figure 4 | Structure analysis (*K*=2 to *K*=5) for all individuals (n = 484). Each individual is represented by a vertical line partitioned into K coloured segments according to the proportion of membership (%) in each cluster. For population abbreviations see Table 1.

Table 2 | Hierarchical analysis of molecular variance (AMOVA).

Source of Variation	*d.f*	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
Among groups	2	686959.219	11893.80493	27.8	F_{IS} = 0.21047	<0.05
Among populations within groups	18	342716.546	346.10698	8.09	F_{SC} = 0.11199	<0.05
Among individuals within populations	460	1528192.078	577.644	13.5	F_{CT} = 0.27796	<0.05
Within individuals	481	1042263.5	2166.86798	50.62	F_{IT} = 0.49377	<0.05
Total	961	3600131.344	4280.42425			

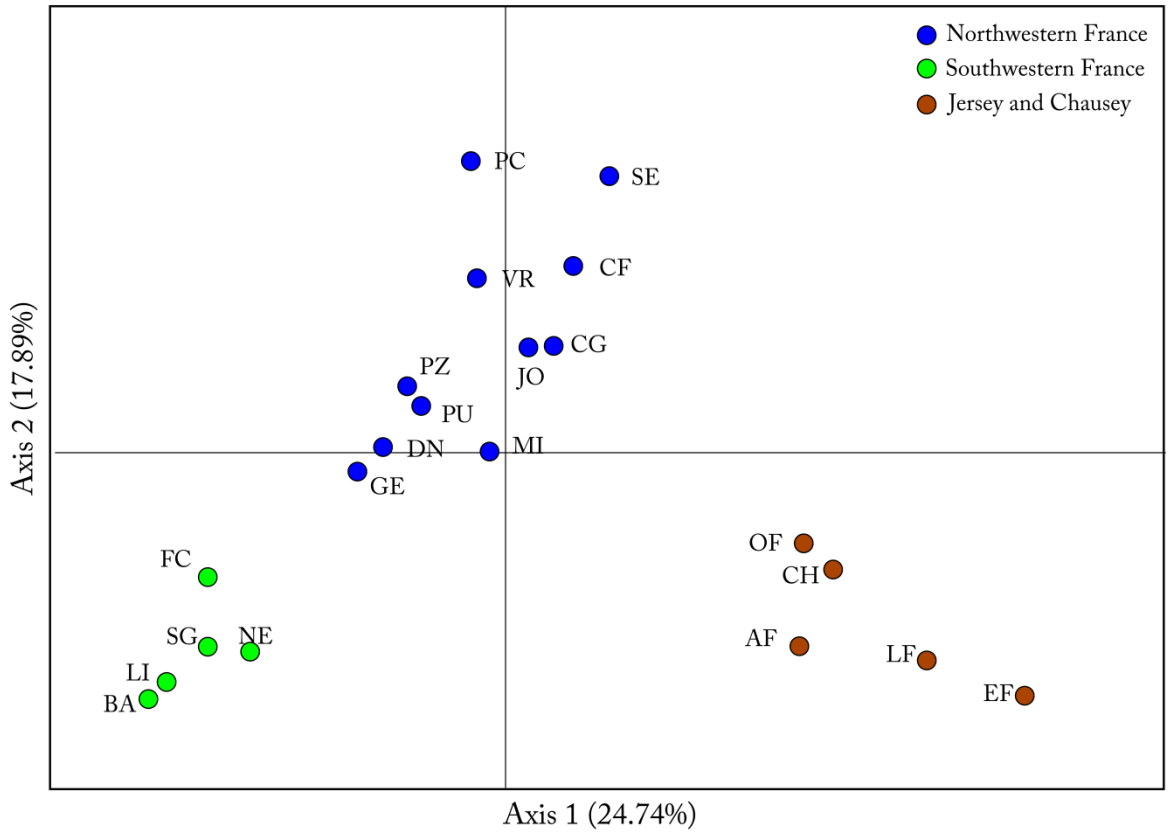

Figure 5 | Principle Coordinate Analysis (PCoA) based on F_{ST} values. Three population groups can be identified; the island populations on Jersey and Chausey (bottom right), the north-western French populations (top cluster) and the south-western populations (bottom left).

DISCUSSION

Our data provides strong evidence that the wall lizard populations on the islands in the English Channel belong to a single origin. Furthermore, the analyses suggest that this mtDNA clade has been isolated from the mainland for a long period of time and should be considered native. The most parsimonious explanation for the origin of the common wall lizard on Jersey and Chausey Islands appears to be that the increasing sea levels 7000 BP isolated island populations from the mainland and from each other, resulting in independent population histories and hence divergence. It remains possible, however, that there is occasional gene flow between islands. For example, the presence of lizards on very small islets in the Chausey archipelago (Walters & Ineich 2006), which are unlikely to be large enough to sustain populations for

thousands of years, might indicate that dispersal occasionally occurs between islands. In addition, the presence of the WFR-H5 haplotype on the island of Chausey, which is the most common haplotype on the mainland, might also provide evidence of occasional gene flow between mainland France and the islands. However, it could also be explained by retention of ancestral genetic variation or a more recent introduction. It is worth noting that a single isolated population on the coast of mainland France (Cap Frehel, CF; Figure 1) also exhibits unique haplotypes, nevertheless it clusters with other mainland populations in all analyses.

Anecdotal evidence suggested that human mediated dispersal might be the most likely explanation for one of the four current locations in Jersey, the population on St. Aubin Fort (Cornish 2011). Although our mtDNA data revealed a different haplotype from other Jersey populations, the nucDNA clusters all Jersey populations together. This suggests that the source population was most likely animals from other Jersey populations and that the difference in haplotype represents a founder effect.

Overall, these results confirm the suspected native status of Jersey and Chausey wall lizards. Thus, the lower genetic diversity of island populations compared to the mainland populations is expected given the lack of gene flow. This might have significant implication for the long-term persistence of the species on Jersey and Chausey Islands. However, since our data suggests that the species have been present on the islands for thousands of years it might have already been subject to a severe bottleneck that purged deleterious recessives (Leberg & Firmin 2008). The species might also have undergone a substantial reduction in abundance more recently. Historical references to the species on Chausey, dated in 1842 (Quatrefages), and subsequently work recorded the species as very common (Joseph-Lafosse 1891;

Gadeau de Kerville 1894, 1897; Gibon 1919). Despite this, the current distribution of the species on Jersey is very restricted (Cornish 2011). One partial explanation for this is that lizards on Jersey were part of a wider pet trade, with lizards being sent from Jersey to England as far back as 1761 (Le Sueur 1976). Indeed, by 1947 the pet trade in lizards had reached such proportions that the local government (States of Jersey) passed the Wildlife Protection (Jersey) Law 1947, which prohibited the buying, selling, exportation or killing of all reptiles and amphibians of Jersey, as a measure to control the increased trade for these animals as pets destined for England (however, none of the contemporary non-native populations in England originate from Jersey (Michaelides *et al.* 2013)). Not only might this explain the current patchy distribution of lizards on Jersey, it might also have contributed to their relatively low genetic variation.

Geographically peripheral populations are often representatives of relatively widespread species within different political boundaries (Bunnell *et al.* 2004). Their conservation value depends upon their genetic divergence from other conspecific populations because of the synergetic effects of isolation, genetic drift, and natural selection. Whether these range-edge populations merit the conservation effort that they are often subject to has been widely debated (Millar & Libby 1991; Hunter & Hutchinson 1994; Lesica & Allendorf 1995). As this study clarified the native status of the wall lizard population on Jersey, it validates its current full protection status under the Conservation of Wildlife (Jersey) Law 2000 (as amended). The law prohibits the unlicensed taking, sale, keeping, injury and destruction of places for shelter (e.g. nest, dens or burrows) and disturbance of any resident animals. Given our results, it is important that Jersey conservation planners recognize the wall

lizard's restricted distribution, vulnerability to future inbreeding depression, susceptibility to disease, predation and the island's ever-increasing urban development when developing species management strategies. For instance, should the granite walls and ramparts of historic fortresses where they are in highest abundance be developed or destroyed, the population's continued survival could be placed at risk. The lizard's long-term conservation status will depend upon increasing habitat connectivity, especially via coastline protection to connect their north-eastern and eastern coast populations on the island.

ACKNOWLEDGEMENTS

We would like to thank Hannah MacGregor for assisting in the field. We also thank Tod Reeder and one anonymous reviewer for useful comments that improved the manuscript.

CHAPTER 2| SUPPLEMENTARY INFORMATION

LIST OF TABLES

LIST OF FIGURES

Table S1 | Details for the ten loci used in the study.

Multiplex	Locus		Primer sequences	Ta*	Product size (bp)	Repeat motif	Range (bp)
1	C150	F	**[6-FAM]**GTCAGCTTTGCAGCACCTTAG	57 °C	193	Di	175-217
		R	GCGATTAGAGAAGGCGTTTG				
	C168	F	**[HEX]**GGTCCGGCTTCAAAGAATAAG		244	Tetra	219-350
		R	CAGAGGACTCGCTCAAGGAC				
	C275-278	F	**[6-FAM]**GCTTAAAATTAATGCTGCTGCTATTGTATC		245	Tetra	210-471
		R	ATAGGTAGAAAATTTATAAACCCTTGG				
2	C164	F	**[6-FAM]**ATCGATGAATGAATGAAGGGCAGT		216	Tetra	170-246
		R	CCAGGCATTGTCAAACTATCTG				
	C038	F	**[HEX]**CAATGTGCAGTGTTGGGTTG		210	Tetra	193-417
		R	ATGTGAGCGACTCCTGGATG				
	C028	F	**[6-FAM]**TTGCTTCTGATACGCCTAGC		287	Tetra	253-413
		R	AGTGTATTGCGACTGTCAATGG				
3	Pm01	F	**[6-FAM]** CCACAGGCATCTGGTTAG	55 °C	128	Tri	119-146
		R	TCCATAAGACTGTAAGACAAGCC				
	Pm05	F	**[HEX]** CAAGAGGGCAGCCTAGTAATG		160	Tetra	135-267
		R	AGATGGGCTCATTTCAACTCC				
	Pm09	F	**[NED]** ACGTGTTTCTGTGCTTTGC		189	Tri	176-220
		R	AGTCAGACGAGAGGTTGCC				
	Pm16	F	**[6-FAM]** GGGATGGAGAAAGATGGCG		192	Tetra	179-217
		R	GCACTTGCCTACTGGTCATAC				

Multiplexes one (1) and two (2) were developed by Heathcote *et al.* (2014) and multiplex three (3) was developed by Richard *et al.*(2012). *Annealing temperature

Table S2 | List of sequence data used in the phylogenetic analysis.

GenBank accession no.	Haplotype code	Region – locality – population ID	Reference
KP118978	WFR-H7	Josselin (JO)	This study
KP118979	JER-H1	Jersey (EF)	This study
KP118980	JER-H3	Jersey (EF, LF, OF), Iles de Chausey (GRI, ANE,LGC,IAO, RIR)	This study
KP118981	JER-H2	Jersey (AF)	This study
KP118982	CHA-H1	Iles de Chausey (GRE)	This study
KP118983	WFR-H4	Nebias (NE), Frontier Cabardes (FC)	This study
KP118984	WFR-H5	Iles de Chausey (GRE), Chateau du Guildo (CG), Dinan (DN), Sees (SE), Vitre (VR), Josselin (JO), Pontchateau (PC), Puybelliard (PU), Pouzagues(PZ), St. Gervais (GE), St. Michel (MI), St. Lizier (LI), St. Girons (SG), Frontier Cabardes (FC)	This study
KP118985	WFR-H9	Cap Frehel (CF)	This study
KP118986	WFR-H8	Bastide (BA)	This study
KP118987	WFR-H1	Cap Frehel (CF)	This study
KP118988	WFR-H2	St. Lizier (LI), Nebias (NE), Frontiers Cabardes (FC)	This study
KP118989	WFR-H3	St. Girons (SG), Bastide (BA)	This study
KP118990	WFR-H6	Cap Frehel (CF)	This study
JQ403290	MS3	France – Montsegur	Schulte et al. 2012 (Amphibia-Reptilia)
JQ403291	LS6	France – Lourdes	Schulte et al. 2012 (Amphibia-Reptilia)
JQ403292	LRo1	France – La Rochelle	Schulte et al. 2012 (Amphibia-Reptilia)
JQ403293	StM1	France – St Malo	Schulte et al. 2012 (Amphibia-Reptilia)
HQ652875	UU91	Germany – Bielefeld (previously assigned to the Southern Alps lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652884	UU30	Germany – Dresden (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652893	UU67	Germany – Mainz previously assigned to the Western France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)

HQ652897	UU76	Germany – Bad Cannstadt (previously assigned to the Tuscan lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652901	UU80	Germany – Stuttgart (previously assigned to the Tuscany lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652921	UU128	Germany – Lorrach (previously assigned to the Romagna lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652932	UU59	Germany – Aschaffenburg (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652941	SD5	Austria (previously assigned to the Tuscany lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
HQ652945	UU57	Germany (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
KF372191	DB16840	France – Vielle -Roche	Salvi et al. 2013 (BMC Evol. Bio)
KF372219	DB13461	France – Massif des Maures	Salvi et al. 2013 (BMC Evol. Bio)
KF372220	DB13460	France – Valle de Gilly	Salvi et al. 2013 (BMC Evol. Bio)
KF372221	DB13430	France – Massif des Maures	Salvi et al. 2013 (BMC Evol. Bio)
KF372222	DBM3	Italy – Viozene	Salvi et al. 2013 (BMC Evol. Bio)
KF372223	DBM1	Italy – Viozene	Salvi et al. 2013 (BMC Evol. Bio)
KF372224	DB15936	Switzerland – Monte Verita	Salvi et al. 2013 (BMC Evol. Bio)
KF372225	DB16837	Italy – Bianzano	Salvi et al. 2013 (BMC Evol. Bio)
KF372230	DB1399	Italy – Ostia Antica	Salvi et al. 2013 (BMC Evol. Bio)
KF372231	DB5938	Italy – Paganico	Salvi et al. 2013 (BMC Evol. Bio)
KF372232	DMM39	Italy – Majelletta	Salvi et al. 2013 (BMC Evol. Bio)
KF372233	DMM40	Italy – Majelletta	Salvi et al. 2013 (BMC Evol. Bio)
DQ001032	mur12	Italy – Friuli-Venezia	Podnar et al. 2007 (J. Mol. Evol)
FJ867365	H1	Italy – Trento, Vercelli	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867366	H2	Italy – Trieste	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867367	H3	Italy – Pavia	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867368	H4	Italy – Pavia	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867369	H5	Italy - Val Germanasca	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867370	H6	Italy – Parma	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867371	H7	Italy – Ferrara, Ravenna	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867372	H8	Italy – Ravenna, Cesena	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867373	H9	Italy – Cesena, Pesaro, Montignano, Senigallia	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867374	H10	Italy – Carpegna, Bolognola, Amatrice, Gran Sasso, L'Aquila, Latina	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867375	H11	Italy – Montifnano, Ancona	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867376	H12	Italy – Pisa	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867377	H13	Italy – Genga, M. te San Vicino	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867378	H14	Italy – Genga	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867379	H15	Italy – M. te San Vicino, Porto S. Elpidio, Macerata, Bolognola, Porto d' Ascoli	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867380	H16	Italy – Viso	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867381	H17	Italy – Caramanico Terme	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867382	H18	Italy – Gran Sasso	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867383	H19	Italy – L' Aquila	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867389	H25	Italy – Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867390	H26	Italy – Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867391	H27	Italy – Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867392	H28	Italy – Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867393	H29	Italy – Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
FJ867394	H30	Italy – Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
JX069775	Mont St Odily	France – Mont St Odily	Gassert et al. 2013 (J. Biogeography)
JX069777	Bitche	France – Bitche	Gassert et al. 2013 (J. Biogeography)
JX069779	Euville	France – Euville	Gassert et al. 2013 (J. Biogeography)
JX069792	Labeaume	France – Labeaume	Gassert et al. 2013 (J. Biogeography)
JX069793	St Remy	France – Saint Remy de Provence	Gassert et al. 2013 (J. Biogeography)
JX069794	Autun	France – Autun	Gassert et al. 2013 (J. Biogeography)
JX069795	St Martin	France – Saint Martin	Gassert et al. 2013 (J. Biogeography)
FR821782	M2	Italy – Pavia	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)

FR821783	M3	Italy – Bereguardo	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
FR821787	N3	Italy – Borgo Montello	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
FR821788	N4	Italy – Borgo Montello	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
AY185095	*P. siculus*	Outgroup	Podnar et al. 2004 (Organisms Div. Evol)
AY185097	*P. melisellensis*	Outgroup	Podnar et al. 2004 (Organisms Div. Evol)
JQ403296	*P. liolepis*	Outgroup	Schulte et al. 2012 (Amphibia-Reptilia)

Table S3 | Historical information on the island populations of the wall lizard.

Sampled Location	Information
St. Aubin Fort (AF)	The fort is located in St Aubin's Bay on the southast coastline of Jersey and dates back to the 16th century (1540s). The population of wall lizard is thought to be a relatively recent introduction (Le Sueur, 1976). There are some records from site managers and gardeners that the lizards have been abundant on the fort since the 1940s. Since then, other sites along the south coast of Jersey have been identified, in both private and public gardens. Smith, (2000) estimated the total minimum number of lizards at St Aubin's Fort to be 54 ± 3.80 individuals.
Fort Leicester (LF)	The fort is built into a hillside above Bouley Bay on Jersey's north-eastern coastline. It was constructed in 1836 as part of an island-wide defence strategy against French invasion. Subsequently the Fort has undergone numerous modifications made by the occupying forces during the Second World War (Hills, 2005a at the end of a small pier used for fisheries and coastal defence. The average number of lizards at the Fort was calculated to be a minimum of 70 ±17.39 individuals (Smith, 2000).
L'Etacquerel Fort (EF)	L'Etacquerel Fort, essentially a gun battery, was developed as part of a late 18th to early 19th century island-wide defense strategy (Hills, 2005b). The Fort is built on a headland on the east side of Fort Leicester, Bouley Bay. The total minimum number of adult lizards calculated was 26 ± 13.83 individuals (Smith, 2000).
Mont Orgueil Castle and Gorey (OF)	Mont Orgueil castle situated on Jersey's eastern coastline was built in several stages from the 13th century onwards. The construction of the castle was undertaken from 1204 as the main defence for the Island. During the 17th century the castle was no longer the primary defence for the Island so it was regarded as the Islands only prison until the end of 17th century, when the castle was stated as a ruin and subsequently abandoned. Eventually repairs were carried out over the 18th to 19th centuries and the castle was open to the public and has been classed as a museum site since 1929. The castle was also altered during the Second World War by German forces but continued as a museum after the occupation. Mont Orgueil castle has the largest population of *P. muralis* on Jersey which is widely distributed around the castles walls and extensive gardens (Hall, 2003).
Chausey Island (CHA)	Iles Chausey archipelago consists of 52 islands totalling approximately 59ha (Walters & Ineich 2006). Thirteen islands have been confirmed the presence of the species. We sampled lizards from Grand Ile (GRI; the largest island, 39h and the largest population), Aneret (ANE), le Grand Colombier (LGC), Grand Epail (GRE), Riche Roche (RIR) and Iles aux Oiseaux (IAO).

Table S4 | Table of null alleles per population per locus.

Population Code	Locus1	Locus2	Locus3	Locus4	Locus5	Locus6	Locus7	Locus8	Locus9	Locus10
AF	**0.33**	0.17	**0.17**	**0.18**	**0.25**	**0.13**	0	0	**0.25**	0
EF	**0.33**	0.3	0.11	No info	0	**0.04**	0	**0.1**	No info	0
LF	**0.33**	0.35	0	No info	0.07	0	0.06	0.04	**0.27**	0.22
OF	**0.32**	**0.25**	0.11	**0.12**	**0.1**	**0.05**	0	0	**0.16**	0.07
CH	**0.27**	0	0.02	**0.12**	0.01	0	0	0	**0.1**	0.06
CF	**0.24**	0	0	No info	0.02	0	No info	0.16	0.04	0.06
CG	0.03	**0.18**	**0.1**	No info	0.2	0	0.07	0	0.13	0.1
DN	**0.01**	**0.04**	**0.16**	0	0	0	0	0.08	**0**	0
SE	0	**0.23**	0	No info	0	0.07	0	0	0	0.02
VR	**0.1**	**0**	0.09	No info	0.05	0.01	**0**	0.05	0.09	0
JO	0.12	0.02	**0.17**	No info	0	**0.01**	**0.01**	0	**0.05**	0
PC	**0.05**	**0.1**	0	0	0	0.02	0	0	**0.06**	0
PU	**0.01**	0.1	0.03	No info	0	0	0.01	0.08	0.01	0.02
PZ	0.02	0.06	0	0	0	**0.02**	**0.05**	0.03	**0**	0.04
GE	0.01	**0.09**	0.01	No info	0.13	0	0	0.09	0	0.05
MI	0	**0**	0.1	No info	0.04	0	0.05	0	**0**	0
BA	0	0.33	0	No info	0	**0.01**	0	0.05	0.04	0.04
LI	0.03	0.07	0.01	0	0	0	0	0	**0**	**0.04**
SG	0.02	**0.42**	0	**0**	0.15	0	0	0	0	0
NE	**0**	**0.24**	0.02	**0**	0.05	0.02	0.03	0.03	0.11	**0.05**
FC	**0.05**	**0.2**	0.02	No info	0.1	0.05	0.01	**0**	0	0.01

Bold values indicated significant deviation from Hardy-Weinberg equilibrium ($p<0.05$).

Table S5 | Matrix of pairwise F_{ST} values.

	AF	EF	LF	OF	CH	CF	CG	DN	SE	VR	JO	PC	PU	PZ	GE	MI	BA	LI	SG	NE	FC
AF	0.000																				
EF	0.251	0.000																			
LF	0.171	0.054	0.000																		
OF	0.160	0.130	0.085	0.000																	
CH	0.128	0.100	0.058	0.076	0.000																
CF	0.199	0.203	0.160	0.152	0.112	0.000															
CG	0.206	0.198	0.149	0.128	0.097	0.082	0.000														
DN	0.221	0.180	0.152	0.141	0.133	0.120	0.080	0.000													
SE	0.259	0.234	0.175	0.144	0.144	0.155	0.182	0.183	0.000												
VR	0.200	0.211	0.147	0.125	0.105	0.116	0.113	0.110	0.094	0.000											
JO	0.146	0.191	0.132	0.101	0.094	0.104	0.106	0.099	0.105	0.053	0.000										
PC	0.248	0.213	0.161	0.152	0.151	0.073	0.086	0.090	0.114	0.083	0.087	0.000									
PU	0.155	0.174	0.127	0.102	0.093	0.098	0.093	0.076	0.108	0.059	0.051	0.069	0.000								
PZ	0.167	0.156	0.109	0.104	0.095	0.078	0.075	0.063	0.091	0.042	0.044	0.052	0.014	0.000							
GE	0.171	0.181	0.130	0.108	0.099	0.113	0.101	0.084	0.121	0.073	0.062	0.091	0.022	0.020	0.000						
BA	0.167	0.140	0.093	0.093	0.078	0.095	0.089	0.070	0.101	0.066	0.063	0.070	0.030	0.023	0.037	0.000					
MI	0.216	0.234	0.172	0.157	0.139	0.170	0.159	0.115	0.200	0.132	0.123	0.157	0.085	0.070	0.069	0.082	0.000				
LI	0.222	0.213	0.161	0.160	0.138	0.154	0.135	0.092	0.197	0.122	0.120	0.152	0.078	0.065	0.066	0.077	0.039	0.000			
SG	0.201	0.196	0.144	0.140	0.127	0.137	0.122	0.083	0.170	0.107	0.095	0.134	0.063	0.053	0.050	0.059	0.034	0.022	0.000		
NE	0.181	0.185	0.130	0.125	0.119	0.131	0.119	0.086	0.159	0.102	0.092	0.132	0.064	0.053	0.051	0.057	0.033	0.024	0.021	0.000	
FC	0.199	0.214	0.156	0.133	0.126	0.127	0.121	0.085	0.165	0.102	0.090	0.113	0.065	0.050	0.055	0.069	0.040	0.043	0.036	0.030	0.000

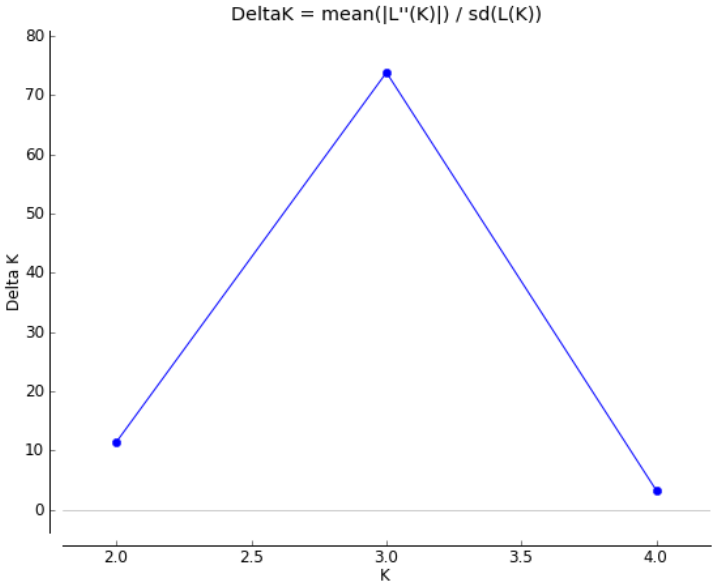

Figure S1 | Plot of Delta K (ΔK). Calculated as in Evanno *et al.* (2005) from K=2 to K=4. Highest Delta K for *K*=3.

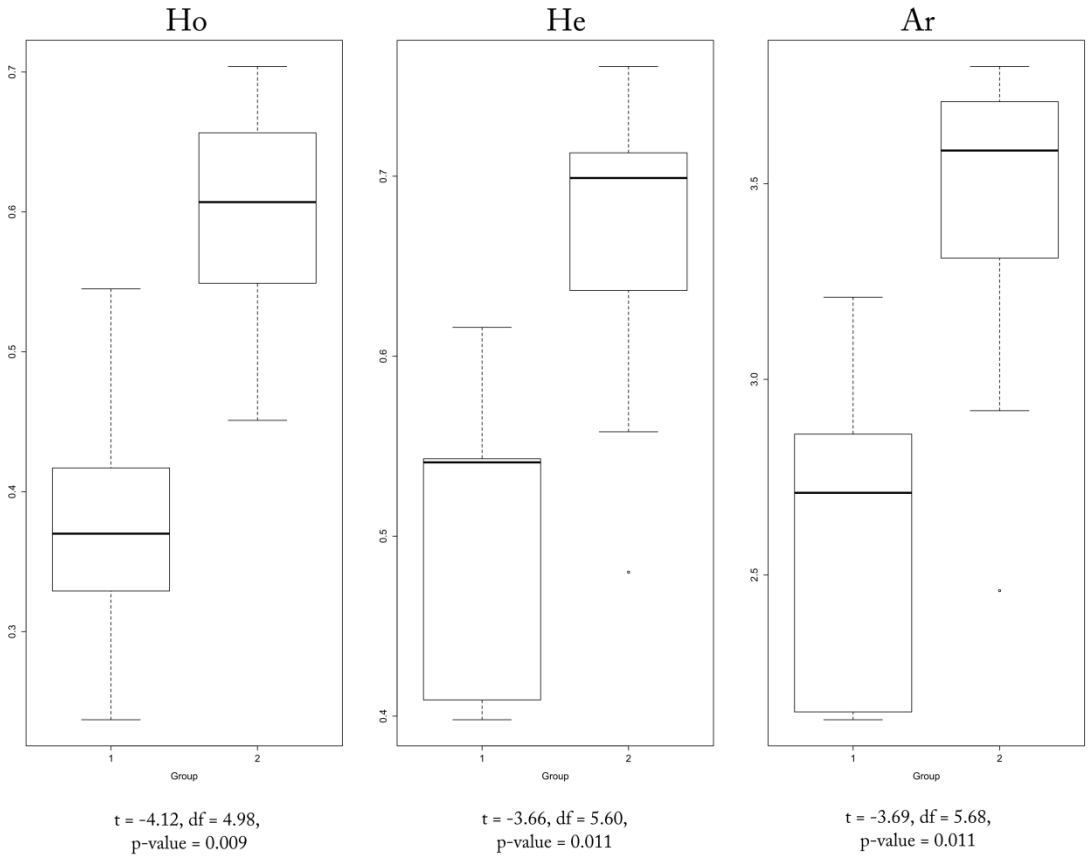

Figure S2 | Plots of genetic diversity indexes between island (group 1) and mainland populations (group 2). Genetic diversity is expressed as H_O, H_E and A_R. Differences in the mean numbers were compared with a Welch Two Sample t-test.

CHAPTER 3

CHAPTER 3

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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 hybridisation 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.

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 Quaternary 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 (Lunt *et al.* 1998; Taberlet *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 & Martin 2010). This suggests that hybridization could result in collapse of evolutionary lineages (e.g., Rhymer & 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 & 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 & 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.

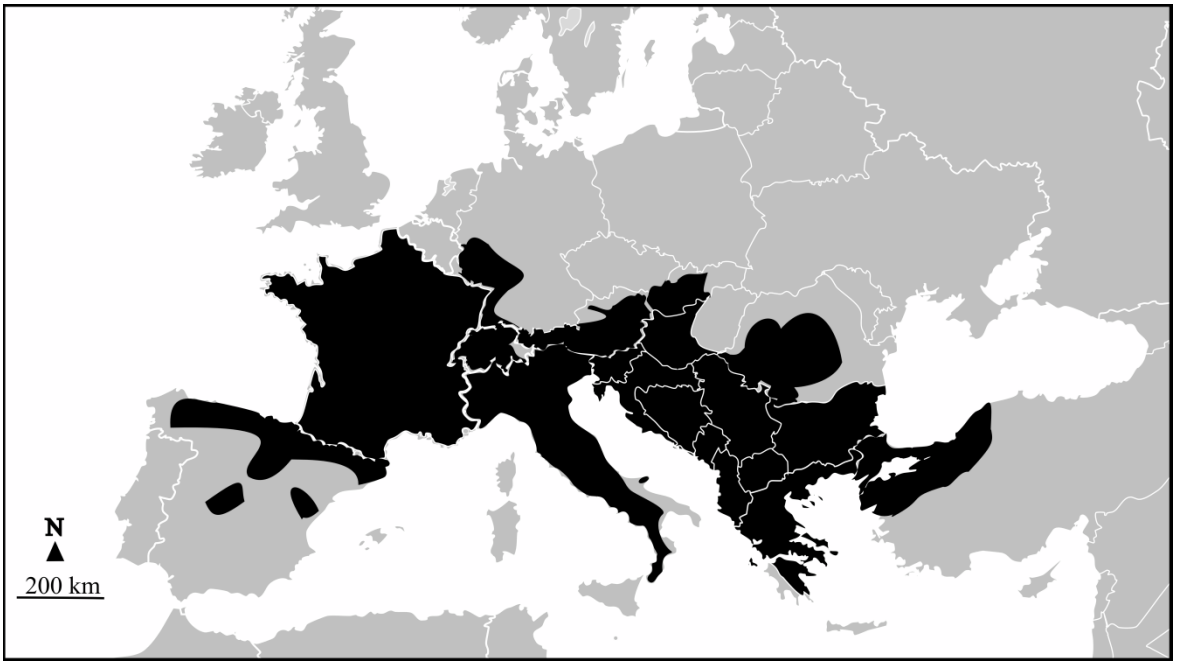

Figure 1 | Distribution of *Podarcis muralis* in the native range.

MATERIALS AND METHODS

Study Species and Sampling

The common wall lizard (*Podarcis 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 (Figure 1; Schulte 2008). *Podarcis muralis* show a strong phylogeographic structure with several genetically and geographically distinct clades (Giovannotti *et al.* 2010; Schulte *et al.* 2012b see below) that likely originated during isolation in glacial refugia (Giovannotti *et al.* 2010). The taxonomy of the species is debated (Gruschwitz & Böhme 1986; Schulte 2008; Glandt 2010), but five or six sub-species are currently recognised, with many additional insular types described (Gruschwitz & 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 (Gleed-Owen 2004; Allan *et al.* 2006; Burke & Deichsel 2008; Schulte *et al.* 2012b). 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 19th 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; T Uller & GM While unpubl). 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 (Figure 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; Figure 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 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.

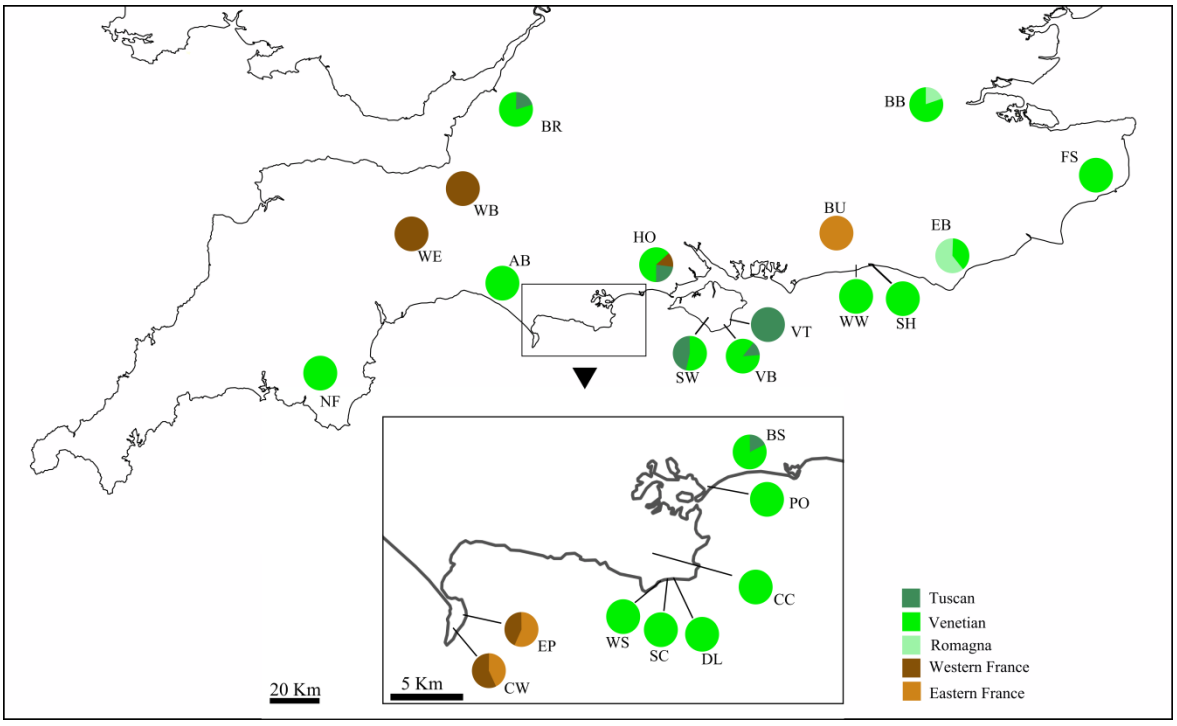

Figure 2 | Distribution of the 23 introduced populations in England. Pie charts indicate the percentage of sampled individuals matched to a specific clade from the native range. For populations abbreviations see Table 1.

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′-AACCGCCTGTTGTCTTCAACTA-3′] and Hpod [3′-GGTGGAATGGGATTTTGTCTG-5′] (Deichsel & Schwiger 2004; Podnar *et al.* 2007; Schulte *et al.* 2012). Amplifications were carried out in a total volume of 15µl consisting of 2µl template DNA, 0.45µl 8pm of each primer (Eurofins), 0.6µl 50mM $MgCl_2$ (Invitrogen), 0.6µl 10mM dNTPs (Invitrogen), 0.06µl Platinium Taq Polymerase (Invitrogen), 1.5µl 10x PCR Buffer (Invitrogen) and 9.34µl PCR grade H_2O. PCR conditions were as follows: an initial denaturation step at 94^{o}C for 1 min, followed by 35 cycles at 94^{o}C for 1 min, 53^{o}C for 45sec and 72^{o}C for 1 min and a final extension step at 72^{o}C for 10min. 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, 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.

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; Podnar *et al.* 2005; Poulakakis *et al.* 2005b; Podnar *et al.* 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.* 2012a) 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 & 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 & 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 & Li 1979). To provide an estimate of the divergence time, we used the published evolutionary rate for *Podarcis peloponnesiaca* and *Podarcis erhardii* (1.55% per million years; Poulakakis *et al.* 2005a*)* 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 & 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 (Figure 3). The phylogenetic tree approach nested these haplotypes within 5 distinct clades from the native range of the species (Figure 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 2-2.5 mya (Supplementary Table 4, see also Bellati *et al.* 2011).

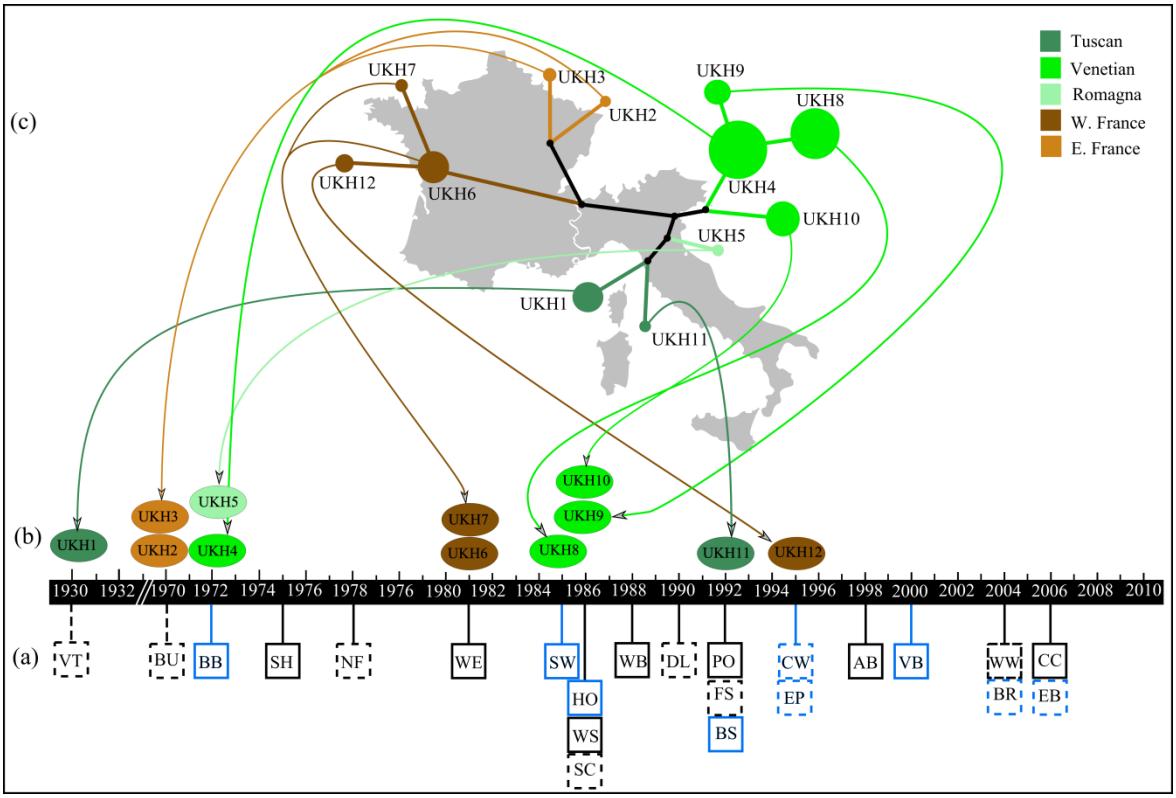

Figure 3 | Haplotype origins and history of introduction of wall lizards in England; (a) The timeline shows introduction* events of the UK populations (blue squares: populations of confirmed mixed origin; dotted squares: introduction date uncertain and approximated from first sighting or record of wall 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 table1 for details on introduction dates.

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

Population	Abbr*	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

* These abbreviations are used in all figures and tables

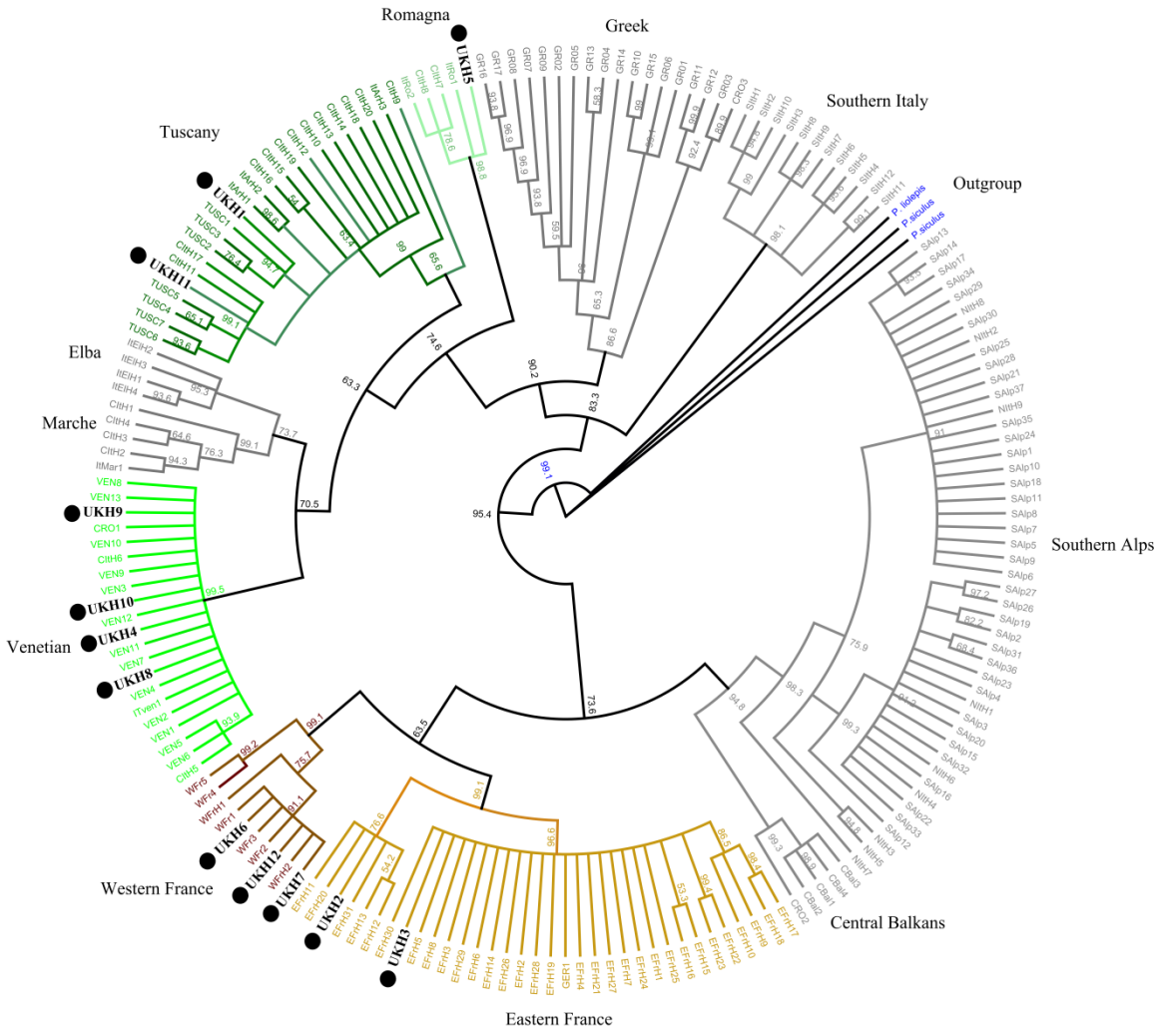

Figure 4 | Bayesian Analysis consensus tree. Derived from mitochondrial cytochrome b sequences of *P.muralis* from 175 published sequences and 12 unique sequences from this study. Bootstrap values are indicated above nodes. Black dots indicate the UK haplotypes that are nested within clades/geographic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

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).

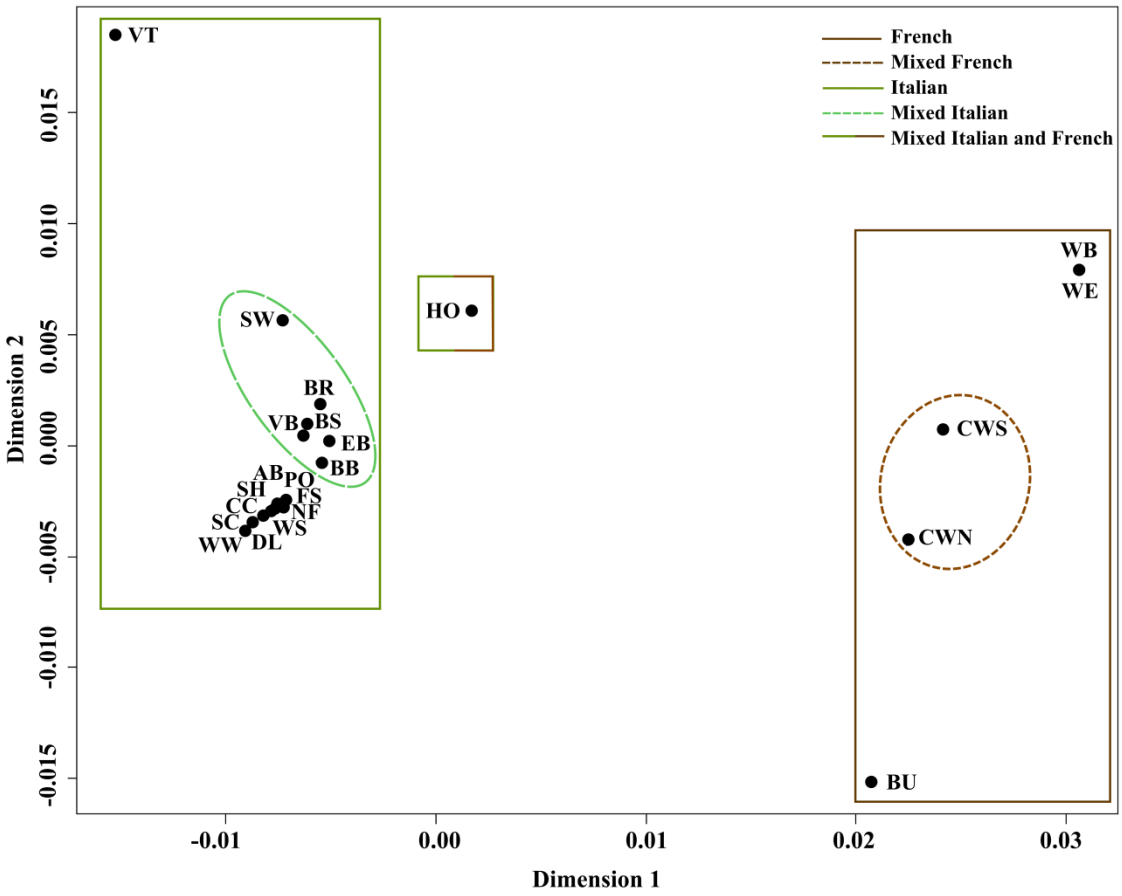

Figure 5 | Multidimensional scaling analysis based on pairwise sequence divergence among populations. The analysis revealed two main clusters; populations with French only haplotypes and populations with Italian only haplotypes. Within each cluster, ellipses indicate populations with multiple clade origin.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

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

Source of variation	*d.f*	Sum of squares	Variance components	Percentage of variation	p value
Among Populations	22	2613.8	5.33	77.50	<0.0001
Within populations	484	749.9	1.55	22.50	
Total	506	3363.7	6.89		

The MDS analysis (Figure 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; Figure 6).

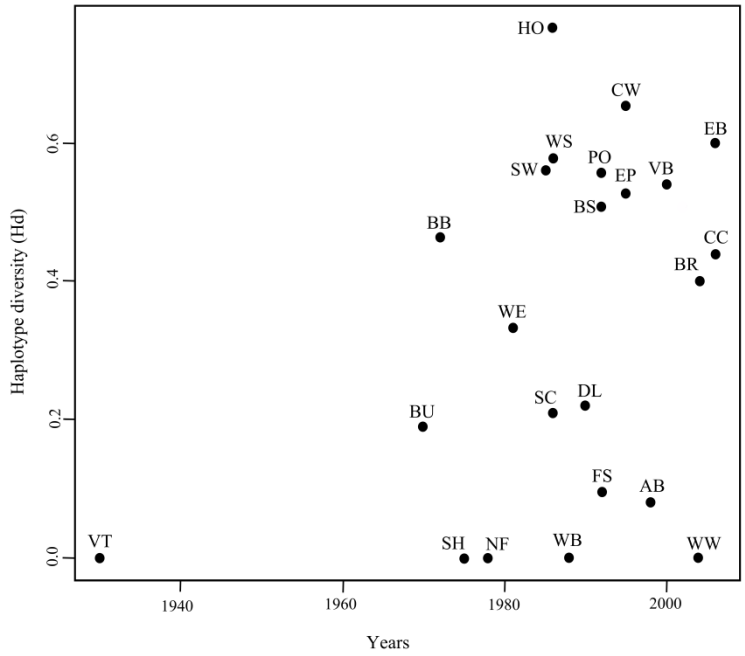

Figure 6 | Year of introduction and haplotype diversity (Hd). There is no evidence for a relationship between the age of a population and Hd. For populations abbreviations see Table 1.

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 & Böhme 1986; Schulte 2008). 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 & 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 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 (Schulte *et al*. 2012b; see also Kolbe *et al.* 2012 for an analysis of four introduced *P. siculus* populations in the USA). Indeed, multiple introductions are increasingly recognised as a common feature of species introductions (Roman & Darling 2007). For example, Kolbe and co-workers (2004) showed that there have been at least eight separate introductions of the brown anole (*Anolis sagrei*) in Florida. Multiple introductions can have substantial impact on genetic and phenotypic variation of introduced populations relative to those in the native range (Dlugosch & Parker 2008; Uller & Leimu 2011), and may even increase the ability of introduced species to adapt to local conditions (e.g. Lavergne & 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 *Lacerta agilis* (Rykena 1991; see also Rykena 1996; Olsson *et al.* 2004).

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, 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; T. Uller & G.M. While, pers. obs.).

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 forty 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 (Figure 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 (T. Uller, unpubl). 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 & 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 intra-specific hybridization in the future.

ACKNOWLEDGEMENTS

We are immensely grateful to Steve Langham (Surrey Amphibian and Reptile Group) for his efforts at mapping the occurrence of *P. muralis* in England and for generously providing detailed information about introduced populations. We are also grateful to all land owners for their permission to catch lizards on their property and to Natural England for permits (20091978; 20102163; 20112817). We are particularly indebted 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 getting access to private gardens and lands, giving us pointers that helped establish the geographic limits of populations, and retrieve details on the introduction history. We are also grateful to Robert Heathcote and Ben Daly for assistance with catching lizards and to Alicia Davies for assistance in the molecular lab. We thank two anonymous reviewers for helpful comments on the manuscript. This research was funded by the British Ecological Society, the National Geographic Society, and the Royal Society of London (all to TU), a FP7 Marie Curie Fellowship (GMW) and a Biotechnology and Biological Sciences Research Council (BBSRC) scholarship (SM).

CHAPTER 3| SUPPLEMENTARY INFORMATION

LIST OF TABLES

Table S1 | First year of introduction for all populations analysed in this paper. Some populations have been subject to multiple introduction events but all data refers to the first known introduction to the location unless for AB where extinction and re-introduction has been confirmed.

Location	Year of Introduction	Source
Abbotsbury (AB)	1998	Interviews with gardeners. An older introduction in 1890s went extinct in the 1960s
Birdbrook (BB)	1972	Interviews with residents/land owner
Boscombe (BS)	1992	Wareham, D.C. 2008. *The reptiles and amphibians of Dorset*. British Herpetological Society
Bristol (BR)	2004*	Unconfirmed date of introduction, first sighting record from Surrey Amphibian and Reptile Group
Bury (BU)	1970*$	Interviews with residents confirm the presence of the lizards in 1970. Exact year of introduction unknown
East Portland (EP)	Unknown	Unknown. Geographic distribution of lizards suggests a separate introduction, but could be expansion of CW#.
Cheyne Weare (CW)	1995*	Surrey Amphibian and Reptile Group
Corfe Castle (CC)	2006*	Unknown year of introduction. First reported in 2006.
Dancing Ledge (DL)	1990$	Surrey Amphibian and Reptile Group
Eastbourne (EB)	Unknown	Unknown year of introduction
Folkstone (FS)	1992*	Interviews with residents/land owner
Holmsley (HO)	1986	Interviews with residents/land owner
Newton Ferrers (NF)	1978*	Interviews with residents/land owner
Poole (PO)	1992	Surrey Amphibian and Reptile Group
Seacombe (SC)	1986$	Surrey Amphibian and Reptile Group
Shoreham (SH)	1975	Surrey Amphibian and Reptile Group
Shorwell (SW)	1985	Interviews with residents/land owner
Ventnor Botanics (VB)	2000	Interview with staff and residents
Ventnor Town (VT)	1930*$	Exact year unknown. Interviews with residents confirm presence of lizards at least as far back as 1930
Wellington (WE)	1981	Interviews with residents/land owner
West Worthing (WW)	2004*	Unknown year of introduction. First reported in 2004.
Winspit (WS)	1986	Surrey Amphibian and Reptile Group
Wembdon (WB)	1988	Interviews with residents/land owner

*= Exact year of introduction unconfirmed, data refers to first confirmed sighting
$= Exact year of introduction not confirmed, data refers to approximate year
#= Photographic (Emma Cockburn, photos taken in 2008) and anecdotal (Steve Langham, Surrey Amphibian and Reptile Group, pers comm) evidence supports the presence of a population of putative Tuscan origin on Portland, but it had not been located at the time of sampling.

Table S2 | GenBank Accession numbers of UK haplotypes

Haplotype	Accession Number
UKH1	JX856982
UKH2	JX856983
UKH3	JX856984
UKH4	JX856985
UKH5	JX856986
UKH6	JX856987
UKH7	JX856988
UKH8	JX856989
UKH9	JX856990
UKH10	JX856991
UKH11	JX856992
UKH12	JX856993

Table S3 | Genetic divergence (Tamura-Nei model) among the 5 main lineages that have been introduced to England.

	VEN	TUS	ROM	WFR	EFR
VEN	-				
TUS	2.9	-			
ROM	2.1	2.9	-		
WFR	4.6	5.4	4.6	-	
EFR	4.4	5.7	4.8	3.5	-

Table S4 | Divergence time (T) among selected nodes.

Node	T (mya)
Clade 1 and Clade 2 (WFR, EFR, CB, SA) (SI, VEN, TUS, ROM, MAR)	4.2
WFR - EFR	3.5
ROM – TUS+VEN+MAR	2.5
TUS-VEN	2

CHAPTER 4

MOLECULAR ECOLOGY

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

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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.

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 secondary 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 are 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 2009a). 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 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; Schulte *et al.* 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 since the oldest populations have adapted to the cooler climate in England (While *et al.* 2015a).

The aim of the present 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 (Figure 1): (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 (Figure 2, Table 1). The native source regions were chosen based on previously identified geographic

regions for mitochondrial clades (with the exception of the Eastern France clade that we did not sample; Schulte *et al.* 2012a; Schulte *et al.* 2012b; Michaelides *et al.* 2013; Salvi *et al.* 2013). Lizards were caught by hand or noosing and a small (ca 5mm) 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) following 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 supplementary Table S1) in a total volume of 11µl reaction mix containing 1µl of genomic DNA, 5µl of Qiagen MasterMix, 0.2µl of each primer (forward and reverse in equal concentrations) and 3.8µl (for multiplex 1,2, 3 and 5) or 3.6µl (for multiplex 4) of PCR grade dH_2O. PCR conditions were as follows: 15min of initialization step at 95°C, 26 cycles of 30sec at 94°C, 90sec at 57°C (for multiplex 1, 2 and 3) or 55°C (for multiplex 4 and 5) and 1min at 72°C and a final extension step of 20min 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.). 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 15µl consisting of 7.5µl of MyTaq HS Mix (Bioline), 0.45µl (8pm) of each primer (Eurofins), 4.6µl PCR grade H_2O and 2µl 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 45sec and 72°C for 1 min and a final extension step at 72°C for 10min. PCR products were purified using the MinElute 96 UF PCR Purification Kit (Qiagen, Valencia, CA). Sequencing reactions were carried out with BIGDye 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 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; Giovannotti *et al.* 2010; Bellati

et al. 2011; Schulte *et al.* 2012b; Gassert *et al.* 2013; Salvi *et al.* 2013; Michaelides *et al.* 2015a) to build a phylogenetic tree and assign each population to a mitochondrial lineage (see details of geographic localities of all sequences used in supplementary Table S2). 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 *P. 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.* 2015a). 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 (Figure 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; Cornuet *et al.* 2010; Cornuet *et al.* 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.

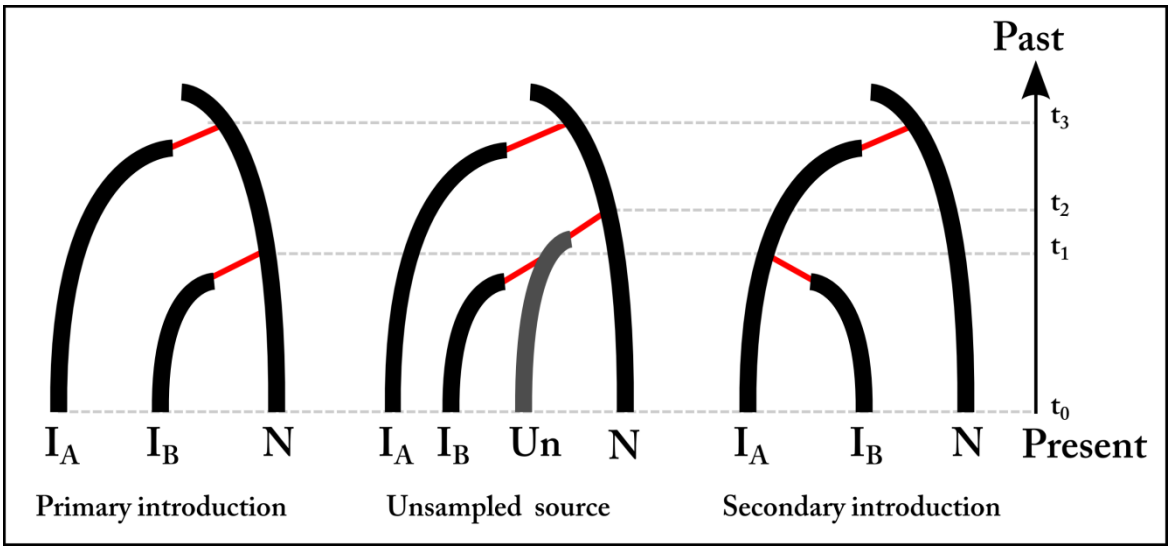

Figure 1 | Graphical representation of *P. muralis* introduction scenarios in England tested by the ABC approach. The "*primary introduction model",* where the introduced population I_B originated independently from the native (N) range, the "*unsampled source model*" where the introduced population 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,t3 represent time of introduction event (in generations). Figure modified from Estoup & Guillemaud (2010).

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 in Supplementary 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; Cornuet *et al.* 2010; Cornuet *et al.* 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, 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 $3x10^6$ 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 non-overlapping 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 analyzed 500 pseudo-observed data set using parameter values drawn from prior distributions (Table S3 in Supplementary 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; firstly using proportions of membership (*Q*) in a defined cluster as a metric of relationship and secondly 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 2015) 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.* 2015a) 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 Figure S1 in Supplementary Information). The geographical distribution of these lineages in our sample is shown in figure 2. Four populations in the native range (in Italy) were found to harbour haplotypes from two different lineages (Figure 2). The median-joining network grouped all sequences into six haplogroups (Figure 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).

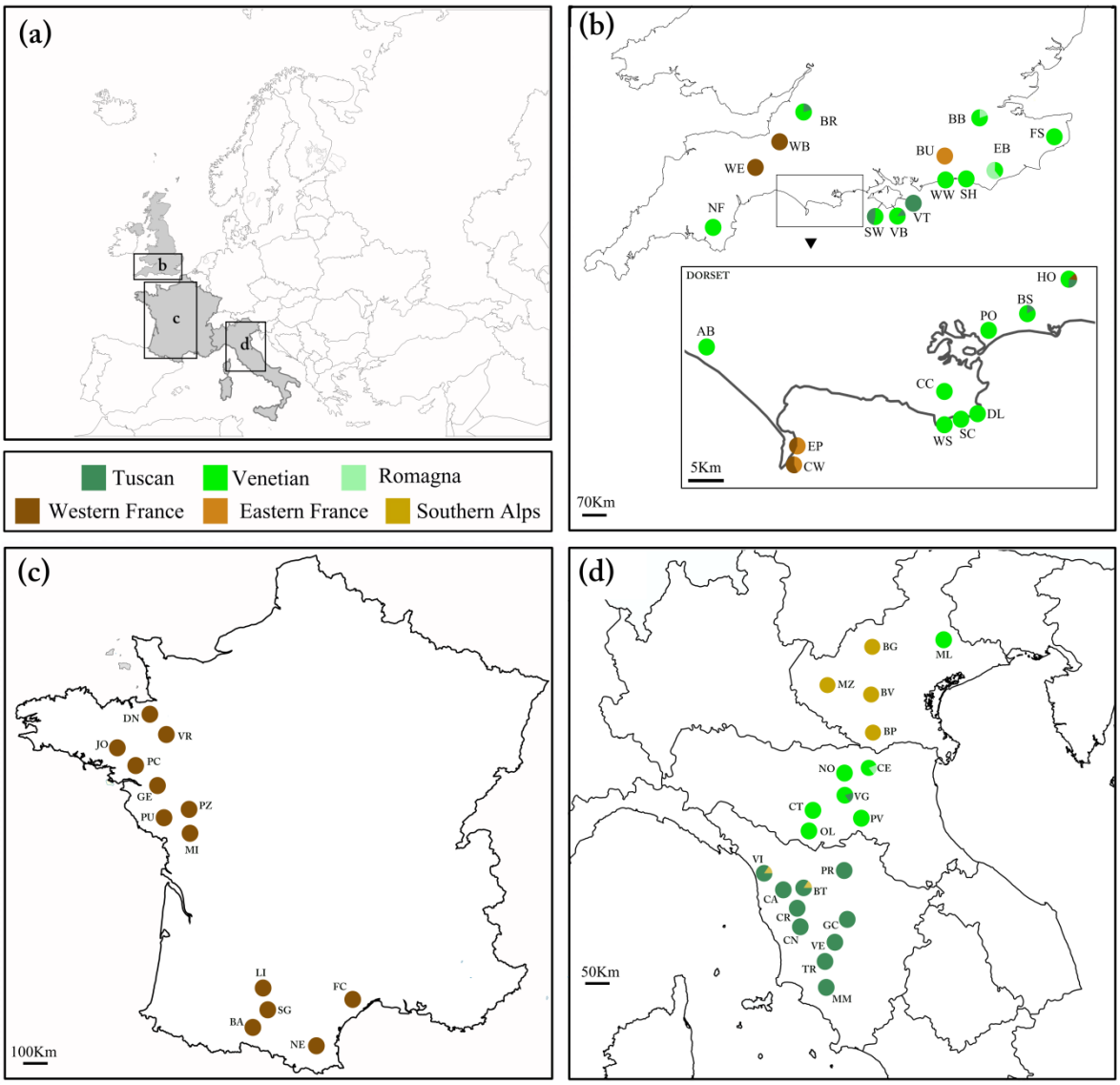

Figure 2 | Sampling locations and mtDNA lineage distribution; a) location of the three sampling regions; b distribution of introduced populations in England (modified from Michaelides *et al.* 2013), c) native populations in France (modified from Michaelides *et al.* 2015), d) native populations in Italy.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

Table 1 | Sampling locations in introduced and native range.

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
Shorwell	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	
Castelarrano	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
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
Pouzagues	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.
**Sequences analysed in Michaelides *et al.* (2015a)
#Lineage abbreviations correspond to Venetian (VEN), Tuscan (TUS), Romagna (ROM), Southern Alps (SAL), Western France (WFR), Eastern France (EFR).
Membership in a genetic cluster as defined by the Structure analysis (see supplementary Table S5 for proportions of membership, Q)
$Data from Michaelides *et al.* (2013).

Genetic structure

The Bayesian clustering analysis in STRUCTURE (see Figure S2 in Supplementary 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 in Supplementary 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 south-western France), whereas the structure of Italian populations showed substantially higher differentiation between native and non-native populations (see Figure S3 in Supplementary Information).

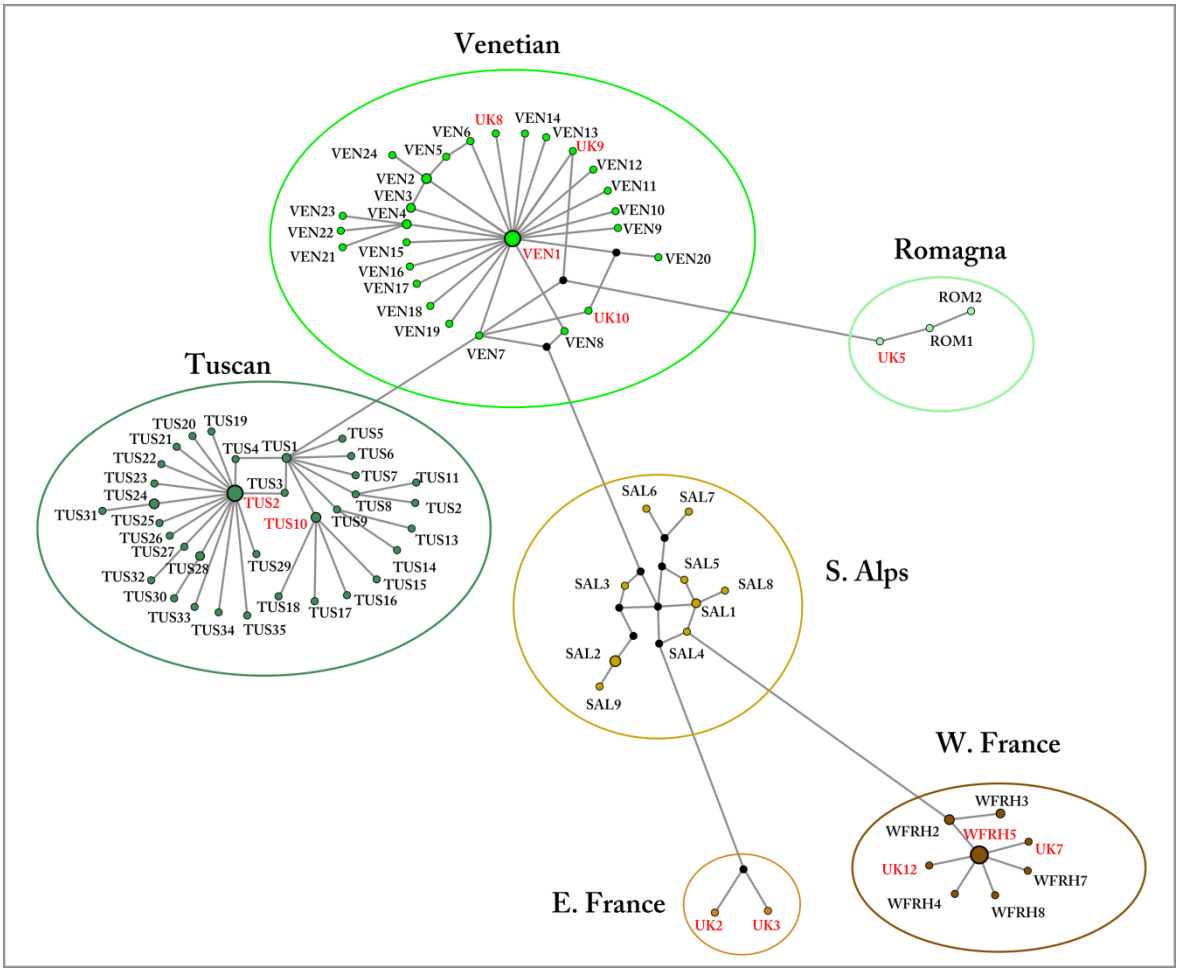

Figure 3 | Median-joining network of mtDNA sequences. Combined sequences analyzed in this study with UK sequences (Michaelides *et al*. 2013) and French sequences (Michaelides *et al*. 2015). Six haplogroups have been identified corresponding to six lineages (Tuscan [TUS], Venetian [VEN], Romagna [ROM], 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).

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 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 harbor 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 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 (Figure 4; see Figure S4a for results for Q = 0.1). The F_{ST} based networks resulted in very similar results (Figures 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.

Table 2 | Posterior probabilities of the selected scenario* for each non-native population tested by ABC.

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]	

* The selected scenario was the one with the significantly highest posterior probability value and with the 95% confidence interval (95% C.I.) not overlapping with the 95% C.I of any other compared scenario. When the 95% C.I. overlap, we all values are reported.
**Populations are ordered according to approximate time of introduction.
***Limited sample size (=5).

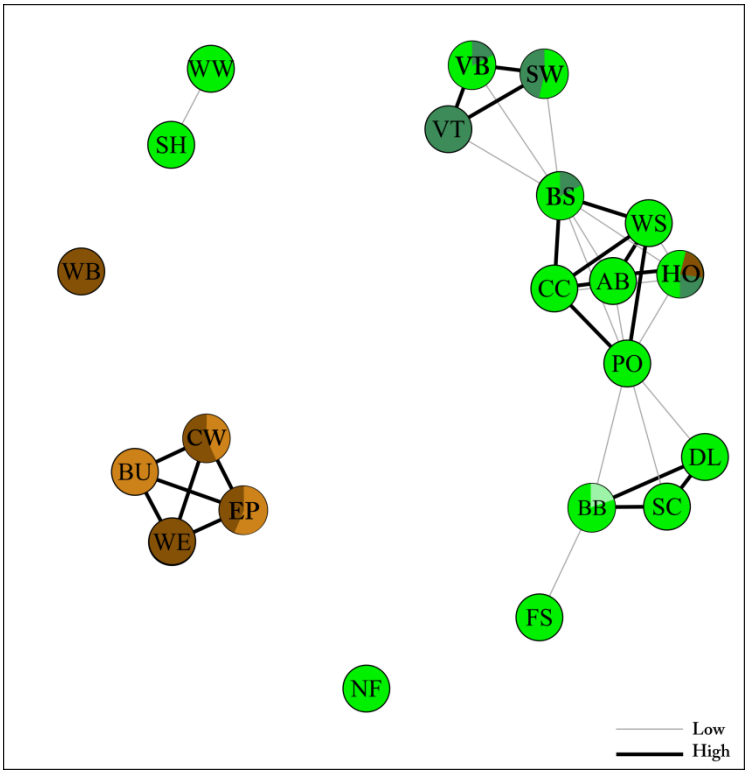

Figure 4 | Network analysis of introduced populations in England. The network is constructed based on average pairwise proportion of membership (Q) in a cluster (*K*) as defined by STRUCTURE. Populations are coloured coded according to mtDNA lineage. The thickness of the line connecting two nodes (populations)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

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%) of 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; Kolbe *et al.* 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 suggests that the commercial pet trade at that time did not make use of the existing population on the Isle of Wight (which is of Tuscan *P. m. nigriventris* origin), nor the previously extensive import from Jersey (which was

officially banned in the 1940s; Michaelides *et al.* 2015a), 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 appear 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 out 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 non-deliberate 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.* 2015a). Non-deliberate introductions are also unlikely, given that the majority 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; Schulte *et al.* 2012c). Somewhat surprisingly, the data in Table 2 does 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, it suggests active translocation of animals of two or three introduced populations within a limited geographic region from the mid-80's, 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 origin show they have adapted to the cooler climate in England (While *et al.* 2015a). 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.,

Anolis 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) harbors 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 harbors 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 *P. 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 1990's. 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 paper by their comments. Funding for this project was obtained by the British Ecological Society, the National Geographic Society and the Royal Society (all to TU). SNM is supported by the A.G 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.

CHAPTER 4 | SUPPLEMENTARY INFORMATION

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Figure S2 |Bayesian Clustering Analysis in STRUCTURE. The optimal number of clusters (*K*) was found at *K*=9. Each individual is represented by a vertical line, divided into *K* colours according to the proportion of membership to each genetic cluster (for population abbreviation see Table 1).

Figure S3 | Bayesian Clustering Analysis in STRUCTURE including both native and introduced populations. The optimal number of clusters (*K*) was found at *K*=6. French-origin introduced and native populations from France belong to two clusters. Italian-origin introduced and native populations from Italy belong to four clusters (substantial differentiation among native and introduced). Each individual is represented by a vertical line, divided into *K* colours according to the proportion of membership to each genetic cluster (for population abbreviation see Table 1).

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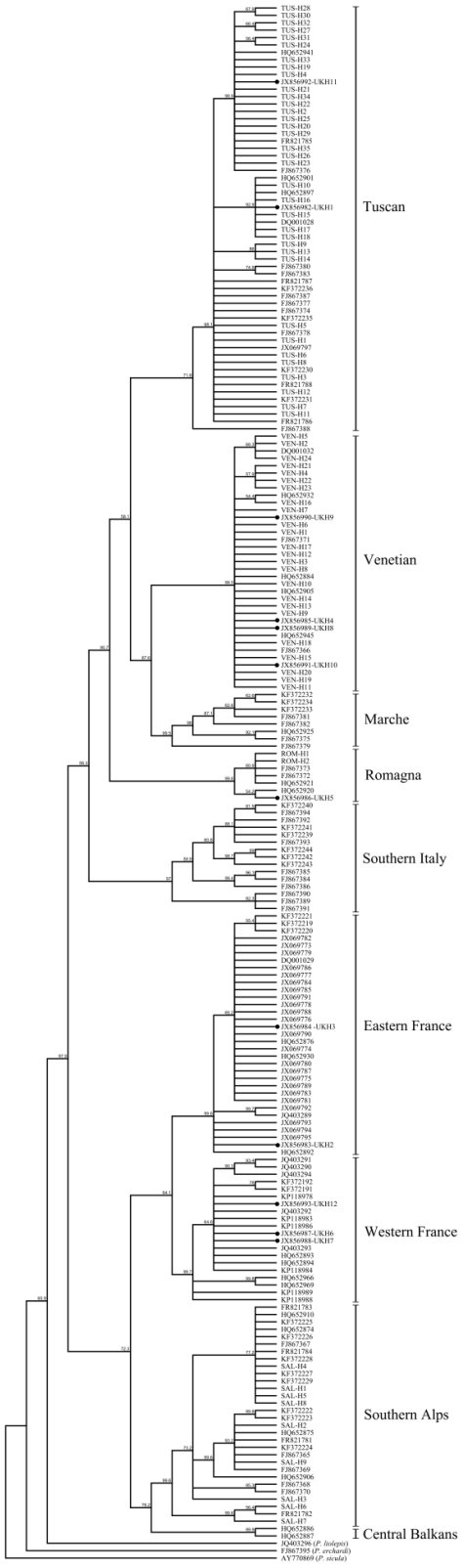

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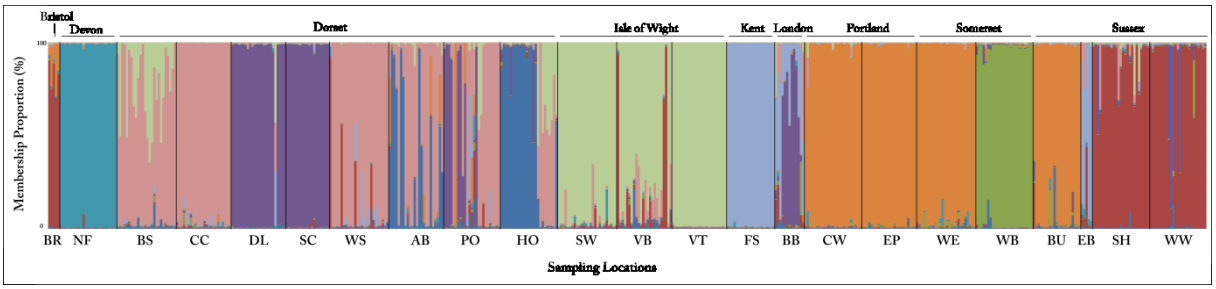

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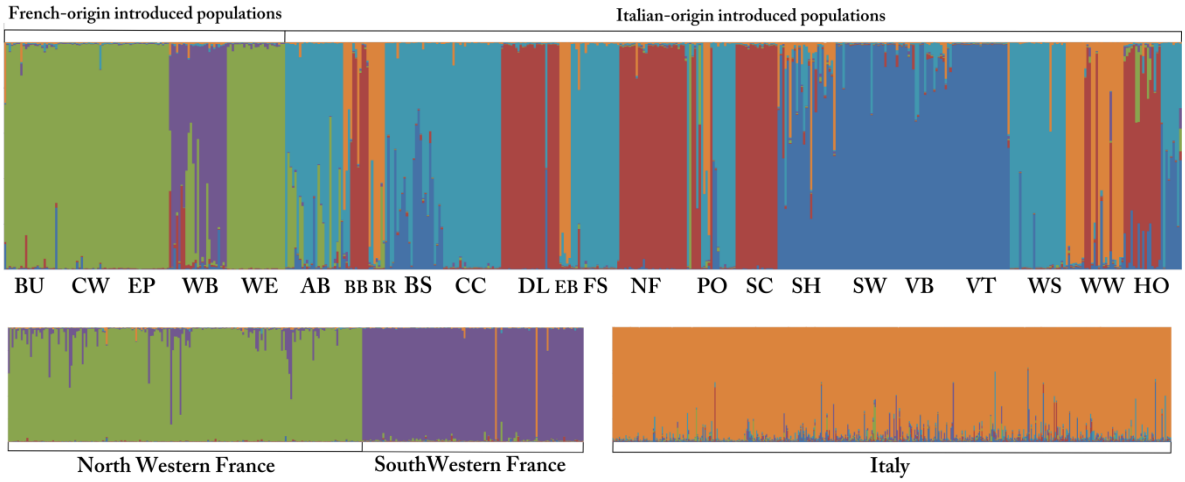

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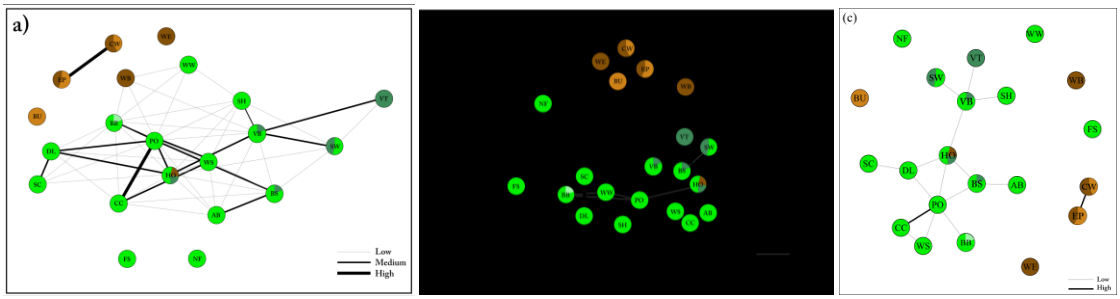

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Table S1 | Microsatellite marker multiplexes used to genotype all individuals in this study.

Multiplex	Locus		Primer sequence (5'-3')	Product size (bp)	Repeat motif	Range (bp)
1	PmurC150	F	**[6-FAM]**GTCAGCTTTGCAGCACCTTAG	193	CA	171-217
		R	GCGATTAGAGAAGGCGTTTG			
	PmurC168	F	**[HEX]**GGTCCGGCTTCAAAGAATAAG	244	TTTC	210-400
		R	CAGAGGACTCGCTCAAGGAC			
	PmurC275_278	F	**[NED]**GCTTAAAATTAATGCTGCTATTGTATC	245	TATC	219-655
		R	ATAGGTAGAAAATTTATAAACCCTTGG			
2	PmurC164	F	**[6-FAM]**ATCGATGAATGAAGGGCAGT	216	GATA	166-246
		R	CCAGGCATTGTCAAACTATCTG			
	PmurC038	F	**[HEX]**CAATGTGCAGTGTTGGGTTG	210	TATC	165-629
		R	ATGTGAGCGACTCCTGGATG			
	PmurC028	F	**[NED]**TTGCTTCTGAATACGCCTAGC	287	TATC	249-545
		R	AGTGTATTGCGACTGTCAATGG			
3	PmurC356	F	**[6-FAM]**GATCTTCAGATGAAGGGTAGTTAGAT	159	GTTA	138-178
		R	ATGAAGACAAACAGGCTTGG			
	PmurC109	F	**[HEX]**AGGAGCCCAGCAGCTGAA	309	GTA	295-355
		R	TTTACATAGACCTGCGGGTATGG			
	PmurC103	F	**[NED]**CCAGGTCTTGTGATCGAGTG	350	GATA	316-480
		R	CCCGACCCAAACTAGTGC			
4	Pm01	**F**	**[6-FAM]** CCACAGGCATCTGGTTAG	-	$(ATT)_{16}$	101-155
		R	TCCATAAGACTGTAAGACAAGCC			
	Pm05	**F**	**[HEX]** CAAGAGGGCAGCCTAGTAATG	-	$(AGAT)_{10}$	135-415
		R	AGATGGGCTCATTTCAACTCC			
	Pm09	**F**	**[NED]** ACGTGTTTCTGTGCTTTGC	-	$(ATT)_{17}$	176-203
		R	AGTCAGACGAGAGGTTGCC			
	Pm16	**F**	**[6-FAM]** GGGATGGAGAAAGATGGCG	-	$(TCTT)_{16}$	161-225
		R	GCACTTGCCTACTGGTCATAC			
5	Pm02	**F**	**[HEX]** TTGGGAAGAAGGGGAAGGG	-	$(AACC)_{7}$	156-280
		R	ATGGCCGCTAGGTCAAGTG			
	Pm19	**F**	**[6-FAM]** CAGCCACAAGGTGAACCAG	-	$(AGGC)_{11}$	144-220
		R	TGTGAGGTCAGAGGCATGG			
	Pm14	**F**	**[NED]** GCAGGATCAGAGCGCAATC	-	$(GCAG)_{7}$	138-186
		R	TGTGGCATGTTGAGACACC			

Table S2 | List of all cytochrome b (*cyt-b*) sequence data used in the phylogenetic analysis. Information on sampling location, GenBank accession numbers and references.

a/a	Haplotype code	GenBank accession number	Region - Locality - Population ID	Reference
1	*P. sicula*	AY185095	Outgroup	Podnar et al. 2004 (Organisms Div. Evol)
2	mur8	DQ001028	Italy - Tuscany, Firenze	Podnar et al. 2007 (J. Mol. Evol)
3	mur9	DQ001029	Germany - Offenburg	Podnar et al. 2007 (J. Mol. Evol)
4	mur12	DQ001032	Italy - Friuli-Venezia	Podnar et al. 2007 (J. Mol. Evol)
5	H1	FJ867365	Italy - Trento, Vercelli	Giovannotti et al. 2010 (Ital. J. Zool.)
6	H2	FJ867366	Italy - Trieste	Giovannotti et al. 2010 (Ital. J. Zool.)
7	H3	FJ867367	Italy - Pavia	Giovannotti et al. 2010 (Ital. J. Zool.)
8	H4	FJ867368	Italy - Pavia	Giovannotti et al. 2010 (Ital. J. Zool.)
9	H5	FJ867369	Italy - Val Germanasca	Giovannotti et al. 2010 (Ital. J. Zool.)
10	H6	FJ867370	Italy - Parma	Giovannotti et al. 2010 (Ital. J. Zool.)
11	H7	FJ867371	Italy - Ferrara, Ravenna	Giovannotti et al. 2010 (Ital. J. Zool.)
12	H8	FJ867372	Italy - Ravenna, Cesena	Giovannotti et al. 2010 (Ital. J. Zool.)
13	H9	FJ867373	Italy - Cesena, Pesaro, Montignano, Senigallia	Giovannotti et al. 2010 (Ital. J. Zool.)
14	H10	FJ867374	Italy - Carpegna, Bolognola, Amatrice, Gran Sasso, L'Aquila, Latina	Giovannotti et al. 2010 (Ital. J. Zool.)
15	H11	FJ867375	Italy - Montifnano, Ancona	Giovannotti et al. 2010 (Ital. J. Zool.)
16	H12	FJ867376	Italy - Pisa	Giovannotti et al. 2010 (Ital. J. Zool.)
17	H13	FJ867377	Italy - Genga, M. te San Vicino	Giovannotti et al. 2010 (Ital. J. Zool.)
18	H14	FJ867378	Italy - Genga	Giovannotti et al. 2010 (Ital. J. Zool.)
19	H15	FJ867379	Italy - M. te San Vicino, Porto S. Elpidio, Macerata, Bolognola, Porto d' Ascoli	Giovannotti et al. 2010 (Ital. J. Zool.)
20	H16	FJ867380	Italy - Viso	Giovannotti et al. 2010 (Ital. J. Zool.)
21	H17	FJ867381	Italy - Caramanico Terme	Giovannotti et al. 2010 (Ital. J. Zool.)
22	H18	FJ867382	Italy - Gran Sasso	Giovannotti et al. 2010 (Ital. J. Zool.)
23	H19	FJ867383	Italy - L' Aquila	Giovannotti et al. 2010 (Ital. J. Zool.)
24	H20	FJ867384	Italy - Gargano	Giovannotti et al. 2010 (Ital. J. Zool.)
25	H21	FJ867385	Italy - Gargano	Giovannotti et al. 2010 (Ital. J. Zool.)
26	H22	FJ867386	Italy - Gargano	Giovannotti et al. 2010 (Ital. J. Zool.)
27	H23	FJ867387	Italy - Latina	Giovannotti et al. 2010 (Ital. J. Zool.)
28	H24	FJ867388	Italy - Matese	Giovannotti et al. 2010 (Ital. J. Zool.)
29	H25	FJ867389	Italy - Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
30	H26	FJ867390	Italy - Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
31	H27	FJ867391	Italy - Monti Alburni	Giovannotti et al. 2010 (Ital. J. Zool.)
32	H28	FJ867392	Italy - Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
33	H29	FJ867393	Italy - Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
34	H30	FJ867394	Italy - Pollino	Giovannotti et al. 2010 (Ital. J. Zool.)
35	P. erhardi	FJ867395	Outgroup	Giovannotti et al. 2010 (Ital. J. Zool.)
36	M1	FR821781	Italy - Verona	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
37	M2	FR821782	Italy - Pavia	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
38	M3	FR821783	Italy - Bereguardo	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
39	M4	FR821784	Italy - Pavia	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
40	N1	FR821785	Italy - Calci	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
41	N2	FR821786	Italy - Borgo Montello	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
42	N3	FR821787	Italy - Borgo Montello	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
43	N4	FR821788	Italy - Borgo Montello	Bellati et al. 2011 (J. Zool. Syst. Evol. Res)
44	UU97	HQ652874	Germany – Bramsche, Ueffeln	Schulte et al. 2008 (Z. Feldherptol),

			(previously assigned to the Southern Alps lineage)	Schulte et al. 2012 (Global Ecol. Biogeogr)
45	UU91	HQ652875	Germany – Bielefeld (previously assigned to the Southern Alps lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
46	UU92	HQ652876	Germany (previously assigned to the Eastern France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
47	UU30	HQ652884	Germany – Dresden (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
48	UU89	HQ652886	Germany – Altenhain (previously assigned to the Central Balkans lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
49	UU90	HQ652887	Germany – Altenhain (previously assigned to the Central Balkans lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
50	UU115	HQ652892	Germany - Darmstadt (previously assigned to the Eastern France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
51	UU67	HQ652893	Germany – Mainz previously assigned to the Western France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
52	UU70	HQ652894	Germany – Mainz previously assigned to the Western France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
53	UU76	HQ652897	Germany – Bad Cannstadt (previously assigned to the Tuscan lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
54	UU80	HQ652901	Germany – Stuttgart (previously assigned to the Tuscany lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
55	UU50	HQ652905	Germany - Mannheim (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
56	UU2	HQ652906	Germany – Tuebingen (previously assigned to the Southern Alps lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
57	UU136	HQ652910	Germany – Bielefeld (previously assigned to the Southern Alps lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
58	UU127	HQ652920	Germany – Loerrach (previously assigned to the Romagna lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
59	UU128	HQ652921	Germany – Loerrach (previously assigned to the Romagna lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
60	UU101	HQ652925	Germany (previously assigned to the Marche lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
61	UU144	HQ652930	Germany (previously assigned to the Eastern France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
62	UU59	HQ652932	Germany – Aschaffenburg (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
63	SD5	HQ652941	Austria (previously assigned to the Tuscany lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
64	UU57	HQ652945	Germany (previously assigned to the Venetian lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
65	P. liolepis	HQ652946	Outgroup	Schulte et al. 2012 (Global Ecol. Biogeography)
66	Noe3	HQ652966	Germany Noerten-Hardenberg, Lower	Schulte et al. 2008 (Z. Feldherptol),

			Saxony (previously assigned to the Western France lineage)	Schulte et al. 2012 (Global Ecol. Biogeogr)
67	Noe1	HQ652969	Germany Noerten-Hardenberg, Lower Saxony (previously assigned to the Western France lineage)	Schulte et al. 2008 (Z. Feldherptol), Schulte et al. 2012 (Global Ecol. Biogeogr)
68	Labeaume	JQ403289	France - Labeaume	Schulte et al . 2012 (Amphibia-Reptilia)
69	Montsegur	JQ403290	France - Montsegur	Schulte et al . 2012 (Amphibia-Reptilia)
70	Lourdes	JQ403291	France - Lourdes	Schulte et al . 2012 (Amphibia-Reptilia)
71	La Rochelle	JQ403292	France - La Rochelle	Schulte et al . 2012 (Amphibia-Reptilia)
72	St. Malo	JQ403293	France - St. Malo	Schulte et al . 2012 (Amphibia-Reptilia)
73	Amboise	JQ403294	France - Amboise	Schulte et al . 2012 (Amphibia-Reptilia)
74	Remich	JX069773	Luxembourg - Remich	Gassert et al. 2013 (J. Biogeography)
75	Larochette	JX069774	Luxembourg - Larochette	Gassert et al. 2013 (J. Biogeography)
76	Mont St Odily	JX069775	France – Mont St Odily	Gassert et al. 2013 (J. Biogeography)
77	Esch-sur-Sure	JX069776	Luxembourg - Esch-sur-Sure	Gassert et al. 2013 (J. Biogeography)
78	Bitche	JX069777	France – Bitche	Gassert et al. 2013 (J. Biogeography)
79	Ansembourg	JX069778	Luxembourg - Ansembourg	Gassert et al. 2013 (J. Biogeography)
80	France – Euville	JX069779	France – Euville	Gassert et al. 2013 (J. Biogeography)
81	Burscheid	JX069780	Luxembourg - Burscheid	Gassert et al. 2013 (J. Biogeography)
82	Urft	JX069781	Germany - Urft	Gassert et al. 2013 (J. Biogeography)
83	Willerwilz	JX069782	Luxembourg - Willerwilz	Gassert et al. 2013 (J. Biogeography)
84	Vianden	JX069783	Luxembourg - Vianden	Gassert et al. 2013 (J. Biogeography)
85	Lux. City	JX069784	Luxembourg - Lux. City	Gassert et al. 2013 (J. Biogeography)
86	Beaufort	JX069785	Luxembourg - Beaufort	Gassert et al. 2013 (J. Biogeography)
87	Kautenbach	JX069786	Luxembourg - Kautenbach	Gassert et al. 2013 (J. Biogeography)
88	Dudelange	JX069787	Luxembourg - Dudelange	Gassert et al. 2013 (J. Biogeography)
89	Wasserbillig	JX069788	Luxembourg - Wasserbillig	Gassert et al. 2013 (J. Biogeography)
90	Braubach	JX069789	Luxembourg - Braubach	Gassert et al. 2013 (J. Biogeography)
91	Wark valley	JX069790	Luxembourg - Wark valley	Gassert et al. 2013 (J. Biogeography)
92	Anhee	JX069791	Belgium - Anhee	Gassert et al. 2013 (J. Biogeography)
93	Labeaume	JX069792	France - Labeaume	Gassert et al. 2013 (J. Biogeography)
94	St Remy	JX069793	France - Saint Remy de Provence	Gassert et al. 2013 (J. Biogeography)
95	Autun	JX069794	France - Autun	Gassert et al. 2013 (J. Biogeography)
96	St Martin	JX069795	France - Saint Martin	Gassert et al. 2013 (J. Biogeography)
97	Alba Fucens	JX069797	Italy - Alba Fucens	Gassert et al. 2013 (J. Biogeography)
98	UKH1	JX856982	England - Ventnor Town (VT), Holmsley (HO), Ventnor Botanical Garden (VB), Shorwhel (SW)	Michaelides et al. 2013 (Biol. Inv)
99	UKH2	JX856983	England - Bury (BU)	Michaelides et al. 2013 (Biol. Inv)
100	UKH3	JX856984	England - Bury (BU), East Portland (EP), Cheyne Weare (CW)	Michaelides et al. 2013 (Biol. Inv)
101	UKH4	JX856985	England - Abbotsbury (AB), Birdbrook (BB),Corfe Castle (CC), Eastbourne (EB), Folkestone (FS), Holmsley (HO), Newton Ferrers (NF), Poole (PO), Seacombe (SC), Shoreham (SH), Shorwell (SW), Ventnor Botanical Garden (VB), Winspit (WS)	Michaelides et al. 2013 (Biol. Inv)
102	UKH5	JX856986	England - Birdbook (BB), Eastbourne (EB)	Michaelides et al. 2013 (Biol. Inv)
103	UKH6	JX856987	England - East Portland (EP), Cheyne Weare (CW), Holmsley (HO), Wembdon (WB), Wellington (WE)	Michaelides et al. 2013 (Biol. Inv)
104	UKH7	JX856988	England - Wellington (WE)	Michaelides et al. 2013 (Biol. Inv)
105	UKH8	JX856989	England - Boscombe (BS), Corfe Castle (CC), Dancing Ledge (DL), Holmsley	Michaelides et al. 2013 (Biol. Inv)

			(HO), Poole (PO), Seacombe (SC), Shorwell (SW), Winspit (WS)	
106	UKH9	JX856990	England - Winspit (WS), West Worthing (WW)	Michaelides et al. 2013 (Biol. Inv)
107	UKH10	JX856991	England - Abbotsbury (AB), Bristol (BR), Boscombe (BS), Corfe Castle (CC), Dancing Ledge (DL), Folkestone (FS), Holmsley (HO), Poole (PO), Ventnor Botanical Garden (VB), Winspit (WS)	Michaelides et al. 2013 (Biol. Inv)
108	UKH11	JX856992	England - Bristol (BR), Boscombe (BS)	Michaelides et al. 2013 (Biol. Inv)
109	UKH12	JX856993	England - East Portland (EP), Cheyne Weare (CW)	Michaelides et al. 2013 (Biol. Inv)
110	DB16840	KF372191	France - Vieille-Roche	Salvi et al. 2013 (BMC Evol. Bio)
111	DB16841	KF372192	France - Vieille-Roche	Salvi et al. 2013 (BMC Evol. Bio)
112	DB13461	KF372219	France - Massif des Maures	Salvi et al. 2013 (BMC Evol. Bio)
113	DB13460	KF372220	France - Valle de Gilly	Salvi et al. 2013 (BMC Evol. Bio)
114	DB13430	KF372221	France - Massif des Maures	Salvi et al. 2013 (BMC Evol. Bio)
115	DPM1	KF372222	Italy - Viozene	Salvi et al. 2013 (BMC Evol. Bio)
116	DPM3	KF372223	Italy - Viozene	Salvi et al. 2013 (BMC Evol. Bio)
117	DB15936	KF372224	Switzerland - Monte Verita	Salvi et al. 2013 (BMC Evol. Bio)
118	DB16837	KF372225	Italy - Bianzano	Salvi et al. 2013 (BMC Evol. Bio)
119	DB16838	KF372226	Italy - Bianzano	Salvi et al. 2013 (BMC Evol. Bio)
120	DPM36	KF372227	Italy - Peschiera del Garda	Salvi et al. 2013 (BMC Evol. Bio)
121	DPM37	KF372228	Italy - Peschiera del Garda	Salvi et al. 2013 (BMC Evol. Bio)
122	DPM38	KF372229	Italy - Peschiera del Garda	Salvi et al. 2013 (BMC Evol. Bio)
123	DB1399	KF372230	Italy - Ostia Antica	Salvi et al. 2013 (BMC Evol. Bio)
124	DB5938	KF372231	Italy - Paganico	Salvi et al. 2013 (BMC Evol. Bio)
125	DPM39	KF372232	Italy - Majelletta	Salvi et al. 2013 (BMC Evol. Bio)
126	DPM40	KF372233	Italy - Majelletta	Salvi et al. 2013 (BMC Evol. Bio)
127	DPM41	KF372234	Italy - Majelletta	Salvi et al. 2013 (BMC Evol. Bio)
128	DPM15	KF372235	Italy - Bassiano	Salvi et al. 2013 (BMC Evol. Bio)
129	DPM16	KF372236	Italy - Bassiano	Salvi et al. 2013 (BMC Evol. Bio)
130	DPM25	KF372239	Italy - Pollino National Park	Salvi et al. 2013 (BMC Evol. Bio)
131	DB5937	KF372240	Italy - Pollino National Park	Salvi et al. 2013 (BMC Evol. Bio)
132	DPM21	KF372241	Italy - Pollino National Park	Salvi et al. 2013 (BMC Evol. Bio)
133	DPM7	KF372242	Italy - Fago del Soldato	Salvi et al. 2013 (BMC Evol. Bio)
134	DPM8	KF372243	Italy - Fago del Soldato	Salvi et al. 2013 (BMC Evol. Bio)
135	DPM6	KF372244	Italy - Fago del Soldato	Salvi et al. 2013 (BMC Evol. Bio)
136	WFR-H7	KP118978	France - Josselin (JO)	Michaelides el al. 2015 (PLoS One)
137	WFR-H4	KP118983	France - Nebias (NE), Frontier Cabardes (FC)	Michaelides el al. 2015 (PLoS One)
138	WFR-H5	KP118984	France - Dinan (DN), Sees (SE), Vitre (VR), Josselin (JO), Pontchateau (PC), Puybelliard (PU), Pouzagues(PZ), St. Gervais (GE), St. Michel (MI), St. Lizier (LI), St. Girons (SG), Frontier Cabardes (FC)	Michaelides el al. 2015 (PLoS One)
139	WFR-H8	KP118985	France - Bastide (BA)	Michaelides el al. 2015 (PLoS One)
140	WFR-H2	KP118988	France - St. Lizier (LI), Nebias (NE), Frontiers Cabardes (FC)	Michaelides el al. 2015 (PLoS One)
141	WFR-H3	KP118989	France - St. Girons (SG), Bastide (BA)	Michaelides el al. 2015 (PLoS One)
142	ROM-H1	KP972470	Italy - Cento (CE)	This study
143	ROM-H2	KP972471	Italy - Cento (CE)	This study
144	SAL-H1	KP972472	Italy - Buti (BT), Badia Polesine (BP)	This study
145	SAL-H2	KP972473	Italy - Bassano Di Grappa (Campesse) (BG), Mizzole (MZ), Barbarano	This study

			Vicentino (BV)	
146	SAL-H3	KP972474	Italy - Buti (BT)	This study
147	SAL-H4	KP972475	Italy - Badia Polesine (BP)	This study
148	SAL-H5	KP972476	Italy - Barbarano Vicentino (BV)	This study
149	SAL-H6	KP972477	Italy - Badia Polesine (BP)	This study
150	SAL-H7	KP972478	Italy - Viareggio (VI)	This study
151	SAL-H8	KP972479	Italy - Badia Polesine (BP), Mizzole (MZ)	This study
152	SAL-H9	KP972480	Italy - Bassano Di Grappa (Campesse) (BG)	This study
153	TUS-H1	KP972481	Italy - Montemassi (MM), Prato (PR)	This study
154	TUS-H2	KP972482	Italy - Buti (BT), Travale (TR), Colle di Val'Elsa (VE), Greve in Chianti (GC), Montemassi (MM), Chianni (CN), Crespina (CR)	This study
155	TUS-H3	KP972483	Italy - Greve in Chianti (GC)	This study
156	TUS-H4	KP972484	Italy - Chianni (CN)	This study
157	TUS-H5	KP972485	Italy - Viareggio (VI)	This study
158	TUS-H6	KP972486	Italy - Greve in Chianti (GC)	This study
159	TUS-H7	KP972487	Italy - Greve in Chianti (GC)	This study
160	TUS-H8	KP972488	Italy - Buti (BT)	This study
161	TUS-H9	KP972489	Italy - Buti (BT)	This study
162	TUS-H10	KP972490	Italy - Prato (PR), Vignola (VG)	This study
163	TUS-H11	KP972491	Italy - Buti (BT)	This study
164	TUS-H12	KP972492	Italy - Viareggio (VI)	This study
165	TUS-H13	KP972493	Italy - Viareggio (VI)	This study
166	TUS-H14	KP972494	Italy - Prato (PR)	This study
167	TUS-H15	KP972495	Italy - Calci (CA)	This study
168	TUS-H16	KP972496	Italy - Prato (PR)	This study
169	TUS-H17	KP972497	Italy - Prato (PR)	This study
170	TUS-H18	KP972498	Italy - Prato (PR)	This study
171	TUS-H19	KP972499	Italy - Montemassi (MM)	This study
172	TUS-H20	KP972500	Italy - Travale (TR)	This study
173	TUS-H21	KP972501	Italy - Travale (TR)	This study
174	TUS-H22	KP972502	Italy - Greve in Chianti (GC)	This study
175	TUS-H23	KP972503	Italy - Crespina (CR)	This study
176	TUS-H24	KP972504	Italy - Montemassi (MM), Travale (TR), Colle di Val'Elsa (VE)	This study
177	TUS-H25	KP972505	Italy - Crespina (CR)	This study
178	TUS-H26	KP972506	Italy - Colle di Val'Elsa (VE)	This study
179	TUS-H27	KP972507	Italy - Calci (CA)	This study
180	TUS-H28	KP972508	Italy - Chianni (CN), Crespina (CR)	This study
181	TUS-H29	KP972509	Italy - Viareggio (VI)	This study
182	TUS-H30	KP972510	Italy - Buti (BT)	This study
183	TUS-H31	KP972511	Italy - Chianni (CN)	This study
184	TUS-H32	KP972512	Italy - Calci (CA)	This study
185	TUS-H33	KP972513	Italy - Montemassi (MM)	This study
186	TUS-H34	KP972514	Italy - Chianni (CN)	This study
187	TUS-H35	KP972515	Italy - Colle di Val'Elsa (VE)	This study
188	VEN-H1	KP972516	Italy - Cento (CE), Castelarrano (CT), Nonantola (NO), Olina (OL), Pian Di Venola (PV), Vignola (VG), Motta di Livenza (ML)	This study
189	VEN-H2	KP972517	Italy - Olina (OL), Vignola (VG), Nonantola (NO)	This study
190	VEN-H3	KP972518	Italy - Nonantola (NO), Vignola (VG)	This study

191	VEN-H4	KP972519	Italy - Nonantola (NO), Pian Di Venola (PV)	This study
192	VEN-H5	KP972520	Italy - Pian Di Venola (PV)	This study
193	VEN-H6	KP972521	Italy - Pian Di Venola (PV)	This study
194	VEN-H7	KP972522	Italy - Castelarrano (CT)	This study
195	VEN-H8	KP972523	Italy - Castelarrano (CT)	This study
196	VEN-H9	KP972524	Italy - Olina (OL)	This study
197	VEN-H10	KP972525	Italy - Olina (OL)	This study
198	VEN-H11	KP972526	Italy - Olina (OL)	This study
199	VEN-H12	KP972527	Italy - Nonantola (NO)	This study
200	VEN-H13	KP972528	Italy - Motta Di Livenza (ML)	This study
201	VEN-H14	KP972529	Italy - Cento (CE)	This study
202	VEN-H15	KP972530	Italy - Pian Di Venola (PV)	This study
203	VEN-H16	KP972531	Italy - Nonantola (NO)	This study
204	VEN-H17	KP972532	Italy - Motta Di Livenza (ML)	This study
205	VEN-H18	KP972533	Italy - Nonantola (NO)	This study
206	VEN-H19	KP972534	Italy - Nonantola (NO)	This study
207	VEN-H20	KP972535	Italy - Cento (CE)	This study
208	VEN-H21	KP972536	Italy - Cento (CE)	This study
209	VEN-H22	KP972537	Italy - Vignola (VG)	This study
210	VEN-H23	KP972538	Italy - Olina (OL)	This study
211	VEN-H24	KP972539	Italy - Pian Di Venola (PV)	This study

Table S3 | Prior distributions of the historical, demographic and mutation parameters used in ABC analyses. Effective population sizes (N) are expressed in number of diploid individuals and times of events (t) in numbers of generations going back to the past.

Interpretation	Parameters	Prior distribution
Effective stable size in introduced population (I_A)	N1	Uniform [10;100]
Effective stable size in introduced population (I_B)	N2	Uniform [10;1000]
Effective stable size in source population (N)	N3	Uniform [10;10000]
Unsampled population (U_N)	N4	Uniform [10;1000]
Bottleneck event duration	B_D	Uniform [1;5]
Effective population size of founders	N1b, N2b, N4b	Uniform [1;50]
Introduction time	t1	Uniform [1;25]*
Introduction time	t2	Uniform [5;30]*
Introduction time	t3	Uniform [15;40]*

*Introduction times (in generations) were modified for each population accordingly based on historical data on approximate date of introduction and assuming a two-year generation time for *P. muralis* (see Michaelides *et al*. 2013).

Table S4 | Confidence in scenario choice by estimating type I and II errors. Type I is the probability of excluding the selected scenario when it is actually the true scenario. Type II is the probability of selecting the scenario when it is not the true scenario.

Population	Selected Scenario	Type I error #	Type II error #
Ventnor (VT)	Oldest population in UK, considered as primary introduction		
Bury (BU)	Primary introduction	0.51 (0.53)	0.29 (0.33)
Birdbrook (BB)	Primary introduction	0.14 (0.11)	0.15 (0.10)
Shoreham (SH)	Primary introduction	0.51 (0.51)	0.15 (0.10)
Newton Ferrers (NF)	Unsampled source	0.38 (0.42)	0.47 (0.49)
Wellington (WE)	Primary introduction	0.50 (0.47)	0.30 (0.30)
Shorwell (SW)	Secondary introduction	0.26 (0.11)	0.13 (0.11)
Holmsley (HO)	Secondary introduction	0.08 (0.08)	0.19 (0.11)
Winspit (WS)	Secondary introduction	0.23 (0.12)	0.11 (0.10)
Seacombe (SC)	Secondary introduction	0.17 (0.12)	0.11 (0.10)
Wembdon (WB)	Primary introduction*	0.52 (0.51)	0.28 (0.29)
Dancing Ledge (DL)	Secondary introduction	0.18 (0.10)	0.06 (0.06)
Poole (PO)	Secondary introduction	0.08 (0.07)	0.10 (0.06)
Folkstone (FS)	Secondary introduction	0.06 (0.06)	0.08 (0.06)
Boscombe (BS)	Secondary introduction	0.09 (0.06)	0.12 (0.06)
East Portland (EP)	Primary introduction	0.45 (0.45)	0.30 (0.31)
Cheyne Weare (CW)	Secondary introduction*	0.48 (0.48)	0.27 (0.27)
Abbotsbury (AB)	Secondary introduction	0.10 (0.04)	0.04 (0.04)
Ventor Botanical Gardens (VB)	Secondary introduction	0.08 (0.03)	0.09 (0.03)
West Worthing (WW)	Primary introduction	0.44 (0.43)	0.28 (0.28)
Bristol (BR)	Unsampled source*	0.28 (0.26)	0.41 (0.59)
Corfe Castle (CC)	Secondary introduction	0.03 (0.02)	0.07 (0.03)
Eastbourne (EB)	Unsampled source*	0.31 (0.30)	0.36 (0.37)

* Selected scenario with the highest posterior probability but with overlapping 95% confidence intervals
# Estimates of type I and type II errors by direct estimate and logistic regression (in parenthesis)

Table S5 | Proportion of membership (Q) in each cluster. #K represents a defined cluster and #pop represents the number of populations in each cluster. Q-values > 0.1 and are indicated in bold.

Population	Cluster (K)									#K
	1	2	3	4	5	6	7	8	9	
AB	0.00	0.01	0.05	**0.27**	0.00	0.00	0.00	**0.64**	0.02	2
BB	0.03	0.01	0.01	0.01	0.04	**0.47**	**0.33**	0.08	0.03	2
BR	0.01	0.01	0.04	**0.93**	0.01	0.00	0.00	0.01	0.00	1
BS	0.00	0.00	0.00	0.01	0.00	0.00	0.00	**0.72**	**0.26**	2
BU	0.02	0.00	**0.92**	0.02	0.02	0.01	0.00	0.00	0.00	1
CC	0.01	0.01	0.00	0.00	0.01	0.01	0.01	**0.96**	0.00	1
CW	0.00	0.01	**0.96**	0.00	0.00	0.00	0.00	0.01	0.01	1
DL	0.00	0.00	0.00	0.01	0.00	**0.94**	0.01	0.02	0.02	1
EB	0.00	0.04	0.00	0.01	**0.11**	0.01	**0.76**	0.07	0.01	2
EP	0.00	0.00	**0.98**	0.00	0.00	0.00	0.00	0.00	0.00	1
FS	0.00	0.00	0.00	0.00	0.00	0.00	**0.98**	0.00	0.00	1
HO	0.00	0.00	0.00	**0.61**	0.00	0.04	0.00	**0.20**	**0.14**	3
NF	0.00	**0.98**	0.00	0.00	0.01	0.00	0.00	0.00	0.00	1
PO	0.01	0.01	0.07	0.02	0.02	**0.31**	0.04	**0.50**	0.04	2
SC	0.00	0.00	0.00	0.00	0.01	**0.97**	0.01	0.00	0.00	1
SH	0.00	0.00	0.00	0.01	**0.89**	0.02	0.01	0.04	0.02	1
SW	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.03	**0.94**	1
VB	0.00	0.01	0.00	0.01	**0.14**	0.01	0.00	0.08	**0.75**	2
VT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.99**	1
WB	**0.96**	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	1
WE	0.01	0.01	**0.95**	0.01	0.01	0.00	0.00	0.00	0.00	1
WS	0.00	0.00	0.00	0.00	0.09	0.01	0.04	**0.85**	0.00	1
WW	0.02	0.00	0.00	0.01	**0.84**	**0.11**	0.00	0.00	0.00	2
#Pop	1	1	4	3	4	5	3	6	5	

Table S6 | Introduction history of *P. muralis* populations in England.

Population	Year *	Introduction scenario	Introduction history
Ventnor (VT)	1930	Primary	Oldest known extant population in the UK. Unknown source. First officially recorded in 1960's but robust support for an introduction before 1930 from interviews with local residents. Originates from Tuscany (Italy) where the same haplotypes are very common
Bury (BU)	1970	Primary	Unknown introduction history. Residents well aware of lizards from at least 1970. Originates from Eastern France but does not match well haplotypes from the easternmost and southern distribution of this lineage
Birdbrook (BB)	1972	Primary	Primary introduction of animals bought in a pet shop. Evidently from Italy, most likely close to Bologna, where populations with both VEN and ROM haplotypes co-occur in the same location
Shoreham (SH)	1975	Primary	Primary introduction of 32 animals from northern Italy. At least two separate source populations were used. Genetic analyses suggest both are from north-eastern Italy, which is consistent with morphology and colouration.
Newton Ferrers (NF)	1978	Unsampled	Independent introduction probably originated as escapees or deliberate introduction of pets when the owners moved house
Wellington (WE)	1981	Primary	Deliberate introduction from Machecoul, Southwest of Nantes (France)
Shorwell (SW)	1985	Secondary	Deliberate introduction, well supported mixture of animals from VT and another mainland population (likely Shoreham, SH)
Holmsley (HO)	1986	Secondary	Mixture of both Italian and French origin individuals. ca 12 escapees from outdoor enclosures

Winspit (WS)	1986	Secondary	Deliberate introduction. Genetic analyses showed four different haplotypes (not found in a single native population) supporting multiple introductions of Italian origin individuals.
Seacombe (SC)	1986	Secondary	Deliberate introduction. Genetically close to WS and other nearby locations on the Dorset coast supporting its status as a secondary introduction.
Wembdon (WB)	1988	Primary / unsampled	Deliberate independent introduction from three locations in western France with seven Females and four males introduced in 1988 or 1989 (from Dordogne) and another six (probably 4F, 2M) later.
Dancing Ledge (DL)	1990	Secondary	Deliberately introduction. Genetically close to WS and other nearby locations on the Dorset coast supporting its status as a secondary introduction.
Poole (PO)	1992	Secondary	Deliberate introduction and a mixture of French and Italian origin individuals.
Folkstone (FS)	1992	Secondary	Unknown history, possibly a secondary introduction from BB or a primary introduction from a source not used for other locations in England.
Boscombe (BS)	1992	Secondary	Deliberate introduction. Genetic analysis suggest multiple introductions (two lineages present
East Portland (EP)	1995	Primary	Deliberate introduction from a captive-bred stock (partly) originating from Brittany (North Western France). Spatial distribution at sampling suggested two separate releases (one in CW), but could also be a single release. Haplotypes from both Eastern and Western France lineage are present suggesting multiple introductions.
Cheyne Weare (CW)	1995	Secondary /Primary / unsampled	Deliberate introduction from a captive-bred stock (partly) originating from Brittany (North Western France). Spatial distribution at sampling suggested two separate releases (one in EP), but could also be a single release. Haplotypes from both Eastern and Western France lineage are present suggesting multiple introductions.
Abbotsbury (AB)	1998	Secondary	Deliberate introduction, most likely using offspring of individuals originally from HO
Ventor Botanical Gardens (VB)	2000	Secondary	Deliberate release. A mixture of animals from VT and another mainland population (likely Shoreham, SH).
West Worthing (WW)	2004	Primary / unsampled	Unknown introduction, possible a separate release or escapees from a captive source
Bristol (BR)	2004	Primary / unsampled	Unknown introduction, a very small populations of animals of Italian origin
Corfe Castle (CC)	2006	Secondary	Unknown introduction, animals of Italian origin. Presence of haplotypes found in other Dorset populations suggests origin from a previously established or captive bred population
Eastbourne (EB)	2006	Primary (unsampled)	Unknown introduction, Italian origin and clearly distinct from populations in Dorset but presence of Romagna mitochondrial haplotype may suggest partly originating from BB.

* Time of introduction or first record

CHAPTER 5

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Loss of genetic diversity and increased embryonic mortality in non-native lizard populations

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ABSTRACT

Many populations are small and isolated which limits genetic variation and increases the risk of mating with close relatives. Inbreeding depression is suspected to contribute to extinction of wild populations, but the historical and demographic factors that contribute to reduced population viability are often difficult to isolate. Replicated introduction events in non-native species can offer insights because it is possible to study how genetic variation and inbreeding depression are affected by demographic events (e.g., bottlenecks), genetic admixture and the extent and duration of isolation. Using detailed knowledge about the introduction history of 21 non-native populations of the wall lizard *Podarcis muralis* in England we show greater loss of genetic diversity (estimated from microsatellite loci) in older populations and in populations from native regions of high diversity. Loss of genetic diversity was accompanied by higher embryonic mortality in non-native populations, suggesting that introduced populations are sufficiently inbred compromising their long-term viability. However, there was no bivariate relationship between population genetic diversity and average embryonic mortality. Similarly, at the individual level, there was no correlation between female heterozygosity and clutch size, infertility, or hatching success, or between embryo heterozygosity and mortality. Thus, the usefulness of marker-based assessment of population viability appears questionable and cannot replace direct estimates of inbreeding depression.

INTRODUCTION

Low genetic diversity connects the historical processes that make populations vulnerable to extinction with inbreeding depression (Newman & Pilson 1997; Saccheri *et al.* 1998). Inbreeding depression is manifested as a loss of individual fitness due to homozygosity of deleterious alleles (Keller & Waller 2002). Populations also need genetic variation to evolve; hence low genetic diversity may compromise the long-term prospects for populations to adapt and persist. Documentation of genetic diversity and its relationship to estimates of individual and population viability is therefore a central concern in conservation biology (Keller & Waller 2002; Frankham 2003; Reed & Frankham 2003).

The links between the demographic and ecological processes that reduce population size, genetic diversity and inbreeding depression have been surprisingly difficult to establish. As a result, the extent to which inbreeding depression is a serious threat to population persistence remains controversial (Frankham 1995; Keller & Waller 2002). One logistical challenge is that data on severe inbreeding depression is often difficult to acquire from wild animals as it may be manifested as mortality early in life, making it cryptic or mistakenly classified as parental infertility (Hemmings *et al.* 2012). Other measures, such as weakly developed secondary sexual characters or low clutch size, may also be signs of inbreeding depression (Runemark *et al.* 2013). However, these are well-known to vary as a result of the local environment and the selective history of populations making it possible to confuse inbreeding depression to relaxed selection on reproductive characters. Direct estimates of early mortality in

contexts where direct environmental effects can be ruled out are therefore particularly useful for establishing that inbreeding depression is occurring.

The processes that compromise population viability should be particularly pronounced in biological invasions, where loss of genetic diversity may impact on establishment success, population growth and adaptive potential (Nei *et al.* 1975; Sakai *et al.* 2001; Lee 2002; Roman & Darling 2007; Dlugosch & Parker 2008). In such non-native populations, genetic diversity is strongly influenced by their origin and the historical and demographic features of their introduction (Taylor & Keller 2007; Keller & Taylor 2008). For example, single introductions from a phylogeographically structured native range followed by secondary introductions should cause significant loss of genetic diversity (e.g. *Linepithema humile*, Tsutsui *et al.* 2000; *Silene latifolia*, Taylor & Keller 2007; *Diabrotica virgifera virgifera*, Ciosi *et al.* 2008) compared to introductions involving a high number of founders and multiple introductions (Kolbe *et al.* 2004; Roman & Darling 2007; Dlugosch & Parker 2008; Uller & Leimu 2011). Knowledge of the colonization history is therefore important to infer why some, but not other, populations lose genetic diversity.

The common wall lizard, *Podarcis muralis* provides an opportunity to study how introduction history, admixture, and isolation shape genetic diversity and inbreeding depression. Native to southern and western Europe, the species has been repeatedly introduced to England, Germany and North America (Allan *et al.* 2006; Burke & Deichsel 2008; Schulte *et al.* 2012b; Michaelides *et al.* 2013). A comprehensive analysis of the colonization history of non-native populations in England revealed nine independent introduction events from two native geographic regions (France

and Italy), with evidence of multiple introductions, secondary introductions and admixture (Michaelides *et al*. 2013, Michaelides *et al*. 2015). Most of the populations are the result of pet escapees or deliberate release of captive-bred animals and/or their offspring (Lever 1977; Michaelides *et al.* 2013). These populations have been established from a small number of founders and are isolated with very little or no gene flow. Thus, genetic drift and inbreeding are expected to have a significant effect on their genetic structure, with loss of genetic diversity being associated with inbreeding depression. By genotyping 1546 native and non-native animals (using 16 microsatellite markers), here we test if genetic diversity of non-native populations is shaped by their geographic and genetic origin, and introduction history (primary vs. secondary and single vs. multiple introductions, admixture, year of introduction and propagule size). For 11 native and 13 non-native populations we also collected data on female fecundity, infertility and hatching failure to test if loss of genetic diversity was associated with loss of individual fitness.

MATERIAL AND METHODS

Study species

In its native range, *P. muralis* shows a complex phylogeographic structure with several genetically and geographically distinct clades that likely originated during isolation in southern glacial refugia in Italy (Apennine Peninsula), the Balkans and on the Iberian Peninsula (Gruschwitz & Böhme 1986; Giovannotti *et al.* 2010; Schulte *et al.* 2012b; Gassert *et al.* 2013; Salvi *et al.* 2013). In the UK, the species has been repeatedly released and there are more than 25 extant populations, the majority being of Italian origin (Michaelides *et al.* 2013; Michaelides *et al.* 2015b). We have

previously shown that about 40% of these populations harbor haplotypes from genetically and geographically distinct lineages and that both primary and secondary introductions are common (Michaelides *et al.* 2013; Michaelides *et al.* 2015b). Combined with historical information of year of introduction (or first sighting/record) and, for seven populations, the number of founders (propagule size), these data provide a valuable opportunity to test how introduction history shapes genetic variation in the non-native range. Capture of gravid females and experimental incubation of eggs under standardized conditions in the laboratory further allow quantification of fecundity, fertility, and embryonic mortality between native and non-native populations, and to test for a relationship between these estimates of fitness and individual heterozygosity.

Sampling and molecular laboratory work

We sampled 21 non-native and 45 native populations between 2008 and 2014 (Michaelides *et al.* 2013; Michaelides *et al.* 2015b). We extracted genomic DNA from tail tissue preserved in ethanol (70-90%) with DNeasy 96 plate kit (Qiagen, Valencia, CA) following manufacturer's instructions (with overnight lysis) and genotyped all individuals at 16 microsatellite loci (Richard *et al.* 2012; Heathcote *et al.* 2014). The selected microsatellite set included markers that were developed using individuals from the two focal lineages and geographic regions (France and Italy). This ensured reliable and accurate estimation of genetic diversity (Queiros *et al.* 2015). Multiplexed polymerase chain reactions (PCRs) were carried out in a total volume of 11µl reaction mix containing 1µl of genomic DNA, 5µl of Qiagen MasterMix, 0.2µl of each primer (forward and reverse in equal concentrations) and 3.8µl (for multiplex 1,2,3 and 5) or

3.6µl (for multiplex 4) of PCR grade dH_2O. PCR conditions were as follows: 15min of initialization step at 95°C, 26 cycles of 30sec at 94°C, 90sec at 57°C (for multiplexes 1 - 3) or 55°C (for multiplexes 4, 5) and 1min at 72°C and a final extension step of 20min 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.). We scored alleles in GENEIOUS 6.1.7 and any ambiguous peaks were repeated to confirm genotype.

Microsatellite analyses

We used MICROCHECKER V.2.2.3 (Van Oosterhout *et al.* 2004) to check for null-alleles, large allele dropouts and scoring errors and FSTAT (Goudet 1995, 2001) to calculate deviations from Hardy-Weinberg equilibrium (at the 0.05 nominal level for multiple tests using sequential Bonferroni corrections). We excluded three loci due to very limited amplification in some populations (i.e., lineage specific loci). Therefore for all subsequent analyses we used 13 microsatellite loci. We calculated observed (H_O) and unbiased expected heterozygosity (H_E) using GENALEX v.6.0 (Peakall & Smouse 2012a), allelic richness (A_R, corrected for sample size) using FSTAT (Goudet 1995, 2001) and genetic differentiation among populations (F_{ST}) in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010).

Genetic diversity in the native and non-native range

We assessed genetic structuring across the native range using a spatial interpolation method employing the *akima* package in R v.3.1.2 (R Development Core Team 2015). To determine how gene flow and genetic drift have influenced population genetic structure within the native and non-native ranges we analyzed the correlation

between geographical distance and genetic differentiation (F_{ST}) using Mantel tests with 9999 permutations using the *ade4* package in R v.3.1.2 (R Development Core Team 2015). We assessed the structure of genetic variation in the two ranges by hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010). We used two-way ANOVA to assess the effects of geographic range (native vs. non-native) and genetic origin (Italian vs. French) on genetic diversity (H_E and A_R). To improve normality of data, we arcsine-square root transformed H_E and square transformed A_R.

Predictors of genetic diversity in the non-native range

We used a GLM with Gaussian distribution on transformed data to test if the origin and introduction history explain variation in genetic diversity in non-native populations. We included the genetic origin (Italian vs. French), the mode of introduction (primary vs. secondary), number of years since introduction (or first observed) and admixture (presence of haplotypes of more than one lineage; yes vs. no). We also tested for the effects of propagule size (founder size) on genetic diversity of the subset of non-native populations for which this was documented or established with high certainty from interviews with, or written accounts by, those involved in the introductions (Michaelides *et al.* 2013).

Fecundity, infertility, and embryonic mortality

We caught 413 gravid females from 11 native and 13 non-native populations during the field seasons 2010-2014 (Supplementary Table S3). Females were housed in individual cages (590 x 390 x 415 mm) at the facilities in Oxford following our standard protocol (While et al. 2015). We collected the first clutch of the season (from

a mating while still in the wild) to generate data on fecundity (C_S, clutch size), infertility (I_N, proportion of infertile eggs) and hatching failure (H_F, the proportion of fertile eggs within a clutch where the embryo died before full term). Infertile eggs can easily be identified on the basis of a lack of calcified egg shell (Olsson & Shine 1997). Eggs that failed to hatch or that did not show heart beat (using a heart rate monitor; Buddy, Avitronics, England) were dissected to confirm the presence of a dead embryo.

We assessed the effects of geographic range (native vs. non-native) and genetic origin (Italian vs. French) on fecundity using a linear mixed model with range, origin and their interaction as a fixed effect, and population as random effect. Infertility and hatching failure were analyzed using generalized linear mixed models (GLMMs) with the same predictors, adding female identity as a random effect, and a binomial error distribution with logit link function. The statistical analysis was carried out using the *nlme* and *lme4* packages (Bates *et al.* 2014; Pinheiro *et al.* 2015) in R v.3.1.2 (R Development Core Team 2015). In addition, for non-native populations we used a GLM with Gaussian distribution on transformed data (arsine square root) to test if average infertility and hatching failure in populations can be explained by their introduction history. We included, genetic origin (Italian vs. French), the mode of introduction (primary vs. secondary), number of years since introduction (or first observed) and admixture (presence of haplotypes of more than one lineage; yes vs. no).

Heterozygosity – fitness correlations (HFCs)

Because loss of genetic diversity is associated with inbreeding which in turn reduces reproductive fitness, a correlation is expected between heterozygosity and fitness (or fitness-related) traits (Reed & Frankham 2003). We assessed the relationship between expected heterozygosity and average clutch size (C_S), infertility (I_N) and hatching failure (H_F) among non-native populations. Populations with less than ten sampled females that we had data on C_S, I_N and H_F were excluded from this analysis to minimize bias in over- or under-estimating the average of the trait.

At the individual level, HFC are statistical associations between individual multilocus heterozygosity and fitness traits. Because spurious HFCs can arise when individuals come from different localities or geographic origins (Slate *et al.* 2004) and since some non-native populations have shown to share demographic history and genetic composition (Michaelides *et al.* 2015b) we used STRUCTURE (Pritchard *et al.* 2000) to assign individuals (females) into demes (K), representing clusters of populations that share close genetic relationships (e.g., because one was established through introduction of individuals of another; Michaelides *et al.* 2015). We ran simulations with a burn-in of 10^5 iterations and a run length of 10^6 iterations from K = 1 to K=11 (for native females) or K=13 (for non-native females). 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 (Earl & vonHoldt 2011). Multiple runs were combined in CLUMPP (Jakobsson & Rosenberg 2007) and each female was assigned into a deme when the proportion of membership (q) for a deme was ≥0.9, Females with a mixed ancestry (0.1<q<0.9) were pooled

together to form the admixed deme (DemeMix). Structure results identified high posterior probability at K=2 for native females (DemeNativeItalian and DemeNativeFrench) and K=4 for non-native females (four demes with females belonging to populations of either Italian-only or French-only populations; DemeIntroITA-A (BS, DL, PO, WS), DemeIntroITA-B (WW, SH), DemeIntroITA-C (VT,VB,SW) and DemeIntroFRA (BU, CW, EP, WE) and a mixed deme, DemeMix (BS, DL, SH, SW, VB, WE, WS); see Table S3 for list of populations and their abbreviations). Therefore, for subsequent analyses we partitioned our data accordingly to determine whether the presence and/or magnitude of HFC varied among the different partitions (demes).

We estimated individual multilocus heterozygosity by calculating the uncorrected homozygosity index (HO, proportion of homozygous loci) and the corrected homozygosity by locus index (HL, weights the contribution of each locus to the homozygosity index depending on allelic variability) in CERNICALIN (Aparicio *et al.* 2006). We performed these calculations separately in each deme (DemeNativeItalian, DemeNativeFrench, DemeIntroItalianA-C, DemeIntroFrench and DemeIntroMix). Since both indices were highly correlated so we only report results for HL (see Results).

Identity disequilibrium (ID, a correlation in heterozygosity and/or homozygosity across loci (Weir & Cockerham 1973)) is considered a fundamental cause of HFC (Szulkin *et al.* 2010). We therefore estimated ID and its significance using the parameter g_2 (David *et al.* 2007). HFC emerge from variance in individual inbreeding and should only exist if $g_2>0$ (Szulkin *et al.* 2010), therefore we assessed the

significance of departure from zero based on 1000 permutations in RMES (David et al 2007) for each deme.

We analyzed the effects of female heterozygosity (F_{HL}) on clutch size (C_S) and hatching failure (H_F) within each deme, and for each fitness trait separately (we did not perform the corresponding analysis on infertility due to the small number of females we had data for). We used Poisson generalized linear models on C_S and binomial GLMMs on H_F including F_{HL} as fixed effect and female ID as a random effect (to control for overdispersion; Bolker *et al.* 2009). We converted the results of each HFC analysis to *r*, the equivalent of the Pearson product moment correlation coefficient, which is a common measurement of effect size (Coltman & Slate 2003). We used the z-values from each model to calculate *r* which was subsequently transformed into Zr (Fisher's transformation) as described in Coltman and Slate (2003). Since we used HL (homozygosity by locus) for the HFC estimates, we reversed the sign of the effect to match results from published meta-analyses (e.g. Chapman *et al.* 2009). We then used univariate analyses and calculated the average effect size across fitness traits (all effect sizes treated as independent data) and the average effect sizes for each fitness trait separately.

Finally, because non-native populations of Italian origin were found to have lost genetic diversity and have increased hatching failure (see Results) we used a subset of females from non-native populations of Italian ancestry to test if high offspring homozygosity is associated with embryonic mortality. For this analysis we used 31 females and clutches that had at least one embryo that hatched and one that died early. Embryos (dead and alive) were genotyped at 13 microsatellite loci and the

homozygosity indices were also calculated in CERNICALIN (Aparicio *et al.* 2006). We then fitted a GLMM with offspring heterozygosity (O_{HL}), femaleID as a random effect and a binomial error distribution with logit link function. P-values were obtained by LRTs of the full model with O_{HL} against the model without O_{HL}. All statistical analyses were carried out in R v.3.1.2 (R Development Core Team 2015) using the *lme4* package (Bates *et al.* 2014).

RESULTS

There was a clear spatial genetic structure in the native range, with the Italian region showing higher levels of genetic diversity (H_E and A_R, Figure 1). Most of the variation was found within populations with only 10-15% of variation between ranges and origins (Table 1). Significant isolation-by-distance pattern was observed within both the native and non-native populations (Mantel tests, $p < 0.05$, Figure 2).

Table 1 | Analysis of Molecular Variance (AMOVA) in the native and non-native range.

Range	Source of variation	*d.f.*	Sum of squares	Percentage of variation
Native range	Among groups (Italy – France)	1	568.14	10.6
	Among populations within groups	40	971.45	7.65
	Within populations	1940	8705.85	81.75
	Total	1981	10245.44	
Non-native range	Among groups (Italy – France)	1	332.87	14.55
	Among populations within groups	18	805.03	15.82
	Within populations	926	3533.18	69.63
	Total	945	4671.09	

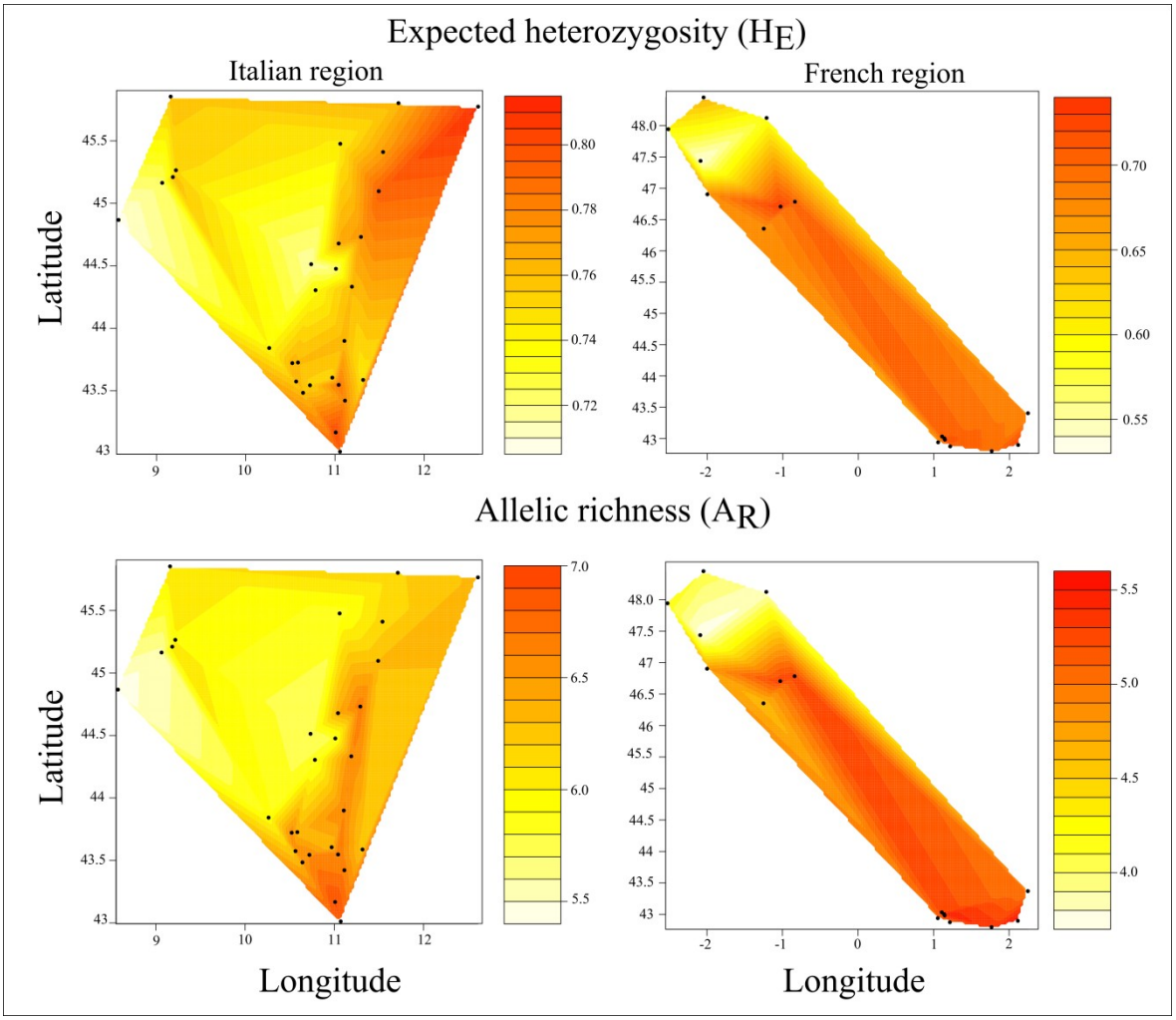

Figure 1 | Genetic diversity in the native range of *P. muralis*. Spatial interpolation of expected heterozygosity (HE) and allelic richness (AR) in each native region (Italy and France).

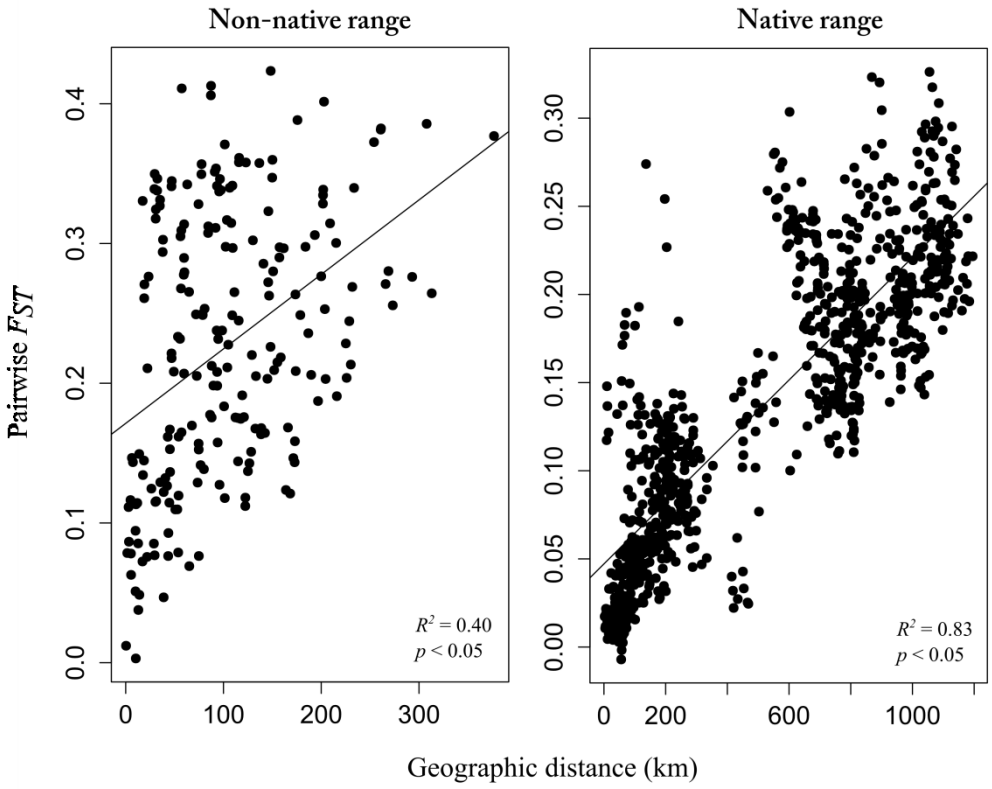

Figure 2 | Correlation between genetic (F_{ST}) and geographic distances. Evidence of isolation by distance in both the non-native and native range. Mantel tests after 9999 permutations. Note different scales on the axes for the two plots.

Genetic diversity (expressed as H_E and A_R) was substantially lower in the non-native populations of Italian origin compared to their native range, whereas non-native populations of French origin showed only weak loss of diversity compared to their native range (post-hoc comparison between French native and French non-native being statistically significant only for A_R; Table 2 and Figure 3). Of the predictors of genetic diversity only the number of years since introduction was statistically significant for H_E (but not for A_R; Table 3). For the subset of populations for which we had data on propagule size, we found a significantly positive correlation between the number of founders and genetic diversity for H_E (R = 0.85, p = 0.01, Figure 4) with borderline statistical significance for A_R (R = 0.74, p = 0.058, Figure 4).

Table 2 | GLM results for predictors of genetic diversity (expected heterozygosity, H_E, and allelic richness, A_R).

Source of variation	*d.f.*	*F*	*P*
H_E ~ Range * Origin			
Range (Native – Non-native)	1,61	77.32	**<0.001**
Origin (Italy – France)	1,61	27.04	**<0.001**
Range : Origin	1,61	11.44	**<0.001**
A_R ~ *Range * Origin*			
Range (Native – Non-native)	1,61	177.95	**<0.001**
Origin (Italy – France)	1,61	71.90	**<0.001**
Range : Origin	1,61	24.53	**<0.001**

Table 3 | GLM results for the predictors of genetic diversity (expected heterozygosity (H_E) and allelic richness (A_R)) in the non-native range. Statistically significant p-values are in bold.

Variable	*d.f.*	*F*	*p*
H_E ~ origin + mode of introduction + admixture + years			
Origin (Italy – France)	1,19	0.13	0.72
Mode of introduction (Primary – Secondary)	1,19	1.29	0.27
Admixture (Yes – No)	1,19	0.01	0.92
Years	1,19	5.75	**0.03**
A_R ~ origin + mode of introduction + admixture + years			
Origin (Italy – France)	1,19	0.21	0.64
Mode of introduction (Primary – Secondary)	1,19	0.43	0.52
Admixture (Yes – No)	1,19	0.03	0.85
Years	1,19	3.18	0.09

Females from non-native populations had significantly larger clutches ($F_{1,411}$ = 6.17, p = 0.02, Figure 3). Infertility was low overall and the incidence of infertility did not differ significantly between ranges and origins (range: $Z_{1,409}$ = -1.07, p = 0.29; origin: $Z_{1,409}$ = -0.57, p = 0.57). In contrast, hatching failure was affected by the interaction between range and origin (Z = -3.88, p<0.001), with significantly higher hatching failure in non-native populations of Italian origin than in their native counterparts (Table 4, Figure 3). Across the non-native range, none of the predictors (region of origin, admixture, mode of introduction and years since introduction) significantly affected hatching failure or fertility (Supplementary Table S6).

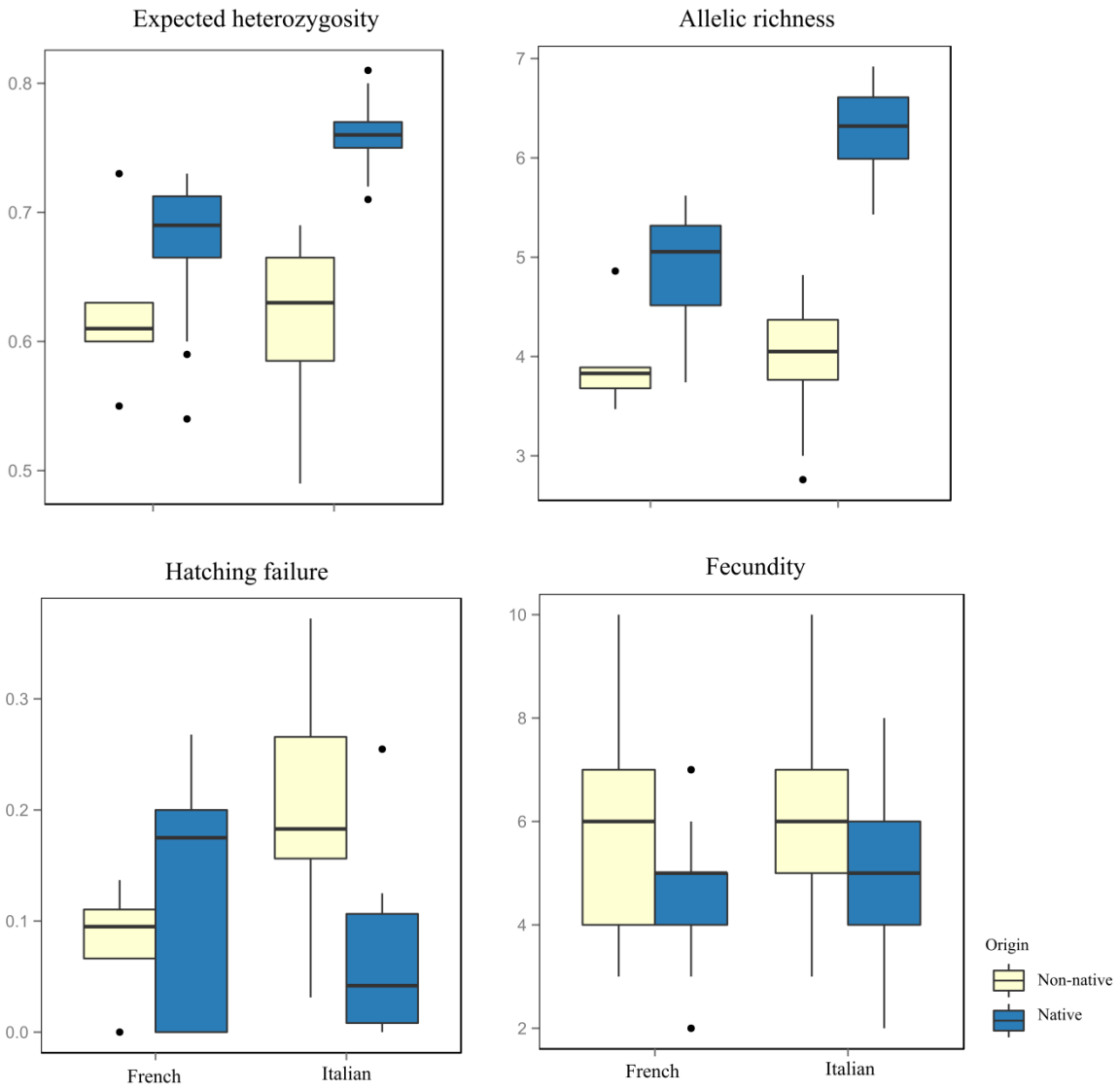

Figure 3 | Genetic diversity and fitness related traits (hatching failure, fecundity (clutch size)) in native and non-native populations of French and Italian ancestry. (A) Expected heterozygosity; **(B)** Allelic richness; **(C)** Hatching failure; **(D)** Fecundity. Different letters above the plots indicate significantly different pairwise comparisons.

Table 4 | GLMM results assessing the effects of range and genetic origin on hatching failure. Statistically significant p-values are in bold.

Variable	Parameter estimate (SE)	*p*	Random effects	Variance	SD
Range (Native – Non-native)	1.3187 (0.7825)	0.09	Population	0	0
Origin (Italian – French)	2.2596 (0.4866)	**> 0.001**	FemaleID	9.827	3.135
Origin:Range	-4.0069 (0.9536)	**> 0.001**			

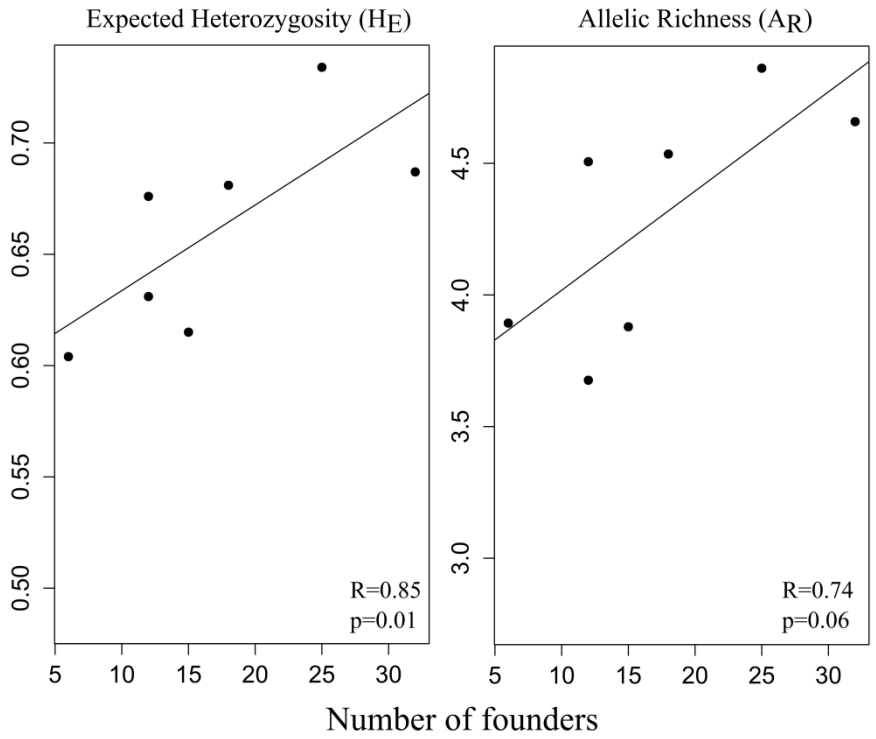

Figure 4 | Correlation between number of founders and genetic diversity.

Population average expected heterozygosity (H_E) in non-native populations was not significantly correlated with clutch size or hatching failure, but populations with higher heterozygosity had significantly lower incidence of infertility (Figure S1). At the individual level, HFCs are expected to emerge from variance in inbreeding, measured with the g_2 statistic, within the various partitions identified by Structure (at K = 2; DemeNativeItalian, DemeNativeFrench and at K = 4; DemeIntroItalianA-C, DemeIntroFrench and DemeIntroMix). We found positive values for all demes except one (DemeIntroFrench) but significant values only for the DemeIntroItalian-B (g_2 = 0.067, p = 0.04, see also Supplementary Table S7). Generalized Linear Mixed Models of HFCs indicated no significant association between female heterozygosity (F_{HL}) and fitness traits (H_F, C_S, we did not perform the corresponding analysis on infertility due to the small number of females we had data for) in any of the data partitions (Table 5). The overall average effect size on all demes combined was low (Žr = 0.039) and

the 95% confidence interval included zero (Table 5). Finally, within clutches, embryos that died before hatching where not more homozygous than their successfully hatched siblings (χ^2=0.01, p=0.91; Supplementary Table S7).

Table 5 | GLM and GLMM results assessing the effects of individual homozygosity (HL) on hatching failure (HF) and fecundity (CS). Female ID was fitted as a random effect in all models on hatching failure. Effect sizes (Žr) are calculated from the z-values as described by Coltman and Slate (2003) with the direction of the effect size reversed. Significant p-values are indicated in bold.

Fitness trait	Model	Factor	Estimate	±SE	z-value	*p*	Žr
Hatching failure (HF)	*ModelDemeIntroITA-A*	Intercept	-1.8055	1.2029	-1.501	0.133	
	(N=29, Rand. eff. Var= 3.681, SD= 1.919)	HL_Deme	0.2191	2.5263	0.087	0.931	-0.0171
	ModelDemeIntroITA-B	Intercept	-0.792	0.365	-2.169	**0.030**	
	(N=31) [ran as GLM]	HL_Deme	-0.901	0.866	-1.040	0.298	0.1919
	ModelDemeIntroITA-C	Intercept	-2.380	1.222	-1.947	0.051	
	(N=58, Rand. eff. Var= 5.35, SD= 2.31)	HL_Deme	0.888	2.767	0.321	0.748	-0.0433
	ModelDemeIntroFRA	Intercept	-8.029	3.128	-2.567	**0.010**	
	(N=61, Rand. eff. Var= 118.4, SD= 10.88)	HL_Deme	-4.126	8.170	-0.505	0.614	0.0665
	ModelDemeIntroMix	Intercept	-2.032	1.891	-1.075	0.283	
	(N=31, Rand. eff. Var= 8.33, SD= 2.88)	HL_Deme	-4.548	5.329	-0.854	0.393	0.1607
	ModelDemeNativeITA	Intercept	-8.047	2.110	-3.800	**0.000**	
	(N=77, Rand. eff. Var= 60.37, SD= 7.77)	HL_Deme	-1.198	6.323	-0.189	0.840	0.0220
	ModelDemeNativeFRA	Intercept	-1.471	2.318	-0.634	0.530	
	(N=30, Rand. eff. Var= 14.28, SD= 3.78)	HL_Deme	-8.005	6.657	-1.202	0.229	0.2293
Clutch size (CS)	*ModelDemeIntroITA-A*	Intercept	1.7271	0.2024	8.531	**0**	
	(N=29)	HL_Deme	0.2365	0.4243	0.557	0.577	-0.1070
	ModelDemeIntroITA-B	Intercept	1.7952	0.1589	11.294	**0**	
	(N=31)	HL_Deme	0.1935	0.3683	0.525	0.599	-0.0973
	ModelDemeIntroITA-C	Intercept	1.8109	0.1650	10.979	**0**	
	(N=58)	HL_Deme	-0.0682	0.3830	-0.178	0.856	0.0238
	ModelDemeIntroFRA	Intercept	1.7427	0.1638	10.635	**0**	
	(N=61)	HL_Deme	-0.0851	0.4326	-0.198	0.843	0.0258
	ModelDemeIntroMix	Intercept	1.8726	0.198	9.455	0	
	(N=31)	HL_Deme	0.0482	0.5448	0.089	0.929	-0.0165
	ModelDemeNativeITA	Intercept	1.6417	0.117	13.979	**0**	
	(N=77)	HL_Deme	0.0132	0.4061	0.033	0.974	-0.0038
	ModelDemeNativeFRA	Intercept	1.6689	0.2252	7.409	0	
	(N=30)	HL_Deme	-0.4206	0.6544	-0.643	0.52	0.1212
						Over all mean Žr	0.0397
						95%CI	±0.111
						HF_Mean Žr	0.087
						95%CI	±0.520
						CS_Mean Žr	-0.007
						95%CI	±0.545

DISCUSSION

Marginal populations, such as non-native populations, are often founded by a small number of animals, have restricted gene flow and, as a consequence, may suffer from inbreeding. Our analyses of non-native wall lizard populations in England showed widespread loss of genetic diversity following introduction and high incidence of embryonic mortality. Despite this, we failed to establish individual-level correlations between heterozygosity and various measures of fitness.

Following the introduction to a new area, populations should undergo a reduction in genetic variation and display increased differentiation among populations due to founder effects, bottlenecks, and genetic drift (Nei *et al.* 1975; Dlugosch & Parker 2008). As predicted, we found a consistent loss of genetic diversity in non-native populations. This was more pronounced in lizards introduced from Italy compared to lizards from France. The lineages diverged from each other approximately 2-3 MYA (Gassert *et al.* 2013; Michaelides *et al.* 2013) and genetic diversity is higher in Italy than in France. Thus, non-native populations from the native region with higher genetic diversity have lost proportionally more genetic variation. This could imply that bottlenecks have been more severe for non-native Italian populations, but it is also possible that it reflects a sampling effect or perhaps an extinction threshold that eliminates populations with lower diversity, making the diversity in extant non-native populations of French and Italian origin similar in magnitude. The higher genetic diversity in Italy compared to France likely reflects historical processes that periodically separated populations in refugia. In particular, there appears to have been multiple refugia within Italy, leading to contemporary zones of secondary

contact following range expansion in the region of Italy from which the UK populations originated (Giovannotti *et al.* 2010; Gassert *et al.* 2013; Salvi *et al.* 2013). Thus, the patterns of diversity in northern Italy may be quite heterogeneous (as indicated by our data), which could contribute to an apparent greater loss of diversity in the non-native range if animals were sourced from relatively low-diversity regions within northern Italy. Consequently, our study emphasizes how the phylogeographic structure in the native range can shape patterns of genetic diversity in the non-native range (Taylor & Keller 2007).

Propagule size is the most consistent predictor of genetic diversity in introduced populations (Dlugosch & Parker 2008; Simberloff 2009; Uller & Leimu 2011; Blackburn *et al.* 2015). This was confirmed in our study where, although information regarding the number of founders was only available for seven populations, diversity increased significantly with the number of animal released. Older populations also harbored less genetic variation than more recently established populations, which may reflect a prolonged period of isolation and absence of gene flow. It is also possible that natural selection contributes to the loss of diversity given the evidence that populations established several to many decades ago have adapted to the colder climate in the UK (While *et al.* 2015a). In contrast there was no evidence for further reduction in diversity in secondary introductions, which is expected to be a characteristic of sequential founder events (Clegg *et al.* 2002). Genetic admixture is common in biological invasions (Kolbe *et al.* 2004; Genton *et al.* 2005; Kolbe *et al.* 2007; Facon *et al.* 2008) and can increase genetic diversity and sometimes create novel combinations of alleles in the new range (Ellstrand & Schierenbeck 2000). However, in our study there was also no evidence that multiple introductions and

admixture, occurring from genetically (and phenotypically) differentiated lineages in the native range, had higher overall nuclear genetic diversity.

Small populations are likely to exhibit mating between close relatives, which may result in inbreeding depression (Keller & Waller 2002). Hatching failure is a common outcome of inbreeding depression in captive birds and reptiles (Bensch *et al.* 1994), and has been directly linked to loss of genetic variation in wild birds (Briskie & Mackintosh 2004; Heber & Briskie 2010; Hemmings *et al.* 2012). In our study, non-native populations of Italian origin showed substantially increased hatching failure compared to non-native populations of French origin. Because eggs were incubated at constant temperatures in the laboratory and hence environmental conditions were standardized across clutches, these effects are highly likely to be due to expression of deleterious recessives. The reasons why hatching failure only was higher in non-native populations of Italian origin is unknown (and low sample size for French populations suggest this result needs to be treated with caution). However, it is consistent with a greater reduction in genetic diversity relative to the native range than French populations, suggesting that populations of Italian origin experienced stronger bottlenecks events. Indeed, the severity of the bottleneck has been shown to significantly influence the degree of hatching failure in birds (Briskie & Mackintosh 2004; Heber & Briskie 2010). It is worth noting that the high levels of early mortality are consistent between years and hence likely to reflect a significant genetic load.

An approach to quantify the effects of genetic erosion on fitness is to estimate correlations between molecular variation and fitness (or fitness-related) traits among and within populations (Szulkin *et al.* 2010). Heterozygosity-fitness correlations

(HFCs) at the population level reveal "ambient inbreeding" shared by all members of the population which is due to fixation of deleterious alleles (fixation load). In a meta-analysis, Reed and Frankham (2003) showed that 19% of the variation in fitness among populations was a result of significant correlations between molecular variation and population fitness. Despite this, and a good sample size relative to other studies, we did not find a statistically significant correlation between population genetic diversity and average clutch size or hatching failure among non-native populations. However, populations with low genetic diversity had increased incidence of infertility, although the absolute levels of infertility were still low (less than 8% of eggs) compared to the very high incidence of embryonic mortality (more than 30%) in some non-native populations. It is unclear why this result was stronger for infertility than for embryo mortality, but it could reflect that inbreeding depression primarily affects sperm production or sperm viability in males. Indeed, inbreeding depression is often manifested in low sperm viability in captivity, and has been demonstrated in wild populations of rabbits (Gage *et al.* 2006). Recent evidence for male effects on offspring through epigenetic modifications of sperm (e.g., Lambrot *et al.* 2013; Radford *et al.* 2014) also raises the possibility that inbred males may produce sperm with compromised genomic or epigenomic stability, which may contribute to early mortality. In addition, mating with close relatives could result in infertility if there is cryptic female choice based on ejaculate characteristics, as has been demonstrated in sand lizards (Olsson *et al.* 1996). Further studies of sperm production, sperm viability and post-copulatory discrimination of males in native and non-native populations are needed to test these hypotheses.

Within groups of non-native populations that cluster together genetically because of a shared ancestry (i.e., demes), individual multilocus heterozygosity can be correlated to fitness traits if there are differences in the inbreeding among individuals (Szulkin *et al.* 2010). This variation generates identity disequilibrium (ID, the correlation in heterozygosity and/or homozygosity across loci; Weir & Cockerham 1973; Szulkin *et al.* 2010). Only one of the four demes showed significantly positive estimates for g_2, but even here we failed to detect an association between female heterozygosity and clutch size or hatching failure (the incidence of infertility was too low for a meaningful test). The average effect sizes across demes suggested that the effect is close to zero. Within clutches, we also did not find any differences in heterozygosity between embryos that died early in development and their successfully hatched siblings.

How can we reconcile the consistent loss of genetic diversity and increased hatching failure in non-native populations with the lack of a bivariate relationship between individual-level heterozygosity and hatching failure? In wild populations, there are many known examples of individual multilocus heterozygosity and fitness correlations (reviewed in Chapman *et al.* 2009). However, effects are relatively weak and effect sizes generally small. Our study ended up using only 13 microsatellite markers. Significant HFCs have been reported with fewer markers (e.g., Chapman *et al.* 2009; Brommer *et al.* 2015; Velando *et al.* 2015), but the neutral markers used might not be sufficient to capture them adequately (Balloux *et al.* 2004; Miller & Coltman 2014), especially as g_2 values suggested at most moderate level of inbreeding. Thus, failing to detect ID and/or HFC's should not be taken as evidence that inbreeding depression is absent (Kardos *et al.* 2014). Nevertheless, it is striking

that the very high incidence of embryonic mortality in particular did not correlate with female or embryo heterozygosity. One possibility is that these effects are largely driven by inbreeding depression in males, as discussed above. If so, we may not be able to detect HFC by focusing on females even if there is substantial evidence for inbreeding depression, as suggested by the high incidence of infertility and embryonic mortality in some non-native populations. It is also possible that some populations with low heterozygosity have undergone purging of deleterious mutations (e.g Pujol *et al.* 2009; Facon *et al.* 2011b). This would imply that not all populations or individuals with low heterozygosity should show high incidence of inbreeding depression. However, the efficiency of purging depends on many genetic and demographic factors (Keller & Waller 2002) and the time necessary to lessen inbreeding depression could be highly variable (Chapman *et al.* 2009).

In conclusion, the levels of genetic diversity in non-native populations of *P. muralis* reflect their origin and phylogeographic structuring in the native range, with substantial loss of diversity in non-native populations from native regions with high genetic variation. Older populations and populations founded by a low number of individuals had lower genetic diversity. Embryonic mortality was higher in non-native populations of Italian origin. Although this is consistent with the greater loss of genetic diversity for Italian-origin populations, there was no evidence that heterozygosity across microsatellite markers is significantly correlated with inbreeding depression at the population or individual levels. Combined these results suggest that the marker-based assessments of population viability may be of limited reliability and should not replace more direct estimates of individual viability, such as early mortality.

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Sampling location	Abbr	Latitude (°N)	Longitude (°E)	N_S	Mode of introduction	Region of origin	Admixture	Years since introduction	H_O	H_E	A_R
Abbotsbury	AB	50.66651	-2.59958	25	Secondary	Italy	NO	16	0.55	0.62	3.88
Birdbrook	BB	51.46246	0.03513	13	Primary	Italy	YES	42	0.61	0.68	4.54
Boscombe	BS	50.71992	-1.84248	25	Secondary	Italy	YES	22	0.56	0.57	3.65
Bury	BU	50.9090	-0.56406	20	Primary	France	NO	44	0.51	0.55	3.47
Corfe Castle	CC	50.63232	-2.05138	25	Secondary	Italy	NO	8	0.60	0.63	4.04
Cheyne Weare	CW	50.52856	-2.43771	25	Secondary	France	YES	19	0.59	0.61	3.89
Dancing Ledge	DL	50.59916	-1.99351	25	Secondary	Italy	NO	24	0.63	0.64	4.19
East Portland	EP	50.53162	-2.43566	25	Primary	France	YES	19	0.55	0.60	3.83
Folkestone	FS	51.08085	1.16976	21	Secondary	Italy	NO	22	0.50	0.55	3.22
Holmsley	HO	50.78682	-1.69622	25	Secondary	Mix	YES	28	0.61	0.68	4.51
Newton Ferrers	NF	50.3159	-4.03624	25	Primary	Italy	NO	36	0.52	0.56	3.00
Poole	PO	50.71528	-1.98498	25	Secondary	Italy	NO	22	0.50	0.68	4.82
Seacombe	SC	50.6220	-1.96287	18	Secondary	Italy	NO	28	0.61	0.65	4.20
Shoreham	SH	50.82762	-0.25674	25	Primary	Italy	NO	39	0.57	0.69	4.66
Shorwell	SW	50.64459	-1.35461	25	Secondary	Italy	YES	29	0.56	0.60	3.89
Ventnor Botanical Garden	VB	50.58544	-1.24676	25	Secondary	Italy	YES	14	0.54	0.68	4.56
Ventnor Town	VT	50.59364	-1.2075	25	Primary	Italy	NO	84	0.39	0.49	2.76
Wembdon	WB	51.13197	-3.02225	25	Primary	France	NO	26	0.70	0.73	4.86
Wellington	WE	50.97848	-3.22494	25	Primary	France	NO	33	0.57	0.63	3.68
Winspit	WS	50.59106	-2.01245	25	Secondary	Italy	NO	28	0.53	0.62	4.05
West Worthing	WW	50.82038	-0.36085	25	Primary	Italy	NO	10	0.55	0.62	3.88

Table S2 | Sampled locations in the native range of *P. muralis.* Details on geographic coordinates, samples size (Ns), expected (H_E) and observed (H_O) heterozygosity and allelic richness (A_R).

Sampling region	Sampling location	Abbr	Latitude (°N)	Longitude (°E)	N_S	H_O	H_E	A_R
France	Bastide	BA	42.93933	1.05599	25	0.63	0.67	5.08
	Dinan	DN	48.45435	-2.04734	25	0.62	0.65	4.08
	Fronteirs Cabardes	FC	43.36959	2.24849	25	0.66	0.68	4.99
	Saint Gervais	GE	46.90274	-1.99874	25	0.61	0.68	4.80
	Josselin	JO	47.9539	-2.54648	25	0.57	0.60	4.20
	Saint Lizier	LI	43.00326	1.13879	20	0.69	0.72	5.48
	Luzenac	LU	42.76318	1.76341	20	0.66	0.70	5.37
	Saint Michel	MI	46.35321	-1.25171	25	0.67	0.67	4.62
	Nebias	NE	42.89679	2.11586	25	0.66	0.73	5.62
	Oust	OU	42.87438	1.21588	20	0.71	0.70	5.03
	Pontchateau	PC	47.4369	-2.08903	25	0.53	0.54	3.74
	Puybelliard	PU	46.70644	-1.02946	22	0.72	0.73	5.30
	Pouzagues	PZ	46.78435	-0.83917	25	0.66	0.70	5.27
	Roquelau	RQ	43.03235	1.10822	25	0.71	0.71	5.26
	Saint Girons	SG	42.98224	1.14627	25	0.70	0.72	5.41
	Vitre	VR	48.12401	-1.2144	20	0.53	0.59	3.83
Italy	Bassano Di Grappa (Campesse)	BG	45.80208	11.71101	25	0.67	0.77	6.19
	Badia Polesine	BP	45.09714	11.49063	25	0.66	0.80	6.30
	Brunoria	BRU	45.16411	9.06425	21	0.61	0.73	5.57
	Buti	BT	43.72659	10.58541	25	0.69	0.76	6.34
	Barbarano Vicentino	BV	45.41062	11.53904	25	0.67	0.78	6.15
	Calci	CA	43.7219	10.52198	25	0.71	0.77	6.60
	Cantugno	CAN	45.21037	9.18403	20	0.69	0.76	6.08
	Certaldo	CD	43.54765	11.04154	19	0.75	0.79	6.71
	Cento	CE	44.73153	11.29081	25	0.67	0.78	6.68
	Castelfiorentino	CL	43.60576	10.96971	12	0.66	0.75	6.32
	Chianni	CN	43.48423	10.64247	25	0.71	0.76	6.51
	Cantalupo	CP	44.8634	8.55294	25	0.59	0.71	5.43
	Crespina	CR	43.57478	10.56486	24	0.66	0.77	6.57
	Castelarrano	CT	44.51326	10.73318	25	0.65	0.72	5.70
	Greve in Chianti	GC	43.58758	11.31481	25	0.69	0.75	6.24
	Lemna	LEM	45.85846	9.15869	19	0.64	0.76	6.10
	Motta Di Livenza	ML	45.77502	12.61037	22	0.72	0.82	6.33
	Montemassi	MM	42.99218	11.06385	25	0.76	0.80	6.91
	Mizzole	MZ	45.47677	11.05915	25	0.63	0.75	5.86
	Nonantola	NO	44.67887	11.04116	25	0.69	0.77	6.39
	Olina	OL	44.30501	10.78226	16	0.67	0.74	5.83
	Peccioli	PE	43.54448	10.72014	25	0.70	0.75	6.61
	Prato	PR	43.89949	11.10575	25	0.68	0.77	6.62
	Pian Di Venola	PV	44.33314	11.18937	25	0.62	0.76	6.61
	Settimo	SET	45.26502	9.21842	20	0.67	0.75	5.99
	Travale	TR	43.16743	11.00843	25	0.72	0.81	6.92
	Colle di Val d'Elsa	VE	43.42148	11.11181	25	0.75	0.78	6.66
	Vignola	VG	44.47634	11.01046	22	0.57	0.72	5.81
	Viarregio	VI	43.84268	10.26326	25	0.66	0.74	5.92

Table S3 | Populations used in the hatching failure analyses. Average hatching failure (H_F), average fecundity (clutch size, C_S), average infertility (I_N), expected heterozygosity (H_E) and allelic richness (A_R).

Sampling Location	Abbr	Range	Origin	N_C[#]	N_{GF}[§]	H_F	I_N	C_S	H_E	A_R
Bury	BU	Non-native	France	3	3	0	0.00	4.33	0.55	3.47
Cheyne Weare	CW	Non-native	France	53	34	0.09	0.04	5.30	0.61	3.89
East Portland	EP	Non-native	France	34	22	0.12	0.05	5.85	0.60	3.83
Wellington	WE	Non-native	France	13	6	0.10	0.00	7.69	0.63	3.68
Boscombe	BS	Non-native	Italy	4	4	0.03	0.00	5.25	0.57	3.65
Dancing Ledge	DL	Non-native	Italy	14	11	0.34	0.00	6.71	0.64	4.19
Poole	PO	Non-native	Italy	8	7	0.16	0.06	6.13	0.68	4.82
Shoreham	SH	Non-native	Italy	27	19	0.12	0.00	6.30	0.69	4.66
Shorwell	SW	Non-native	Italy	12	12	0.21	0.08	6.50	0.60	3.89
Ventnor Botanical Gardens	VB	Non-native	Italy	40	32	0.18	0.02	6.18	0.68	4.56
Ventnor Town	VT	Non-native	Italy	61	37	0.27	0.05	5.87	0.49	2.76
Winspit	WS	Non-native	Italy	16	12	0.17	0.00	6.06	0.62	4.05
West Worthing	WW	Non-native	Italy	15	11	0.37	0.00	6.07	0.65	4.19
Dinan	DN	Native	France	4	4	0	0.00	4.75	0.65	4.08
Josselin	JO	Native	France	10	10	0.18	0.03	4.40	0.60	4.20
Seiches-sur-le-Loir	LL	Native	France	2	0	0.20	0.00	5.00	NA	NA
Pontchateau	PC	Native	France	4	4	0	0.06	4.50	0.54	3.74
Pouzagues	PZ	Native	France	14	12	0.27	0.00	4.93	0.70	5.27
Certaldo	CD	Native	Italy	4	4	0.13	0.00	4.75	0.79	6.71
Castelfiorentino	CL	Native	Italy	5	5	0	0.07	4.80	0.75	6.32
Greve in Chianti	GC	Native	Italy	29	27	0.03	0.02	5.24	0.75	6.24
Peccioli	PE	Native	Italy	8	8	0	0.02	4.88	0.75	6.61
Prato	PR	Native	Italy	15	15	0.25	0.03	5.80	0.76	6.62
Colle di Val d'Elsa	VE	Native	Italy	18	18	0.05	0.03	4.94	0.78	6.67

[#] Number of clutches per population (N_C)
[§] Number of genotyped females (N_{GF})

Table S4 | GLM results for the effects of number of founders and years since establishment on genetic diversity (expected heterozygosity, H_E, and allelic richness, A_R).

H_E ~ *Number of founders + Years*

Source of variation	*d.f.*	*F*	*P*
Number of founders	1,6	3.81	0.12
Years	1,6	0.05	0.84

A_R ~ *Number of founders + Years*

Number of founders	1,6	3.48	0.14
Years	1,6	0.09	0.78

Table S5 | GLM results for the predictors of hatching failure (H_F) and infertility (I_N) in the non-native range.

H_F ~ origin + mode of introduction + admixture + years

Variable	*d.f*	*F*	*p*
Origin (Italy – France)	1,12	3.790	0.09
Mode of introduction (Primary – Secondary)	1,12	0.245	0.63
Admixture (Yes – No)	1,12	0.074	0.79
Years	1,12	0.399	0.55

I_N ~ origin + mode of introduction + admixture + years

Origin (Italy – France)	1,12	0.0398	0.85
Mode of introduction (Primary – Secondary)	1,12	0.0325	0.86
Admixture (Yes – No)	1,12	2.9603	0.12
Years	1,12	1.6545	0.23

Table S6 | Values of g2 indicating variance in inbreeding within demes. Significant deviations from zero are indicated in bold.

Partition	N	g_2	*p*	s.d
DemeNativeItalian	77	0.021	0.20	0.03
DemeNativeFrench	30	0.017	0.27	0.03
DemeIntroITA-A (BS, DL, PO, WS)	32	0.0387	0.07	0.03
DemeIntroITA-B (WW, SH)	31	0.0670	**0.04**	0.04
DemeIntroITA-C (VT,VB,SW)	58	0.0066	0.31	0.01
DemeIntroFRA (BU,CW,EP,WE)	61	-0.0087	0.66	0.02
DemeIntroMix (BS,DL,SH,SW,VB,WE,WS)	31	0.0147	0.33	0.03

Table S7 | GLMM results assessing the effects of individual heterozygosity (O_{HL}) on embryo mortality. Female ID was fitted as random effect in both models.

Fitness trait	Model	Explanatory Term	Estimate ± SE	Z-statistic	*P-value*	*LRT*
Embryo mortality (31 clutches, 160 eggs, 97 hatched)	Model A	Intercept	-1.07 ± 1.20	-0.89	0.375	
		O_{HL}	0.10 ± 1.06	0.10	0.919	
		Female ID	Variance (SD): 0.22 (0.47)			
	Model B	Intercept	-0.45 ± 0.19	-2.37	0.018	χ^2=0.006
		Female ID	Variance (SD): 0.24 (0.49)			*p*=0.93

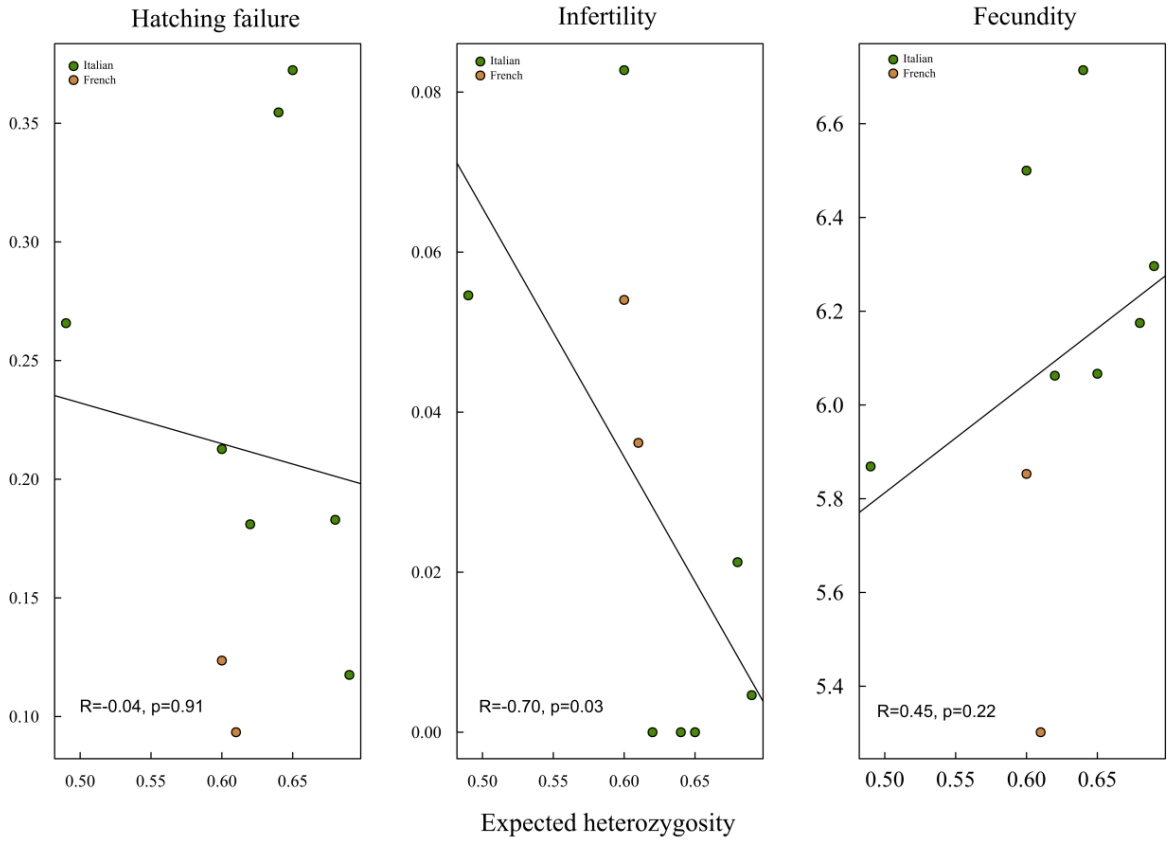

Figure S1 | Correlation between expected heterozygosity and population average fitness trait in non-native populations. Significant correlation only for infertility.

CHAPTER 6

OVERVIEW

In this thesis, I set out to further our understanding on how historical processes shape genetic diversity, and to what extent we can link the colonisation history, genetic diversity, individual fitness and population viability of non-native populations. To achieve this, I used a combination of molecular markers, analytical tools and the common wall lizard *Podarcis muralis* as a study system.

First, I studied populations at the northern range margin of *P. muralis* and aimed to assess the genealogical relationship between island and mainland populations and their genetic architecture **(Chapter 2)**. The results showed that these small and fragmented populations on islands of Jersey and Chausey archipelago are most likely native, with genetic differentiation reflecting isolation following sea level increase, approximately 7000 years ago. Genetic diversity decreases towards range margins in mainland Western Europe (see also Gassert *et al.* 2013), but these island populations exhibit an additional significant reduction in genetic diversity relative to the closest mainland populations. I then moved beyond the native range and aimed to elucidate the geographical origin(s) of human-mediated introductions of *P. muralis* in England **(Chapter 3)**. I traced their origin in five, geographically distinct, native clades with evidence of multiple clades within a single population. Following up with extensive sampling in the native range, I aimed to reconstruct the colonisation history of these non-native populations **(Chapter 4)**. Results revealed (at least) nine separate sources, and widespread primary, but geographically restricted secondary, introductions. Finally, I investigated the level of genetic variation in these non-native populations in relation to the native region and the links to individual fitness and

population viability **(Chapter 5)**. I showed that the phylogeographic structure in the native range and the details of colonisation is reflected in the genetics of non-native populations. Introductions are associated with wide-spread loss of genetic diversity and the high occurrence of embryonic mortality (particularly in populations experiencing greater loss of genetic diversity) could compromise the long term viability of these populations. However, within non-native populations, there was no evidence that lizards with lower heterozygosity across thirteen microsatellite markers showed reduced fitness, despite the high incidence of infertility and embryonic mortality relative to the native range.

All biological invasions share common issues, in terms of the process of colonisation, and common consequences, in terms of ecological and economic impact as well as evolutionary consequences that influence the success of non-native species (Blackburn *et al.* 2011; Chapple *et al.* 2012b). In this Discussion, I put my results into the context of this broader framework of the colonisation process to show how these contribute to our understanding of the causes and consequences of genetic variation after colonisation events. I first give an overview of what we can learn from studies on colonising species and why lizards in particular. I then follow the stages of colonisation addressing emerging questions (Figure 1) with a focus on other studies of lizards, emphasizing where general conclusions are emerging regarding how the different steps affect the genetics of colonising species and pointing out where the most important remaining gaps are.

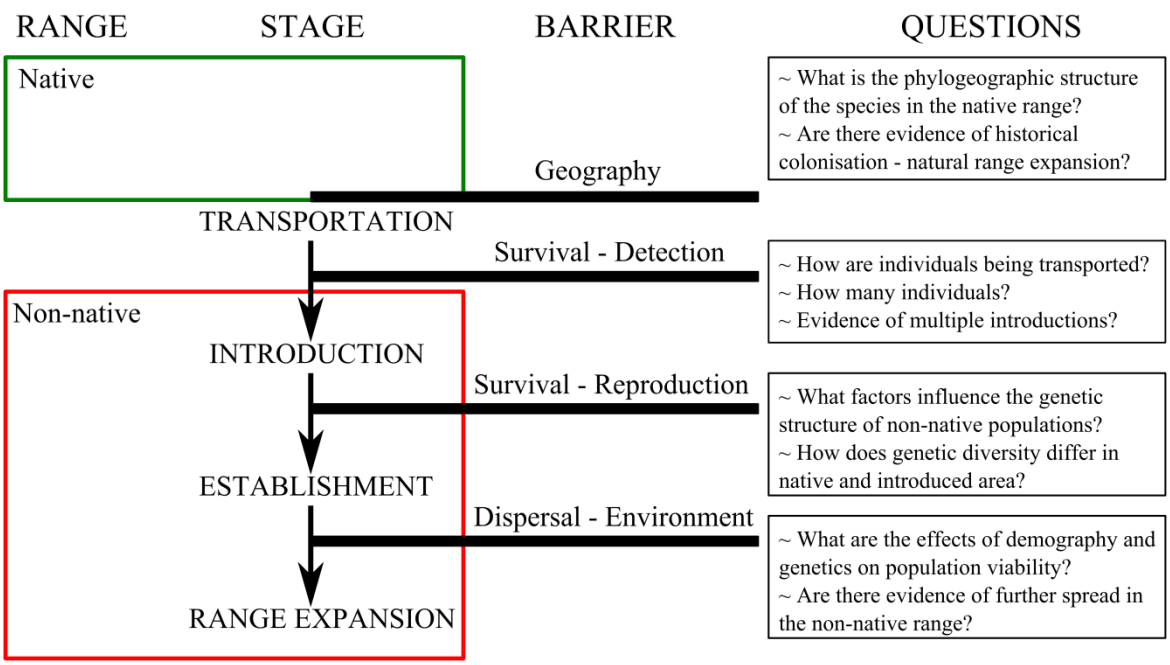

Figure 1 | The stages of colonisation and relevant questions for studies involving non-native species. All questions have been addressed in the present study.

STUDYING COLONISING SPECIES

Colonising species and human-aided introductions in particular, have been long recognised as a major component of global change with detrimental ecological and economic effects (Vitousek *et al.* 1996; Mack *et al.* 2000; Pimentel *et al.* 2000; Sax *et al.* 2005). However, they also considered as excellent study systems to understand ecological and evolutionary process occurring in real-time (Sakai *et al.* 2001; Lee 2002; Sax *et al.* 2007). Studying non-native species can generate important insights into what makes certain populations vulnerable to extinction (Sax & Gaines 2003), the consequences of in- and out-breeding (Rhymer & Simberloff 1996; Verhoeven et al. 2011), and the ability for populations to rapidly adapt to novel environments (Lee 2002). Such results can also be applied to effective management regimes and to define conservation priorities (Sax *et al.* 2007). Traditionally, much attention has

been paid to invasive plants (weeds) and pests and only a few species of introduced reptiles and amphibians (in particular the cane toad *Rhinella marina* in Australia and Anolis lizards in Florida) have so far received extensive scientific study. Most of non-native reptiles, and lizards in particular, have been overlooked by both conservation biologists and evolutionary ecologists, despite the exponential growth in introductions (Kraus 2009).

Lizards are a paraphyletic group of non-avian reptiles with currently 6145 species (The Reptile Database, accessed Sept 2015) that are characterised by a spectacular range of morphologies and ecologies and occupy a wide range of habitats in mainland and island ecosystems (Pianka & Vitt 2003). For example, lizards managed to colonise and diversify on oceanic islands (e.g., marine and land iguanas (*Amblyrhynchus cristatus*, *Conolophus spp.*) on Galapagos achipelago, Parent *et al.* 2008) and some species are found up to 4500m above sea level (e.g. the toad-headed sand lizard, *Phrynocephalus erythrurus* on the Tibetan plateau Jin & Liu 2010). In addition to their naturally worldwide occurrence (apart from Antarctica), lizards have also been introduced (intentionally or accidently) outside their native range, through human interventions. There are now more than 1300 documented reptile introductions with lizards comprising about 630 of these (Kraus 2009).

THE STAGES OF COLONISATION

The colonisation process (natural or human-mediated) involves a series of stages (transport, introduction, establishment and spread) with associated barriers (e.g. environment, geography, survival, reproduction, dispersal) that individuals of a species need to overcome in order to become successful colonisers (Figure 1, Kolar &

Lodge 2001; Blackburn *et al.* 2011; Chapple *et al.* 2012b). Lizards have typically entered the transportation stage either accidentally (via cargo shipments or nursery trade) or have been intentionally transported via the pet trade (Kraus 2009). The latter has been the dominant pathway since the 1950s (Hulme 2009; Kraus 2009) with North America and Europe been the recipients of most introductions. For example, 43 lizard species were introduced via the pet trade in Florida, USA, alone (Krysko *et al.* 2011) contributing to the largest number of established non-native reptiles in the world. The pet trade is also the main route for the introduction of *Podarcis muralis* in England, which often involves escapees from outdoor enclosures or release of captive animals and/or their offspring (**Chapters 3** and **4**).

Pet trade introductions have a number of features that are not shared by other invasion routes (Kraus 2009; Krysko *et al.* 2011). For example, aesthetic preferences may bias the introductions towards certain species, animals from a certain geographic region, or animals with relatively high expression of attractive traits (e.g. chameleons, boas and iguanas in Hawaii and Florida, Kraus 2009; *Podarcis siculus* in US, Kolbe *et al.* 2012). Indeed, the proportion of non-native common wall lizard populations in England that have the, geographically very restricted, dorsal green Italian phenotype is much higher than expected (Michaelides *et al.* 2013). Introductions in West and East Germany suggests that the distribution of non-native lizards strongly reflects availability through restrictions imposed on travel during the communist era, and may also reflect popular 'holiday destinations' in southern Europe (Schulte *et al*., 2013). Interviews support that actively bringing in animals after holidays is the pathway of introduction of several, maybe all, of the UK

introductions of lizards of the brown type from France (Tobias Uller, unpublished data). However, this was not the main cause of introduction of lizards from Italy, which were often acquired through commercial pet trade, again suggesting that the pet trade targets more colourful variants (Michaelides *et al.* 2013; Michaelides *et al.* 2015b).

Once species have successfully arrived at an introduction site their long-term fate will be determined by their ability to accommodate to any change in habitat, predation and so on. Recent research on reptile introductions has identified climate matching and propagule pressure to be the most important predictors of non-native establishment success (Mahoney *et al.* 2015). These are broadly the same factors that best predict establishment success for other vertebrates like birds, amphibians, mammals, and invertebrates (Hayes & Barry 2007; Blackburn *et al.* 2009; Bomford *et al.* 2009; Rago *et al.* 2012). For ectotherms like lizards, the similarity of annual temperature profiles between native and non-native range might be critical for their survival during both active and inactive phases of the year. In England, however, the climatic conditions of the introduced locations have a poor match to the environmental niche in the native range for all source lineages (Schulte *et al.* 2012b). The ability of lizards from north-central Italy to persist, and sometimes thrive is surprising, given the very different spring and summer temperatures in England (While et al. 2015a, Appendix A). This supports previous conclusions that the fundamental climatic niche is poorly represented by the realised niche in the native range for this species and suggests that other factors than climate may be more limiting (Schulte *et al.* 2012b). However, While *et al.*, (2015) recently showed that the

much cooler soil temperature in England indeed imposes strong selection at the incubation duration stage. Non-native lizards of both genetic origins (French and Italian) have adapted to the cooler conditions by retaining their embryos for longer before oviposition and increasing embryonic growth. These adaptive responses promote establishment success and long-term persistence of wall lizards in England.

Besides climate matching and life history traits (for example in birds, Sol *et al.* 2012) successful colonisation is primarily a numbers game (Blackburn *et al.* 2015). Introduced populations are more likely to establish if more individuals are released and/or a higher number of release events occur at the same location (Simberloff 2009; Blackburn *et al.* 2015). The number of introduced individuals is often difficult to know and as we do not have data for any of the extinct populations in England, the extent to which propagule pressure contributes to the success of non-native wall lizards is unknown (but see below for effects on genetic diversity). Out of the ca 50 known introductions, about 30 are still extant, but at least in some of these, extinction is probably best explained by habitat destruction ('shading out'), eradication, or failure to survive or recruit due to cool temperatures. However, undoubtedly population size is fundamental for population persistence (Lande 1998), owing to both demographic and genetic processes (Frankham 2003, 2005a). Large founding populations can result in higher colonisation success by moderating stochastic processes (e.g. genetic bottlenecks, environmental stochasticity), reducing the probability of inbreeding and thus also of inbreeding depression which often associated with small populations (Frankham *et al.* 2002; Simberloff 2009; Blackburn *et al.* 2015). The underlying mechanisms and the contribution of demography and

genetics in the context of colonisation has received research attention (Hufbauer *et al.* 2013; Szucs *et al.* 2014) but their specific role and interaction for the long-term outcomes of colonisation events have not yet been resolved.

THE GENETICS OF COLONISING SPECIES: *PODARCIS MURALIS* AS A MODEL SYSTEM

The complex phylogeographic structure of *P. muralis* in southern Europe is the result of the combined effects of historical process (e.g., range expansion – contraction, isolation in glacier refugia), selection and drift that led to the formation of least five main lineages (Böhme 1986; Giovannotti *et al.* 2010; Bellati *et al.* 2011; Salvi *et al.* 2013), and spatial variation in neutral genetic diversity (Figure 2; **Chapter 4 and 5**). Such genetic structuring in the native range is expected to have consequences for the amount and structuring of genetic diversity also in the introduced range and may lead to non-native populations having higher genetic diversity than their native counterparts (Kolbe *et al.* 2004; Taylor & Keller 2007). Our study of *P. muralis* in the native range identified regions of secondary contact between genetically (and morphologically) differentiated lineages (Michaelides *et al.* 2015; see also While *et al*. 2015b, Appendix B). Such detail is important when inferring the colonisation history of non-native populations since it may help to disentangle admixture resulting from multiple introductions (of genetically and geographically different populations) from introductions whose source are a natural hybrid zone in the native range.

Genetic and phenotypic data in this study suggest that a substantial proportion of non-native populations originating from Italy have their origin from a hybrid zone between the Tuscan and the Venetian lineages (located in northern Tuscany and Bologna-Modena). However, using Approximate Bayesian Computation methods, we

were also able to identify with confidence a high (40%) proportion of multiple introductions, some of which resulted in mixing of lineages that do not naturally co-occur in the native range. Even if there is generally assortative mating between lineages (e.g., Heathcote *et al*. submitted), hybridisation occurs extensively and even the main lineages that diverged ca 2 mya appear genetically compatible (While *et al.* 2015b; Appendix B). As a consequence, several of the introduced populations are best described as hybrid swarms (Harrison 1993). In addition, hybridisation is asymmetric, which results in retention of the exaggerated male secondary sexual characters, a common feature of the Tuscan lineage (While *et al.* 2015b, Appendix B). These post-introduction processes will of course influence the contemporary genetic diversity, but their importance is difficult to establish (Keller & Taylor 2008). The majority of work on the genetics of non-native species therefore focuses on the historical and demographical features of their introduction. This is true also of our work on wall lizards, but it is important to keep in mind that post-introduction processes, such as natural and sexual selection, may substantially contribute to the genetics of contemporary non-native populations by favouring specific phenotypic traits (Sakai *et al.* 2001; Keller & Taylor 2008).

Population genetic theory predicts that genetic diversity in non-native populations will be lost since such populations are often founded by a limited number of animals (Nei *et al.* 1975). Loss of genetic diversity should be particularly pronounced in secondary introductions, which have been subject to two bottlenecks (i.e., sequential founder effects Clegg *et al.* 2002). However, genetic diversity has been demonstrated to sometimes be higher in non-native populations because of multiple introductions

from different parts of the range (Kolbe *et al.* 2004; Kolbe *et al.* 2007; Dlugosch & Parker 2008; Facon *et al.* 2008; Uller & Leimu 2011). Over time, genetic diversity would nevertheless be expected to decrease unless there is continued gene flow.

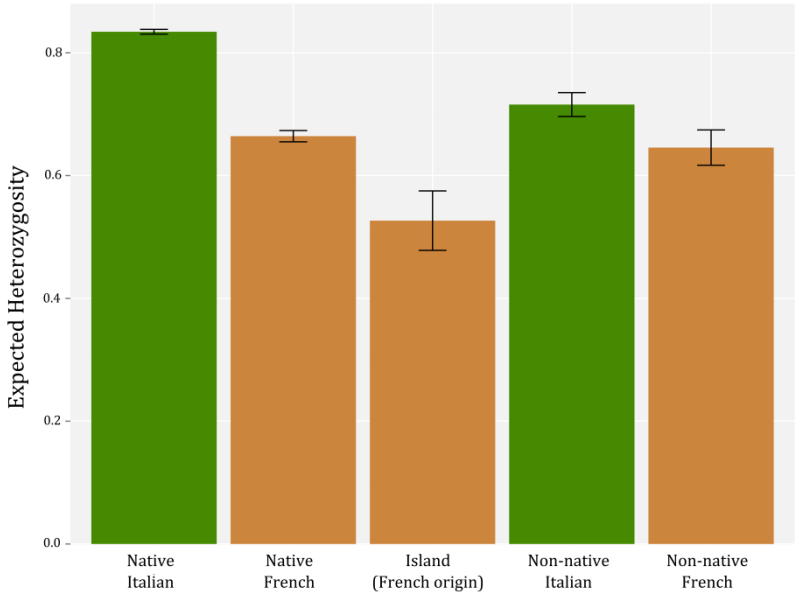

Figure 2 | Genetic diversity across the native and non-native range. Mean expected heterozygosity by region is calculated based on eight microsatellite markers.

We sampled 23 non-native populations of wall lizards in England to test these hypotheses. The level of genetic diversity within non-native populations mainly depended on the region of origin (Figure 2, see also **Chapter 5**). Populations of Italian origin – where native within-population diversity is high – have lost a greater amount of genetic diversity than their French counterparts, but still retain higher genetic variation overall. Thus, both the loss and the absolute levels of genetic diversity in non-native populations depend on the geographic origin. For the populations where the number of introduced animals was known, genetic diversity increased with increased number of founders (**Chapter 5**). Contrary to expectations, secondary introductions (as assessed using ABC) did not have lower genetic diversity than

primary introduction and populations with multiple haplotypes did not have higher levels of nuclear genetic diversity. Although a comparison of genetic diversity (measured by nuclear markers) between primary and secondary introductions has not been addressed in the literature (Dlugosch & Parker 2008), maintaining levels of genetic variability could be explained by subsequent introductions and admixture. Kolbe *et al.* (2007) suggested that introductions generally follow a sequential two-step process; first, populations experience a reduction in genetic variation due to founder events and/or bottlenecks and second, an increased due to introductions of individuals from multiple sources. Approximately half of the secondary introductions in England harbour haplotypes from multiple lineages and hence may follow this pattern. On the other hand, this might be the result of a more recent introduction. Indeed, prolonged periods of isolation and absence of gene flow resulted in low levels of genetic variation for older non-native populations. This is also supported by a comparison across all populations and regions, where island wall lizard populations appear to have the lowest levels of genetic diversity (Figure 2), similar to the oldest established non-native population (in Ventnor, VT, on the Isle of Wight). Taken together, this study shows that, on average, successfully established lizard populations from single or multiple introductions (with/without admixture) that experience bottlenecks lose genetic variation, but not to a large extent and perhaps only consistently so after some time in isolation (perhaps partly due to selection; While *et al.* 2015a, Appendix A).

Long-term establishment and further spread from the site of introduction (range expansion) is the final step in the colonisation process (Figure 1). Inbreeding

depression could be a significant hurdle to long-term population viability, which makes it important to link introduction history and genetic diversity with signs of loss of fitness at the individual and population level. Using a set of neutral markers, I showed both a loss of genetic diversity and an increase in hatching failure for non-native populations (**Chapter 5**). This has been reported from long-term studies of native birds and mammals (Slate *et al.* 2000; Kruuk *et al.* 2002) and to my knowledge this study is the first demonstration of a widespread loss of fitness in a non-native species. Reduced genetic diversity and inbreeding increase the expression of deleterious alleles and hence populations are assumed to show a reduced mean fitness (Keller & Waller 2002; Reed & Frankham 2003). However, despite the significant loss of genetic diversity in introduced populations there was a lack of strong direct correlations between population-level heterozygosity and population averages of fitness traits (hatching, infertility, and clutch size). One possible explanation is low statistical power due to limited sample sizes (nine populations, with varying sample sizes on female data). It could also be that the expression of deleterious alleles has not been fixed in the population or have been purged (e.g. Facon *et al*. 2011).

A relationship between heterozygosity and fitness is also expected at the individual level since mating among relatives increases inbreeding, expression of deleterious alleles through homozygosity and hence inbreeding depression (Keller & Waller 2002). However, there were no significant heterozygosity-fitness correlations (HFCs) in non-native wall lizards. Similarly to this study, the majority of HFC studies have been based on microsatellite data. However, all meta-analyses of HFC studies

(Coltman & Slate 2003; Chapman *et al.* 2009; Miller & Coltman 2014) report a very low average effect size. It has been suggested that HFCs will need a larger number of markers than is commonly applied (Balloux *et al.* 2004; Kardos *et al.* 2014; Miller & Coltman 2014; Miller *et al.* 2014) to capture a higher correlation with genome-wide heterozygosity. Future use of large-scale data sets of SNP loci will likely provide a more confident exploration of HFCs (Miller & Coltman 2014; Miller *et al.* 2014; Kardos *et al.* 2015). However, HFCs rely on among-individual variation in inbreeding and when variance is low, HFC cannot rise (Szulkin *et al.* 2010). The variance is measured through the use of g2, a parameter that estimates the identity disequilibrium defined as the excess of double heterozygotes at two loci relative to the expectation under random association, standardised by average heterozygosity (David *et al.* 2007). Only positive values of g2 give rise to HFCs and it is unclear how often this condition applies for past studies of HFCs (Szulkin *et al.* 2010). When females from non-native populations were pooled to form genetic demes (according to proportion of membership to a deme based on shared ancestry; see **Chapter 5**), g2 was positive for all demes but one (populations of French origin; see also Table S6 in **Chapter 5**) albeit statistically significant only for one deme (DemeIntroITA). This suggests overall only moderate levels of inbreeding in most non-native populations.

The evolutionary and conservation consequences of inbreeding depression are expected to depend on the degree to which it is expressed at specific life-history stage (e.g., during embryonic development, Hemmings *et al.* 2012) and/or be sex-specific (Brekke *et al.* 2010; Losdat *et al.* 2014). In this study, embryonic mortality was not correlated with reduced heterozygosity in females. Within clutches that had both,

hatched and unhatched embryos, there was also no evidence for an effect of embryo/offspring heterozygosity on hatching failure. Inbreeding depression could also be manifested in male reproductive success affecting for example sperm viability (Asa *et al.* 2007; Losdat *et al.* 2014). The incidence of infertility in non-native populations in England may thus reflect inbreeding depression in males that reduces sperm viability. This can be tested, for example, by comparing sperm viability of males from both native and non-native populations. This will also provide scope to explore the potential for post-copulatory mechanisms, for example cryptic female choice, to buffer the negative effects of inbreeding depression in males (Olsson *et al.* 1996; Lovlie *et al.* 2013; Frere *et al.* 2015).

This thesis has established a number of important aspects of the genetics of colonisation in wall lizards. However it is far from complete. In particular, an important question in invasion biology concerns the process of adaptation, and how, for example, small population size, loss of genetic diversity and expression of novel (e.g., through admixture) or cryptic genetic variation in stressful environments influence the ability for populations to respond to selection (Blackburn *et al.* 2015; Bock *et al.* 2015; Dlugosch *et al.* 2015). Although these questions remain very challenging, they may be possible to address through the application of Next Generation Sequencing, which enables the screening of large proportions of the genome for many individuals in a rapidly and cost-effectively approach (Baird et al. 2008). Genome-wide studies may facilitate the identification of genes of functional importance for interesting phenotypes, including behaviours, morphologies and life histories associated with colonisation success (Suarez and Tsutsui 2008). In addition,

more experimental work is needed to decouple the numeric and genetic diversity components of propagule pressure as well as reveal the potential fitness benefits of multiple introductions (Blackburn *et al.* 2015; Bock *et al.* 2015; Dlugosch *et al.* 2015). Given the evidence for rapid local adaptation (While *et al*. 2015a, Appendix A) and a growing understanding of the causes of hybridisation (While *et al*. 2015b, Appendix B) in the common wall lizard, the replicated introductions of *P. muralis* could continue to provide useful study objects.

CONCLUSION

This thesis has established the genetic structure and diversity of native and non-native marginal populations of common wall lizards at the northern climatic limit of the species' distribution. Non-native populations are highly restricted geographically, but the large number of independent introductions has led to a high genetic diversity on a regional scale even if diversity typically is lower within non-native than native populations. I demonstrated that reconstructing the colonisation history is important when aiming to understand the causes and consequences of genetic variation during colonisation. Although non-native populations show, in some cases substantial, loss of individual fitness through early life mortality, estimates of genetic diversity may not be sufficient to identify populations at risk of extinction. Nevertheless, non-native populations in England appear to have maintained sufficiently high population sizes to avoid inbreeding and retained sufficient evolutionary potential to adapt to their new climate, suggesting that the prospects of at least some populations may largely be dictated by availability of suitable habitat than by internal population factors.

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APPENDIX A

PROCEEDINGS B

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Research

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Adaptive responses to cool climate promotes persistence of a non-native lizard

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Successful establishment and range expansion of non-native species often require rapid accommodation of novel environments. Here, we use common-garden experiments to demonstrate parallel adaptive evolutionary response to a cool climate in populations of wall lizards (*Podarcis muralis*) introduced from southern Europe into England. Low soil temperatures in the introduced range delay hatching, which generates directional selection for a shorter incubation period. Non-native lizards from two separate lineages have responded to this selection by retaining their embryos for longer before oviposition—hence reducing the time needed to complete embryogenesis in the nest—and by an increased developmental rate at low temperatures. This divergence mirrors local adaptation across latitudes and altitudes within widely distributed species and suggests that evolutionary responses to climate can be very rapid. When extrapolated to soil temperatures encountered in nests within the introduced range, embryo retention and faster developmental rate result in one to several weeks earlier emergence compared with the ancestral state. We show that this difference translates into substantial survival benefits for offspring. This should promote short- and long-term persistence of non-native populations, and ultimately enable expansion into areas that would be unattainable with incubation duration representative of the native range.

1. Introduction

Non-native populations often encounter novel environments that impose strong directional selection. They therefore provide useful systems to study the process of adaptation [1–6]. However, evidence for adaptive divergence between native and non-native populations is currently limited, in particular for vertebrates [1]. Furthermore, the selective agents responsible are typically inferred from the pattern of phenotypic divergence, rather than being independently demonstrated, which makes it difficult to rule out non-adaptive processes [7]. The most convincing cases of adaptation in non-native species are therefore those where the causes of selection can be identified and the responses mirror locally adapted phenotypes within native ranges.

Recent studies of introduced insects and plants suggest that adaptive responses can facilitate spread into environments that were previously too stressful [8–10]. As climatic similarity is often the best predictor of establishment success for non-native species [11–13], climate is likely to exercise strong selection on many non-native populations. Such populations may therefore remain small and geographically isolated until they evolve tolerance to the new climatic regime, following which rapid range expansion is made possible. Understanding the mechanisms by which populations respond and adapt to novel climatic conditions

is therefore crucial for predicting both the persistence and expansion of non-native species.

The common wall lizard, *Podarcis muralis*, is native to southern and western Europe but has been introduced multiple times to Germany, England and North America over the past 100 years [14–16]. These introduction sites represent different climatic conditions compared with the native range [14]. For example, air temperatures during the main activity season in populations in England are 5–10°C lower than their source regions in Tuscany and western France (electronic supplementary material, figure S1). Although behavioural thermoregulation enables non-native lizards to maintain annual activity patterns similar to those in their native range, these climatic differences put soil temperatures well below what females prefer for their nests (electronic supplementary material, table S1). Such cool conditions retard the rate of embryonic development [17], resulting in delayed hatching and even failure to complete embryogenesis before winter in cool summers [18]. Thus, we should predict strong selection for shorter incubation duration in non-native wall lizard populations, in particular at the cool temperatures rarely encountered in the native range but that are frequent in introduced populations in England.

Incubation duration can be reduced through several different mechanisms. First, embryos may be more advanced at oviposition. By capitalizing on the female's ability to maintain body temperatures substantially higher than those of nests, this reduces the overall time for completion of embryogenesis [19]. Egg retention is evolutionarily labile in lizards and has been shown to vary with climate within and between species [20–24]. Second, embryos can evolve a faster development rate. For example, in the Eastern fence lizard (*Sceloporus undulatus*), embryos from populations in cooler climates have a higher cardiac output and hence a more efficient delivery of nutrients and oxygen, which promotes faster embryogenesis [25]. Finally, embryos may hatch at an earlier developmental stage or at a smaller size and capitalize on residual yolk for completion of growth after emergence [26,27].

Here, we designed a set of common-garden experiments to test the hypothesis that non-native populations have adapted to the cool climate in their non-native range. First, we show that soil temperatures in the non-native range are physiologically stressful and impose significant selection for shorter incubation duration. Second, we test whether non-native populations have responded to this selection and establish the underlying mechanisms of adaptation. Finally, we use developmental reaction norms to infer the implications of shifts in incubation duration for the timing of hatching and recruitment under naturally fluctuating soil temperatures in the non-native range. Our results demonstrate prolonged embryo retention and faster embryonic growth at low temperatures in introduced wall lizards and suggest that these responses will have significant implications for the long-term persistence of this species in England.

2. Material and methods

The common wall lizard is a small (approx. 50–70 mm snout-to-vent length) diurnal lizard. It is native to southern and western Europe, but has established non-native populations in many places in Europe and in North America [14,15,28]. There are around 30 extant populations in southern England, the majority of which are highly geographically restricted with limited ecological impact [15].

(a) Female thermal preference and soil temperature

We first established preference for nest sites under unrestricted thermal conditions and in field enclosures. Unrestricted conditions were established by creating a thermal gradient in a large cage (1070 × 480 × 420 mm) of approximately 45°C to 20°C during the peak in the afternoon, falling to 15°C at night (electronic supplementary material, table S1). Five females were used in this experiment. Females were inspected in the morning and in the afternoon for signs of egg laying. Once they had laid, we recovered their clutch and replaced it with a thermal logger (Thermochron Ibutton, model number DS1921G) that logged the temperature for five consecutive days. A second dataset was generated from females housed in outdoor enclosures where suitable nesting sites with naturally variable thermal properties were available. Five female lizards were placed into the enclosure and allowed to lay eggs. The temperatures of nests were monitored using thermal loggers for 35 days following oviposition. The temperatures of these nest sites were compared to possible nest sites across the enclosure. Further details on these experiments are provided in the electronic supplementary material.

(b) Effects of hatching date on recruitment into adulthood

In 2012, we released 288 hatchlings from females of both French and Italian origin in a disused quarry close to the original non-native populations on the Dorset coast (50.59° N, −2.01° E). All eggs were incubated at a constant 24°C. This temperature is at the high end of the average expected temperature of nests in England but at the low end of temperatures in the native range (electronic supplementary material, table S1; [29,30]). Upon hatching, offspring were weighed to the nearest 0.01 g and their snout-to-vent length and total length were measured to the nearest 0.5 mm with a ruler. They were toe clipped for individual identification. Individuals were released in four batches, the timing of which represented a compromise between limiting the number of days offspring spent in captivity while maximizing the number of hatchlings released at a single field trip. The releases occurred on 15 July (offspring hatching from 9 July until 15 July), 24 July (16 July—24 July), 6 August (25 July—6 August) and 21 August (6 August until 14 August).

Lizards were recaptured in their second year (i.e. first year as adults) by repeated visits during the breeding season from late March until late June ($n = 10$). Upon recapture, individuals were measured for snout-to-vent length and mass (as above) before being temporarily marked with a marker pen before release to avoid unnecessary recapture. Overall, we recorded 41 individuals from the 288 released hatchlings, representing a recapture rate of 14% over the first two years of life. We used the recapture data to test how the timing of hatching affects recruitment into adulthood using models with a categorical variable with three levels representing release batch, lineage (Italian versus French), and their interaction as fixed effects, and included days in the laboratory and mass at hatching as covariates. Because the last release included only 27 offspring from seven clutches, we pooled the last two release batches to avoid having one level represented by very limited data. Furthermore, because our sample size is modest, we could not meaningfully take family effects into account (nine out of 65 families produced two recruits, the rest one or none). We therefore treated all offspring as independent for this analysis.

(c) Establishing differences in incubation duration between native and introduced populations

(i) Experiment at constant temperatures (20°C versus 24°C)

We caught 122 lizards by noosing at the early stages of gestation in early spring 2013 at three locations in Tuscany (Greve in Chianti (43.58° N, 11, 31° E), Prato (43.91° N, 11.10° E), Colle di Val d'Elsa (43.42° N, 11.11° E)), four in western France (Dinan (48.45° N, −2.07° E), Josselin (47.95° N, −2.54° E), Pontchateau (47.43° N, −2.09° E), Pouzagues (46.78° N, −0.44° E)) and four in southern UK (Italian origin: Ventnor Town (50.59° N, −1.21° E), Ventnor Botanical Garden (50.58° N, −1.22° E); French origin: Cheyne Weare (50.53° N, −2.43° E) and East Portland (50.54° N, −2.42° E)). The non-native populations represent at least two separate native sources for both the Italian and the French lineage that correspond well to the sampling locations of native animals [15]. Females had visible mating scars, large follicles or carried recently ovulated eggs (as determined by palpation), which ensured that they were fertile but would complete the large majority of gestation in the laboratory.

Females were housed individually in cages (590 × 390 × 415 mm) with sand as substrate, bricks as shelter, a water bowl, and held at a light cycle of 12 L : 12 D. They were given access to basking lights (60 W) for 8 h d^{-1} and a UV light (EXO-TERRA 10.0 UVB fluorescent tube) for 4 h d^{-1} and they were fed mealworms and crickets daily. Cages were inspected in the morning and in the late afternoon for signs of oviposition (which is easily assessed by visual inspection of females), such that eggs were recovered within 12 h of oviposition. Once laid, clutch size was assessed and the clutch and female were weighed to the nearest 0.01 g. All data in this paper are from the first clutch produced by females in that year (females can lay up to three clutches per year). The median lay date for native females was two weeks earlier than introduced females of both lineages (introduced French females: 30 May, native French females: 13 May, introduced Italian females: 29 May, native Italian females: 13 May).

For 65 clutches (each with more than four eggs), we selected one egg for dissection to assign the embryo to a developmental stage at oviposition according to the scheme for Lacertids [31]. For embryos that exhibited characters of two consecutive stages, we assigned an intermediate stage (e.g. 26.5). All staging was conducted by a single person (J.W.) who was unfamiliar with the coding system and hence embryos were scored blindly with respect to origin (native versus introduced) and lineage (French versus Italian). The remaining eggs were split into two groups and put into small plastic containers filled two-thirds with moist vermiculite (5 : 1 vermiculite : water volume ratio; changed every 20th day) and sealed with clingfilm. Half the clutch was incubated at constant 20°C and the other at constant 24°C in standard refrigerated incubators fitted with water baths to maintain humidity. During incubation, we measured the heart rate of all embryos within each of the 122 clutches as an estimate of cardiac output and hence developmental rate [32]. Following previous work [25,32], heart rate was measured using a digital egg monitor (Buddy, Avitronics, England) in a room at constant temperature set to either 20°C or 24°C (matching the incubation temperature of eggs). This was done twice during incubation, at 35 and 65 days following oviposition in the 24°C treatment and at 20 and 40 days in the 20°C treatment.

Eggs were inspected daily for signs of hatching (i.e. pipping) and the hatching date refers to the day of emergence. At hatching, the offspring snout-to-vent lengths and total lengths were measured to the nearest 0.5 mm with a ruler and mass was recorded to the nearest 0.01 g.

Because of the limited sample size per population (between five and 17 clutches), we pooled populations into the four main categories (Native Italian, Native French, Non-native Italian and Non-native French). We analysed differences in clutch size and the embryonic stage at oviposition between lineages (French versus Italian) and origin (native versus non-native). Because of the very large differences in incubation duration and heart rate in the 20°C and 24°C treatments (122.80 ± 0.60 days versus 56.15 ± 0.28 days, 47.45 ± 0.34 beats min^{-1} versus 76.23 ± 0.51 beats min^{-1}), we analysed these variables separately for the two temperature treatments (heart rate was averaged across the two measurements for each clutch and temperature [25,32]). By contrast, hatchling size variables overlapped between incubation treatments and we therefore fitted a single model with lineage, origin and temperature to the mean hatching mass per clutch and treatment, including female identity as a random effect. All linear models were fitted in R using the car package to generate type III *F*-tests of fixed effects. When the interaction(s) was non-significant, results for main effects are presented from models excluding the interaction.

(ii) Experiment at shifting temperatures

To assess differences in incubation duration at temperatures too low for completion of embryogenesis, we designed a second experiment shifting eggs between temperatures. For this experiment, we only used animals of Italian origin, collecting gravid females in spring 2014 at three locations in Tuscany (Greve in Chianti (43.58° N, 11, 31° E), Peccioli (43.55° N, 10.72° E), Colle di Val'Elsa (43.42° N, 11.11° E)) and the same two focal non-native Italian populations as in the above experiment (Ventnor Town and Ventnor Botanical Garden). All animals were handled and treated as above. Clutches were split into four categories: (i) constant 28°C ($n = 50$), (ii) 15°C for 14 days followed by constant 28°C ($n = 50$), (iii) 18°C for 14 days followed by constant 28°C ($n = 45$) and (iv) 28°C for 14 days, followed by 18°C for 14 days, and finally 28°C until hatching ($n = 40$). The last treatment was included to address if there were any marked differences for embryos exposed to cool temperature in early- versus mid-development [33]. Eggs were inspected for hatching around the same time daily (in the late afternoon) and hatchlings were measured as described above.

We calculated for each clutch the differences in incubation duration between eggs at constant 28°C and eggs at the other treatments, and used these estimates as our response variable. A significant difference between native and non-native populations would be interpreted as faster (or slower) developmental rate at cool temperatures. Such effects may be more or less pronounced if embryos adjust their developmental rate to conditions experienced early in gestation [19,34]. Eggs incubated at 18°C for 14 days before being transferred to 28°C took on average half a day longer to hatch than eggs from the same clutch that were exposed to the 14 days 18°C treatment in mid-gestation (46.5 versus 45.9 days; paired *t*-test, $t = 2.99$, $p = 0.01$, d.f. = 28). However, because we were primarily interested in the overall effect between native and non-native populations, we use the average of the two treatments in our analyses and for fitting thermal reaction norms (see below), which maximizes sample sizes when embryos in one of the 18°C treatments failed to hatch.

To test for differences in preferred body temperature between native and introduced gravid females, in 2014, we recorded the body temperature of 72 individually housed, captive females from both native Italian and non-native UK populations (same sources as described above) eight times a day over a three-day period using an infrared thermometer. This has been demonstrated to be a reliable measure of internal body temperature in small lizards [35]. Measurements began one hour after basking lights came on and continued on the hour until basking lights turned off. At night, temperatures dropped to 15°C across all cages. Thus, there was limited potential for females to alter gestational temperature via shelter site choice. We analysed differences between native and introduced females using linear mixed models

with body temperature as the response variable, origin (native or introduced) as the predictor variable and individual ID and observation day as random effects.

(d) Predicted hatching success in the UK

Temperature-dependent developmental rate at constant temperatures can be used to predict incubation duration at fluctuating temperatures [36–38]. To predict incubation duration of our non-native wall lizard populations, we used our data on developmental rate at constant temperatures at 20°C, 24°C and 28°C and the estimated developmental rate from the experiment switching eggs between 15°C or 18°C and 28°C (all for the Italian lineage; see the electronic supplementary material for further details). Data on developmental rate at higher temperatures were collected from the literature assuming that the developmental rate approaches a species-specific maximum at 35°C, data for which were provided by incubation experiments on a population from northern Spain (belonging to the same lineage as our French populations; [39,40]). Given that the difference between lineages and origins is small already at 24°C and that high temperatures are rarely encountered in the UK (see above), this should not bias our estimates. We fitted a four-parameter Weibull function and verified that it performed well for predicting incubation duration using experimental incubation under a daily fluctuating temperature regime similar to that of natural nests in England (see the electronic supplementary material for details).

We used soil data for locations south of the 53rd parallel north obtained from the British Meterological Office Integrated Data Archive System, Land and Marine Surface Stations Data for the period 2002–2013. We chose this period because of the availability of recent and yearly data across a range of consistent locations that are representative of annual variation, including relatively warm (e.g. 2006) and cool (e.g. 2011) summers, and because it covers the range of known wall lizard introductions in the UK. For each of the sites, soil temperatures are recorded every hour. We used the period from 15 May to 15 September in our analysis as mid-May is representative of the timing of oviposition for non-native lizards in the wild (the median lay date for females captured in the middle or end of gestation and brought to our laboratory for oviposition across years is 16 May; electronic supplementary material, table S2) and hatching past mid-September is unlikely because of the rapid decrease in daily soil temperature maximum (soil temperatures exceed 20°C—the minimum constant temperature for successful hatching in the laboratory—less than 0.3% of the time following 15 September across all years and sites in southern England). In addition, this four-month period is approximately equal to the predicted incubation duration for sites close to extant non-native populations and is in line with observations of newly hatched offspring in populations in England and at the northern native range limit ([41]; T.U. and G.M.W. 2008–2013, personal observation; T. Pashley 2000–2010, personal communication).

To generate expected hatching dates, we calculated the average proportion of time per day spent at different temperatures over this period and used this and the developmental rate data to generate predicted incubation durations for each location and year (see the electronic supplementary material for details).

3. Results

(a) Female thermal preference and soil temperature

Typical nesting sites in the introduced range are substantially cooler than is preferred by females for their nests under unrestricted thermal conditions (electronic supplementary material, table S1). We fitted thermal reaction norms for developmental rate of native Italian lizards to soil temperatures at depths representative of nesting sites in southern England (obtained from the UK Meteorological Office). This predicted incubation times well over three months even in relatively warm years, and failure to complete embryogenesis before winter in cooler years (electronic supplementary material, table S1 and figure S2).

Table 1. Recruitment of offspring into the breeding population as a function of offspring hatching date. Offspring were released in four batches corresponding to offspring hatch date. Because the last release included only 27 offspring from seven clutches, we pooled the last two release batches.

release batch	hatch dates	proportion of offspring recruited (%)
1	9 July to 15 July	16.50
2	16 July to 24 July	20.50
3	25 July to 21 Aug	7.60

(b) Effects of hatching date on recruitment into adulthood

There were strong effects of hatching date on juvenile recruitment into adulthood. Even under benign incubation temperatures that result in hatching in mid- to late summer, a two- to three-week difference in hatching translated into substantially reduced recruitment into the breeding population (logistic linear model: $\chi^2 = 7.4$, $p = 0.02$; table 1). Combined, these results suggest strong selection for earlier hatching in non-native wall lizard populations.

(c) Divergence in incubation duration between native and introduced populations

There was no difference in female snout-to-vent length between origins (native versus non-native; $F_{1,118} = 0.01$, $p = 0.92$) or lineages (French versus Italian; $F_{1,118} = 1.63$, $p = 0.20$). Females from non-native populations produced larger clutches than females from native populations, and Italian females produced larger clutches than French females (origin: $F_{1,117} = 8.73$, $p = 0.004$; lineage: $F_{1,117} = 12.96$, $p < 0.001$; snout-to-vent length: $F_{1,117} = 63.4$, $p < 0.001$). Eggs from French females were heavier than eggs from Italian females and tended to be smaller in non-native populations of both origins (origin: $F_{1,118} = 5.37$, $p = 0.022$; lineage: $F_{1,118} = 3.31$, $p = 0.072$). A total of 521 eggs were produced, of which 468 hatched. Embryonic mortality did not differ between origins ($\chi^2 = 0.35$, $p = 0.55$) or lineages ($\chi^2 = 0.02$, $p = 0.90$).

Incubation duration was strongly affected by incubation temperature, and it was significantly shorter in non-native populations of both Italian and French lineages at 20°C but not at 24°C (table 2 and figure 1). Embryos of the Italian lineage hatched sooner than embryos of the French lineage at both temperatures (table 2 and figure 1). Egg mass did not affect incubation duration at 20°C, but larger eggs had shorter incubation duration at 24°C (table 2). The shifting incubation temperature experiment confirmed shorter incubation duration for non-native animals at both 15°C and 18°C. Eggs from non-native females of Italian origin exhibited a 12.7 ± 0.33 day delay in incubation duration when held at 15°C for 14 days (compared to eggs held at a constant 28°C)

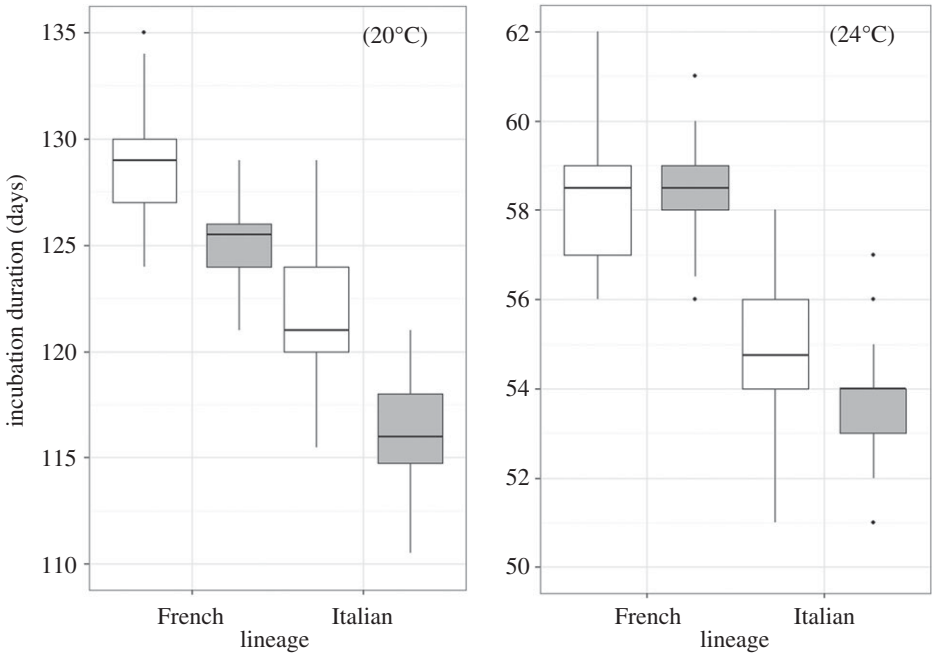

Figure 1. Incubation duration of eggs kept at constant 20°C or 24°C from female wall lizards of French and Italian lineages sampled from native (white fill) and non-native (grey fill) populations.

Table 2. Output from linear models examining the effect of lineage and origin on the incubation duration of eggs kept at 20°C or 24°C. Statistically significant *p*-values are in bold. Main effects reported from model excluding interaction.

	20°C			24°C		
factor	d.f.	*F*	*p*	d.f.	*F*	*p*
lineage	1,96	151.77	**<0.001**	1,99	197.23	**<0.001**
origin	1,96	47.41	**<0.001**	1,99	3.07	0.08
egg mass	1,96	0.83	0.36	1,99	4.27	**0.04**
lineage × origin	1,95	1.68	0.19	1,98	0.74	0.39

compared with a delay of 13.6 ± 0.14 days for eggs from native females ($F_{1,36} = 7.91$, $p = 0.007$). The same pattern was observed at 18°C (non-native populations = 10.9 ± 0.21 day delay; native populations = 11.5 ± 0.18 day delay; $F_{1,36} = 4.14$, $p = 0.049$).

Embryos from non-native populations were significantly more advanced at oviposition compared with embryos from native populations, and this pattern was consistent for both lineages ($F_{1,55} = 11.10$, $p = 0.002$; figure 2). Differences in embryonic stage are unlikely to be explained by exposure to higher temperature before oviposition as there was no difference in gestational body temperature of non-native and native females (tested in the Italian lineage only: $\chi^2 = 0.92$, $p = 0.34$). Heart rate showed a more complex pattern, with a predicted higher heart rate in non-native populations of the French lineage compared with their native populations, but no difference in embryos from the Italian lineage. This lineage-by-origin interaction was significant at 20°C ($F_{1,86} = 7.64$, $p = 0.007$; figure 3) but failed to reach statistical significance in the 24°C treatment ($F_{1,85} = 3.71$, $p = 0.057$; figure 3). Refitting models of incubation duration with embryonic stage at oviposition as an additional predictor confirmed that it significantly contributed to the shorter developmental time at both 20°C ($F_{1,49} = 14.23$, $p < 0.001$) and 24°C ($F_{1,52} = 16.72$, $p < 0.001$). This was not the case for heart rate, which failed to significantly predict incubation duration at either temperature in refitted models ($F_{1,81} = 1,38$, $p = 0.24$ and $F_{1,84} = 1.89$, $p = 0.17$, respectively). In both cases, the difference between non-native and native populations at 20°C remained statistically significant ($p < 0.001$). Finally, Italian offspring were smaller than French offspring ($F_{1,105.71} = 19.52$, $p = 0.001$) and both lineages had smaller offspring at 20°C compared with 24°C ($F_{1,65.96} = 135.32$, $p < 0.001$), but offspring from non-native populations hatched at a similar size to offspring from native populations ($F_{1,104.98} = 1.56$, $p = 0.21$).

(d) Consequences for the timing of hatching

To estimate the consequences of these responses for the timing of hatching, we modelled the predicted incubation duration of non-native and native lizards (of Italian origin) based on naturally fluctuating soil temperatures in sites representative of nesting locations across 34 sites in southern England. Predicted hatch dates for non-native eggs were one to three weeks earlier compared with the ancestral state, which greatly increased

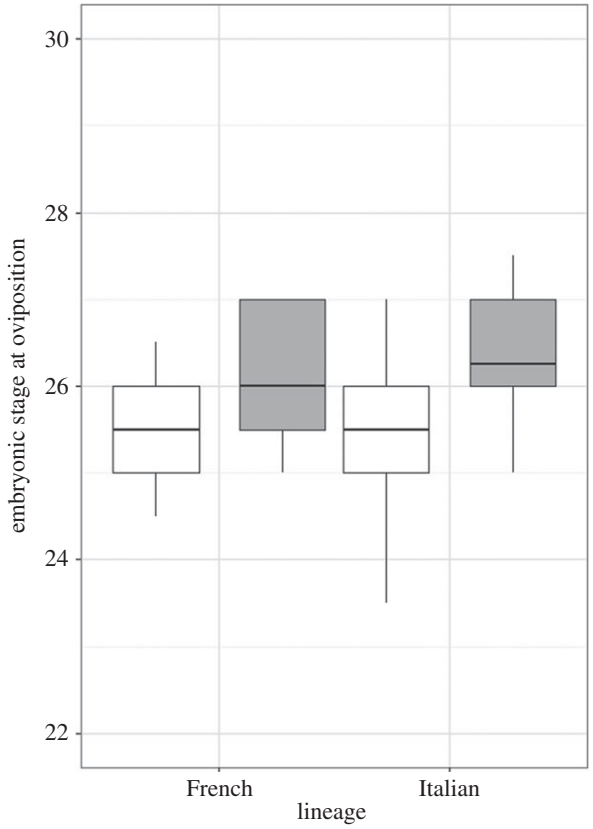

Figure 2. The embryonic stage of development at oviposition for eggs laid by French and Italian female wall lizards from both native (white fill) and non-native (grey fill) populations.

the chances of completing embryogenesis before autumn (figure 4).

4. Discussion

Our common-garden experiments demonstrate parallel reduction in incubation duration of embryos in wall lizard populations following independent introduction to cooler climate. This is consistent with adaptive evolutionary responses to the relatively cool nest temperatures in the introduced range, which necessitates sustained development at temperatures well below 24°C to complete embryogenesis (only 3% of soil temperature recordings at soil depths representative of nests in southern England are above 24°C for the relevant incubation period, i.e. from 15 May to 15 September). While embryo retention and faster developmental rate have evolved repeatedly in lizards in cool climates [21–24], our results demonstrate that such adaptations can arise very rapidly. Extant wall lizard populations in England were introduced less than 100 years ago, with the target populations tracing their origins back to between two and eight decades [15]. Thus, the results are consistent with recent evidence that geographical clines in introduced insects and plants can evolve within tens of generations (e.g. [42,43]), and with studies of vertebrates that have demonstrated adaptive divergence across a similar number of generations [2,3,5,44–47].

The reduction in incubation duration in non-native wall lizards appears to have multiple causes. Embryos in non-native populations are more advanced at the time of egg laying, which reduces the overall time to complete embryogenesis in the nest. We can rule out facultative egg retention as all females were housed under identical conditions and there was no difference in selected body temperature between native and introduced females (in addition, previous work has failed to experimentally demonstrate plasticity in egg retention in *P. muralis* [30], but see [24] for evidence from the skink *Bassiana duperreyi*). However, egg retention cannot fully account for the reduction in incubation duration in non-native populations, nor does it account for the difference between lineages, as both lineage and origin explained significant amounts of variation even when the differences in embryo stage were controlled for statistically. Embryos from non-native populations must therefore also develop faster at and below 20°C. Interestingly, there was no, or limited, increase in development rate at 24°C and 28°C. These responses thus demonstrate adaptive evolution of the slope and curvature of thermal reaction norms, which appears to be common for population divergence in plasticity [48].

What are the mechanisms underlying faster developmental rate in non-native populations? Eggs were somewhat smaller in non-native populations, but egg size cannot explain differences in incubation time between non-native and native populations (in fact, larger eggs hatched earlier at 24°C). Other maternal effects on yolk composition could be involved and the relative contribution to changes in yolk nutrients versus intrinsically upregulated metabolism in embryos warrants further study. The increased heart rate of embryos from non-native populations of the French lineage suggests that a faster development may partly be owing to increased cardiac output [32]. This mechanism has been shown to account for faster developmental rate at high latitudes in *Sceloporus* lizards [25]. However, heart rate itself was a poor predictor of incubation duration across our populations and lineages, suggesting that the divergence between non-native and native populations in the rate of development is not well explained by such simple estimates of nutrient and O_2 delivery to developing tissues. The same applies to differences between lineages. Data from Spanish populations close to the presumed ice age refugia for the French lineage have even slower developmental rates at cool temperatures than our native French populations [39], suggesting that there may be a phylogenetic signal to developmental rate, which persists in non-native populations.

By predicting incubation duration of non-native and native lizards from soil temperatures across the introduced range, we show that the combined effects of egg retention and faster embryonic growth should lead to a one- to three-week earlier emergence compared with the ancestral state. Even two-weeks earlier hatching, which is a common prediction from the data, can make the difference between successful hatching and failing to hatch before the onset of autumn. Our recapture data show that this also constitutes a substantial (e.g. twofold) increase in survival after emergence (see also [30]). This increased survival of early hatched offspring could be the result of several non-mutually exclusive mechanisms, including increased opportunity for growth and production of fat bodies prior to hibernation ([49] see also [50]), positive effects of high embryonic temperature on physiological and morphological traits [51,52] or greater ability to capitalize on seasonally available food sources [53]. Earlier emergence and long-term persistence of non-native populations could be further enhanced if non-native lizards also initiated reproduction earlier than their native counterparts. However, the extent to which there may have been corresponding responses in

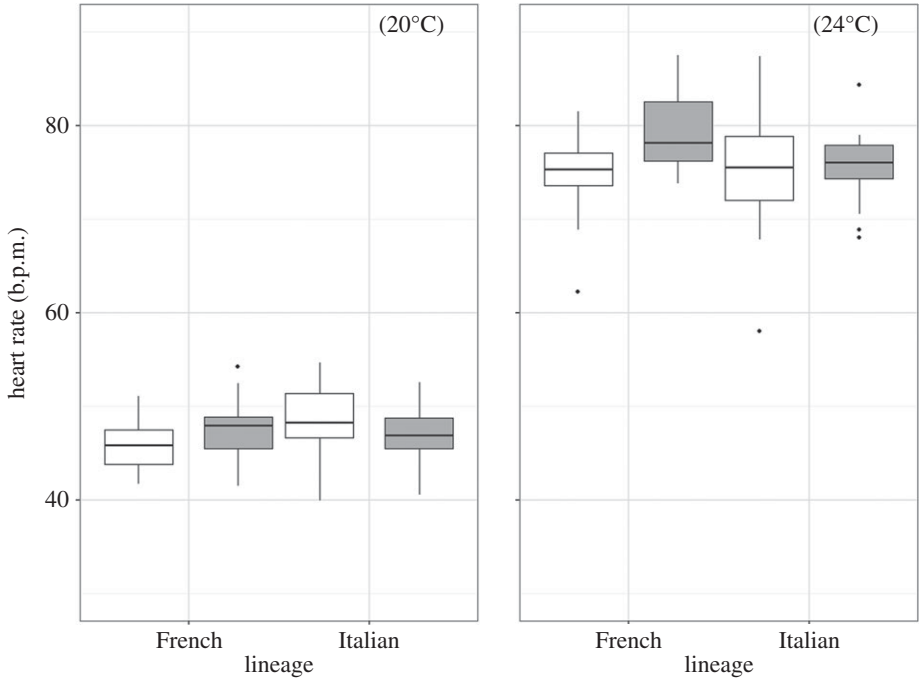

Figure 3. The heart rate (b.p.m.) of developing embryos of eggs kept at a constant 20°C or 24°C from female wall lizards of French and Italian lineages sampled from native (white fill) and non-native (grey fill) populations.

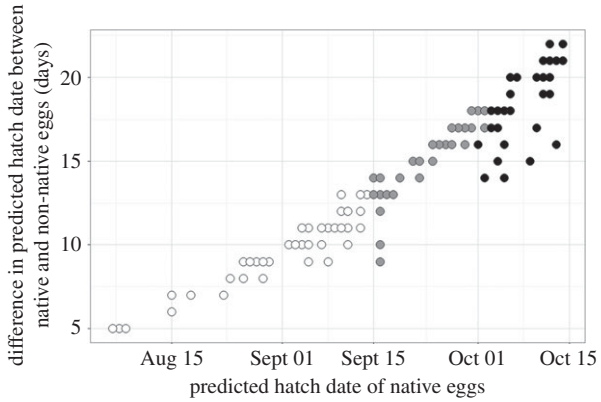

Figure 4. The predicted difference in the timing of hatching of eggs from native versus non-native wall lizard populations of the Italian lineage as a function of the estimated timing of hatching for native developmental rates. Estimates are based on soil temperatures across 34 sites in southern England over a 10-year period and a laying date of 15 May (data truncated at 15 Oct). Assuming hatching is unlikely past 15 Sept (see the electronic supplementary material), white dots represent soil temperatures that would allow successful hatching for both native and introduced lizards, grey dots represent soil temperatures that would allow successful hatching only for lizards from introduced populations and black dots represent soil temperatures that would not allow hatching for either native or introduced lizards.

female characters that promote egg laying early in spring in non-native populations is currently unknown.

The shorter incubation duration should increase short- and long-term persistence of non-native populations and may enable expansion into areas that would be unattainable with incubation durations representative of the native range. A time delay between introduction and range expansion appears to be a common pattern in biological invasions and recent studies of non-native insects and plants have demonstrated how adaptive divergence can facilitate spread into environments that were previously too stressful [8,9]. Wall lizards in England show limited natural dispersal [15] but the ability to recruit from nests with less benign thermal profiles may contribute to their expansion in several locations. Non-native populations with shorter incubation duration may also serve as sources for new introductions and hence make human-mediated range expansion more likely [54].

In summary, prolonged embryo retention and faster embryonic growth at low temperatures in non-native wall lizards suggest rapid adaptation following introduction to a cool climate. We show that these responses have significant effects on recruitment and hence are likely to contribute to the survival and eventual range expansion of the species in its introduced range.

Ethics statement. All work was approved by the University of Oxford's Local Ethical Review Process and the UK Home Office (PPL: 30/2560).

Data accessibility. All data associated with this MS will be made available on dryad (doi:10.5061/dryad.pd351).

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Author contributions. T.U. and G.M.W. conceived of and managed the project, collected and analysed data, and wrote the paper. J.W., G.P., T.H., B.F., B.H. and S.M. collected data for different parts of the project, and N.J.B. assisted with analysis of climatic data. All authors commented on the paper.

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Conflict of interests. We have no competing interests.

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APPENDIX B

ECOLOGY LETTERS

Ecology Letters, (2015) doi: 10.1111/ele.12531

LETTER

Sexual selection drives asymmetric introgression in wall lizards

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Abstract

Hybridisation is increasingly recognised as an important cause of diversification and adaptation. Here, we show how divergence in male secondary sexual characters between two lineages of the common wall lizard (*Podarcis muralis*) gives rise to strong asymmetries in male competitive ability and mating success, resulting in asymmetric hybridisation upon secondary contact. Combined with no negative effects of hybridisation on survival or reproductive characters in F1-hybrids, these results suggest that introgression should be asymmetric, resulting in the displacement of sexual characters of the sub-dominant lineage. This prediction was confirmed in two types of secondary contact, across a natural contact zone and in two introduced populations. Our study illustrates how divergence in sexually selected traits via male competition can determine the direction and extent of introgression, contributing to geographic patterns of genetic and phenotypic diversity.

Keywords

Female choice, hybridisation, introgression, lizards, male–male competition.

Ecology Letters (2015)

INTRODUCTION

Gene transfer between species, sub-species or genetic lineages via hybridisation is increasingly recognised as an important cause of diversification and adaptation (Arnold 2007; Abbott *et al*. 2013; Hedrick 2013). Because hybridisation does not necessarily lead to an even mix of genetic and phenotypic characters of the parental lineages, it can cause new characters to arise or existing characters to be unequally transferred between lineages. This may be particularly likely when phenotypes that have diverged in allopatry confer a fitness advantage to one lineage upon secondary contact, making it advantageous for the other lineage to express the same characters. For example, expression of hetero-specific characters can have a survival advantage, which has been suggested to explain introgression of wing patterns between *Heliconius* butterflies (Pardo-Diaz *et al*. 2012), pest resistance in mice (Song *et al*. 2011) and the evolution of climate adaptation and herbivore resistance in sunflowers (Whitney *et al*. 2006, 2010). Alternatively, characters that confer a reproductive advantage in the competition for mates can enhance hybridisation rates as well as provide hybrids with a selective advantage relative to subdominant pure-bred competitors. In the absence of severe genetic incompatibilities, this may enable secondary sexual characters to rapidly spread from one lineage to another (Parsons *et al*. 1993; Prado *et al*. 2009; Baldassarre *et al*. 2014).

Sexually selected hybridisation has primarily been studied with respect to female choice. While female choice will often restrict gene flow (e.g. Saetre *et al*. 1997; Seehausen *et al*. 2008), increasing evidence suggests that it can also lead to asymmetric rates of hybridisation and introgression of male sexual characters (Wirtz 1999; Stein & Uy 2006; Pfennig 2007). For example, in a hybrid zone between the golden-collared (*Manacus vitellinus*) and white-collared (*Manacus candei*) manakins, females prefer golden-collared males on mixed leks, which results in asymmetric introgression of golden plumage colouration across the hybrid zone (Parsons *et al*. 1993; Stein & Uy 2006). In contrast, evidence that divergence in sexual characters conferring an advantage in male–male competition can promote asymmetric gene flow between lineages is very limited (Hedrick 2013). This is despite that competition between males for resources is important for the evolution of character displacement and reproductive isolation (Grether *et al*. 2013), and hence features frequently in speciation theory (Price 2008). Behavioural experiments suggest that competitive exclusion of males of the sub-dominant lineage may contribute to the golden-collared male mating advantage at mixed leks in manakins (McDonald *et al*. 2001), cause differences in the frequency of hetero-specific pairings between pied and

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collared flycatchers (Vallin *et al*. 2012) and between hermit and Townsend's warblers (Pearson 2000). However, even in these relatively well-studied systems the link between intraspecific sexual selection and genetic and phenotypic introgression remains largely circumstantial.

We studied how behavioural interactions as well as post-copulatory and post-zygotic reproductive isolation influence gene flow between two phenotypically distinct lineages of the common wall lizard, *Podarcis muralis*. This species has formed a number of genetic lineages in Southern Europe reflecting isolation in ice age refugia (Salvi *et al*. 2013). Our focus was on lizards native to Western Europe, which correspond morphologically to the *P. muralis brongniardii* subspecies, and on lizards native to northern Italy (Tuscany), which correspond morphologically to the *P. muralis nigriventris* subspecies (Böhme 1986). These lineages now form a natural contact zone in Liguria (northwestern Italy, see Results) and have also come into secondary contact more recently as a result of human introductions in both Germany and southern England (Schulte *et al*. 2012a; Michaelides *et al*. 2013).

We used an experimental approach to generate predictions regarding how natural and sexual selection should influence the direction of introgression, followed by genetic and phenotypic analyses in all three regions of secondary contact to test these predictions. First, we conducted an extensive phenotyping of lizards from the two main lineages in allopatric native populations to establish the extent to which they exhibit divergence in sexually selected characters. Second, we used experimental populations in outdoor enclosures to test whether such differences translate into an asymmetry in male dominance and realised hybridisation upon secondary contact. Third, we assessed the reproductive compatibility of between lineage crosses and the survival and reproductive competence of F1 hybrids. Finally, we made use of these data to predict the direction of introgression, which we tested using mitochondrial DNA, microsatellites and phenotypic data across three locations (one native and two introduced) of hybridisation.

METHODS

Common wall lizards are small, 45–75 mm snout-to-vent length, diurnal lizards that inhabit a range of natural and anthropogenic habitats. We studied native populations in France, western Germany, and northwestern Italy, which collectively belong to a mitochondrial lineage that we here refer to as the western European lineage, and populations in northern Italy (Tuscany), which we refer to as the Italian lineage (Schulte *et al*. 2012a). The data in this paper also involve 11 introduced populations in England of single native origin, and two introduced populations of mixed origin (i.e. presence of animals of both western European and Italian origin or hybrids), one in England and one in Germany. Further details on the populations are found in Table S1.

Character divergence in allopatry

We collected morphological and colouration data on 793 animals from 31 native populations of pure Western European and Italian origin. We captured all lizards by noosing, weighed them to the nearest 0.01 g and measured their snout-to-vent length, total length, head length and head width to the nearest mm. Using photographs, animals were scored for ventral (blackness) and dorsal (greenness) colouration. Ventral blackness was scored by quantifying the proportion of black to non-black pixels on each lizard's chest (Fig. S1). Dorsal greenness was scored based on an intensity scale from 1 to 10 (1 being *pure brown*, 10 being *pure green*, Fig. S2), which was confirmed to be highly correlated with scores from digital photographs analysed in Photoshop CS4 and with values for green chroma extracted using spectrophotometry [see Supporting Information (SI) for full details]. We also collected data on bite force and male testes mass, which are both commonly under sexual selection in lizards (Olsson & Madsen 1998; see SI for full details and sample sizes).

Patterns of dominance, courtship and paternity upon secondary contact

To generate predictions regarding the direction of hybridisation, we carried out two separate experiments using outdoor enclosures (7 × 7 m), designed to simulate conditions during secondary contact. Each enclosure was fitted with suitable habitat (bricks, wooden pallets) and stocked with 16 animals, four males and four females of each lineage (with the exception of four enclosures in 2010; see SI for details). In 2010, 10 enclosures were stocked with a total of 160 animals of either Italian or Western European origin sourced from introduced populations in England. In 2013, we conducted a similar experiment using eight enclosures stocked with a total of 128 animals captured from native populations in western France or Tuscany. In both experiments, we collected individuals from multiple populations (n = 10 in 2010 and n = 7 in 2013). To reduce population-of-origin effects, animals from the same source population were distributed among all enclosures as evenly as possible. Within this constraint the location of each individual was assigned randomly. The two experiments differed slightly in the distribution of habitat within enclosures, but followed the same protocol for data collection (see SI for full details).

Individuals were captured from the wild prior to females laying their first clutch and were transported to the laboratory. The experiments were conducted following oviposition of the first clutch (females typically lay at least two clutches per season). All individuals of each sex were released into a given enclosure at the same time. Males were released first to allow them to establish territories, followed by females (~ 7 days between the release of males and females). Females released more than 3 days after oviposition were kept cool (~ 10 °C) during this period to avoid progression through the next ovulation cycle. Behavioural interaction data were obtained throughout the experiment from rotating 45 min observation periods per enclosure, conducted by three (in 2010) or two (in 2013) observers in an ethogram (Table S2, see SI for full details). This resulted in a total observation period of ~ 510 h in 2010 and ~370 h in 2013.

Once females were ready to lay, all individuals were recaptured and returned to cages in the laboratory. Cages were inspected in the morning and late afternoon for signs of egg

laying. Eggs were incubated at a constant 24 °C (2010) or 28 °C (2013) in standard refrigerated incubators fitted with water baths to maintain humidity. At hatching, offspring were euthanised (using concussion followed by permanent destruction of the brain) and their tissues used for genetic analysis. DNA was isolated from tail–tip tissue using standard protocols (see SI for full details). Paternity was assigned using microsatellites (Table S3) in CERVUS v 3.0 (Marshall *et al.* 1998) based on the trio (mother, father, and offspring) LOD score and a strict confidence level of 95%.

To confirm whether these patterns were the result of pre- as opposed to post-copulatory mechanisms, we carried out 16 sperm competition trials in the laboratory in which Western European ($n = 6$) and Italian ($n = 10$) females were mated to males of both their own and the other lineage. All trials were carried out in the same type of terrarium used for housing the animals (see above) in the first 5 days following oviposition, which corresponds to the female receptive period under laboratory conditions. Females were introduced to the terrarium and allowed to acclimatise for 20 min after which one of the males was introduced to the female. Once mated (all within an hour) that male was removed and the second male was immediately introduced (all also mated within an hour). The order of males with respect to lineage was reversed each trial. Offspring were genotyped along with their mother and the two potential fathers as described above.

Fertility and viability of F1 hybrids

To test for decreased hybrid fitness we carried out 62 crosses between males and females of the two lineages. We introduced a male of either the same lineage or the other lineage into a female cage 3 days after she had laid her first clutch and left them together for 5 days. Eggs were collected following oviposition, scored for infertility based on presence and calcification of the egg shell (Olsson & Shine 1997) and incubated at 24 °C. Embryonic mortality was scored and assessed using dissection of eggs that did not show any evidence of heart beat (using a digital egg monitor: Buddy; Avitronics, Truro, England). Ninety-six offspring from these crosses were raised to maturity under laboratory conditions. After reaching mature size (~ 5 months after hatching) they were hibernated for 10 weeks at 4 °C. For logistical reasons we were unable to conduct further crosses to establish a F2 generation and therefore assessed reproductive capacity under captive conditions for a subset of animals. We recorded whether or not females produced eggs within 2 months of emergence of hibernation and the resulting clutch size. We also recorded the testes mass of 23 males. These characters should reflect fertility of F1 hybrids, but it should be noted that it does not establish sperm characteristics in males and that incompatibilities may not be evident until the F2 generation; our data may therefore underestimate genetic incompatibilities in hybrids.

Statistical analyses

All data were analysed using R version 3.0.3 (R Development Core Team 2010). We used linear (mixed) models to analyse differences between lineages and sexes in phenotypic characters in both wild-caught animals and experimental crosses and to establish patterns of behaviour and parentage in the experimental enclosures. Detailed description of all models can be found in the SI.

Genetic and phenotypic patterns in regions of secondary contact

We examined phenotypic and genetic patterns of introgression within three separate regions of secondary contact between the Italian and Western European lineage. In the native hybrid zone, we sampled 17 populations from central Tuscany (where animals are known to fall within the Tuscan haplotype lineage; *sensu* Schulte *et al.* 2012a; and exhibit *P. m. nigriventris* phenotypes) to western Liguria (where animals are known to belong to the western European haplotype lineage and exhibit typical *P. m. brongniardi* phenotype) (Böhme 1986) (Fig. 1). Second, we sampled 27 animals from a location in England (Holmsley) that is known to have both Italian and Western European origins (Michaelides *et al.* 2013). Third, we sampled 203 animals from a population in south-western Germany (Mannheim) where animals from the Italian lineage have been introduced in a region where the western European lineage is native (Schulte *et al.* 2012b). In each of these populations, we recorded traits as described above and removed ~ 5 mm of the tail or took buccal swabs for DNA analyses.

In the native hybrid zone, we tested predictions regarding the direction of gene flow using a geographic cline approach (Szymura & Barton 1986; Gay *et al.* 2008). Because microsatellite loci were highly variable and showed few private alleles, typically at low frequencies, we estimated the nuclear genetic cline from a Bayesian hybrid index (HI) based on allele frequencies at all loci using the programme STRUCTURE v 2.3.4 (Pritchard *et al.* 2000). Because phylogeographic studies have established two lineages in this geographic region we conducted all analyses assuming two genetic clusters (i.e. $K = 2$). The simulations, using the admixture model, run with a burn-in of 10^5 iterations and a further run length of 10^6 iterations. Runs were replicated five times and combined using CLUMPP (Jakobsson & Rosenberg 2007). We used the probability that an individual was assigned to the Italian cluster (Q) as our hybrid index. The hybrid index was subsequently used to assign individuals as either pure Western European ($Q \leq 0.1$), pure Italian ($Q \geq 0.9$) or hybrid ($0.1 < Q < 0.9$) (e.g. Baldassarre *et al.* 2014). We also fitted the corresponding cline for haplotypes based on the cytochrome *b* mitochondrial gene. Phenotypic clines using population averages were fitted for three traits: dorsal greenness, ventral blackness and relative head length. These are all quantitative characters with large and well-established differences between lineages (greater in the Italian lineages, e.g. Böhme 1986, Fig. S3; see Results). Relative head length was calculated as the residual score from a regression of head length on snout-to-vent length. Clines were treated separately for males and females.

Genetic and phenotypic clines were fitted using the Metropolis-Hastings Markov chain Monte Carlo algorithm implemented in the package hzar in R version 3.0.3 (Derryberry *et al.* 2014). For the genetic analyses we ran two sets of five models. Each model estimated cline centre (cumulative distance from sampling location Colle di Val D'Elsa in Tuscany, c) and width (1/maximum slope, w), but could also fit

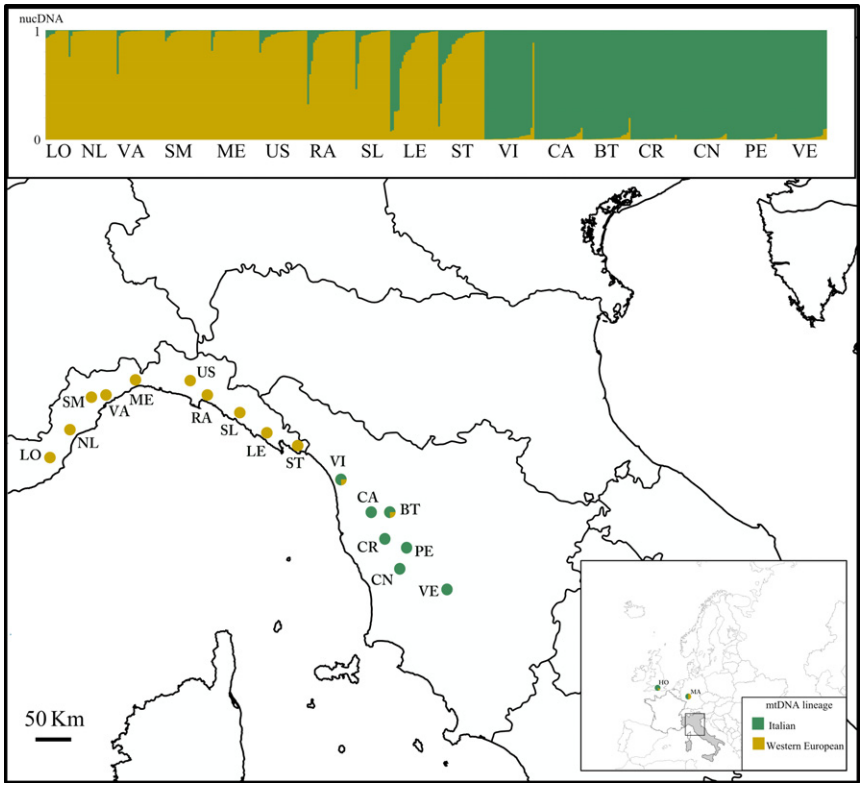

Figure 1 Map of the native hybrid zone in northern Italy. We used 17 populations that created a cline from mid Tuscany up the Ligurian coast. The colour of the dots indicates the association with a particular mitochondrial lineage. Two populations in the middle of the cline contained a mix of haplotypes (VI and BT). At the top of the figure a STRUCTURE output indicates the extent of admixture (using microsatellite nuclear DNA) within these populations as a function of distance from the far western end of the cline (running left to right). Insert shows the location of the geographic region and the two hybrid regions in England (Holmsley) and Germany (Mannheim).

different combinations of the exponential decay curve parameters δ and τ (none, right tail only, left tail only, mirrored tails, or both tails separately). One set of models fixed the cline ends at the empirically observed values, whereas the other set also estimated these values from the data. We also ran the corresponding models for each of the phenotypic traits, but because of small sample size (< 10) for the Westernmost population, the models with fixed cline ends used the value of the closest population (i.e. Noli, NL). For each of the clines we compared models based on the AIC corrected for small sample size (AICc) and selected the model with the lowest AICc as the best-fitting model. The coincidence of cline centres for mtDNA vs. ncDNA, and for ncDNA vs. phenotypic clines was assessed using the maximum-likelihood derived confidence intervals, where we considered non-overlapping confidence intervals as statistically supported differences in cline location. We verified the conclusions by re-fitting models that constrained the cline centre to correspond to that of the cline to which it was compared.

For the two non-native populations we tested for the presence of hybrids and the direction of hybridisation. First, we conducted a principle coordinate analysis (PCoA) to visualise pair-wise individual multi-locus genetic distance calculated in GenAlEx (Peakall & Smouse 2012). For the non-native population in England, we included four non-native populations of pure origin (three of Italian and one of Western European origin) that served as source populations (Michaelides *et al.* 2015). For the non-native population in Germany (Mannheim) we did not include reference populations as the exact origins are unknown. Instead, we pooled individuals into two groups based on the lineage assignment from the cytochrome *b* gene (Western European and Italian). Second, we conducted Bayesian assignment tests to identify individuals of mixed origin. We used the admixture model as implemented in STRUCTURE to assign individuals as either pure Western European ($Q \leq 0.1$), pure Italian ($Q \geq 0.9$) or hybrid ($0.1 < Q < 0.9$). We also did the corresponding analysis in the programme NewHybrids (Anderson & Thompson 2002), which computes posterior probabilities of individual assignment into different categories of hybrids based on their multilocus genotypes (F1 or F2). We used uniform priors with 10^5 sweeps before and 10^6 sweeps after burn-in. The direction of hybridisation was assessed by comparing the outcome of these assignment tests to the mitochondrial haplotype.

RESULTS

Character divergence in allopatry

Lizards from the Italian lineage displayed the characteristic green dorsal and black ventral colouration typically ascribed to *P. m. nigriventris* and had larger heads, stronger bite

force and greater testes mass (Table S4; Fig. 2). Sexual dimorphism was generally greater in the Italian lineage (Table S4, Fig. 2).

Patterns of paternity upon secondary contact

We found highly consistent results across both experiments. Italian males were strongly dominant over Western European males, winning more agonistic interactions (permutation test using QAP – 2010, lineage: $P = 0.019$, snout-to-vent length: $P = 0.012$, QAP – 2013, lineage: $P < 0.001$, snout-to-vent length: $P = 0.05$; Fig. 3a). Across both experiments, dorsal greenness, ventral blackness and head length, phenotypic characters that are exaggerated in Italian males, were all strong phenotypic predictors of dominance (Table S5).

Italian males courted significantly more females (Table S6; Fig. 3b), and had higher reproductive success overall and with females of the opposite lineage, than Western European males (Table S6; Fig. 3c). Accordingly, Western European females produced a significantly higher proportion of hybrid offspring compared to Italian females (Table S6; Fig. 3d). Rerunning models including dominance as a predictor suggested that differences in reproductive success between Italian and Western European males were well explained by dominance and hence consistent with male–male competition (Table S7).

Males were more likely to sire offspring with females from their own lineage under sperm competition (intercept: $\chi^2 = 8.45$, d.f. = 1, $P < 0.01$), but there was no statistical support for a bias in reproductive success with females from the other lineage between Western European and Italian males (proportion of hybrids in Italian clutches = 0.21 ± 012, proportion of hybrids in Western European clutches = 0.42 ± 0.20, lineage: $\chi^2 = 0.22$, d.f. = 1, $P = 0.64$).

Fertility and viability of F1 hybrids

Embryonic mortality was not higher for between-lineage crosses (17%) compared to within-lineage crosses (16%) (male lineage: $\chi^2 = 3.19$, $P = 0.07$, female lineage: $\chi^2 = 3.19$, $P = 0.43$, male lineage × female lineage: $\chi^2 = 0.42$, $P = 0.51$). Animals of pure Western European origin had slower growth rates and were smaller following hibernation than animals of Italian and hybrid origin (Western European offspring = 47.3 ± 1.19 mm, Italian offspring = 52.5 ± 0.78 mm, Hybrid offspring = 51.6 ± 0.64 mm, cross: $\chi^2 = 245.3$, $P < 0.001$, sex: $\chi^2 = 16.8$, $P = 0.32$). We found no significant difference between the crosses in testes mass for a given body size (Western European males = 0.017 ± 0.01 g, Italian males = 0.035 ± 0.01 g, Hybrid males = 0.031 ± 0.01, cross: $\chi^2 = 1.81$, $P = 0.24$, snout-to-vent length: $\chi^2 = 0.07$, $P = 0.72$). Captive-reared females of French origin did not reproduce, but female hybrids were as likely to reproduce as pure-bred Italian females (36% of Italian females reproduced vs. 45% of hybrid females: $\chi^2 = 0.03$, $P = 0.85$), and there was no significant difference in clutch size (Italian females = 3.20 ± 0.58, Hybrid females = 3.55 ± 0.17: $\chi^2 = 0.51$, $P = 0.48$).

Genetic and phenotypic patterns in regions of secondary contact

The results above predict that introgression should be male-driven and asymmetric from the Italian lineage into the

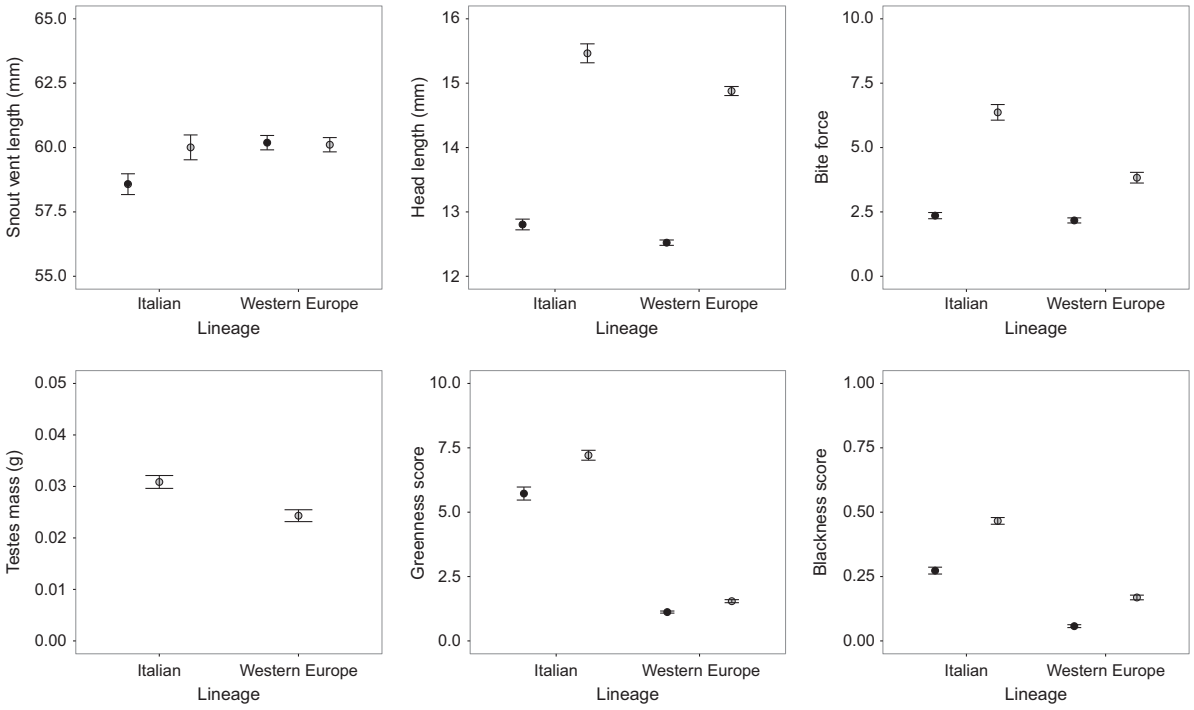

Figure 2 Means ± SE for Western European and Italian animals in morphological and colour phenotypes. Black dots indicate females and grey dots indicate males.

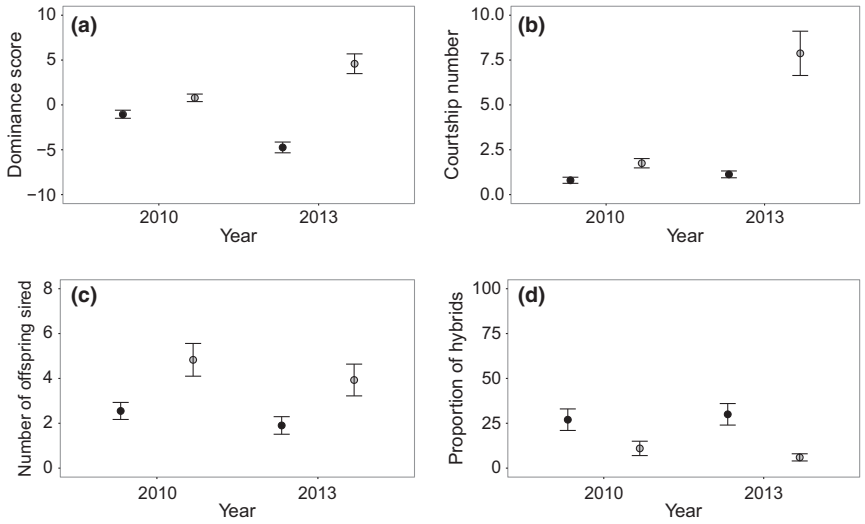

Figure 3 Means ± SE for Western European and Italian lizards in male dominance, number of courtships males initiated, the number of offspring males sired and the proportion of a females clutch that were hybrids. All outputs are the result of our experimental secondary contact zone experiments carried out with non-native (2010) and native (2013) lizards. Black dots indicate Western European lizards and grey dots indicate Italian lizards. Note that the higher reproductive success of Italian males (panel c) is the result both of a greater number of clutches with paternity assignment for Italian females and higher reproductive success with females of the opposite lineage (panel d).

Western European lineage. As predicted, the location of the geographic cline for microsatellites was shifted westwards compared to the cline for mtDNA (Table 1; Fig. 4). A microsatellite cline with its centre constrained to that of the mtDNA cline provided a significantly worse fit to the data (ΔAICc = 43.1). The locations of both genetic clines were significantly different from the cline centres for all three phenotypic traits (dorsal greeness Fig. 4, ventral blackness and relative head length Fig. S4), which were shifted even further to the west (Table 1). Notably, we failed to identify significant levels of genetic admixture for several of the western-most populations with Western European haplotypes (e.g. US, RA, SL; Fig. 1) that were phenotypically very similar to populations identified as being of pure Italian origin (Fig. 4). The best-fitting models for the phenotypic clines differed between traits and, for black ventral colouration, between the sexes (Table S8). There was a significant correlation between genetic differentiation and geographic distance ($r^2 = 0.70$, $P < 0.001$, Fig. S5).

STRUCTURE assigned all individuals in the putative hybrid population in southern England as being of pure Italian origin despite four individuals having mtDNA haplotypes from Western Europe (Fig. S6). The results were corroborated by the output from NewHybrids in which all individuals were assigned as being pure Italian (Table S9) and the PCoA in which hybrid individuals were found within the cluster of pure Italian individuals (Fig. S7). In Mannheim, STRUCTURE identified 23 out of 203 individuals as hybrids ($0.1 < Q < 0.9$; Fig. S8) and NewHybrids tended to classify these as being F2 hybrids (e.g. F1 × F1 hybrids; Table S11). Eight hybrid individuals in Mannheim harboured Italian mtDNA haplotypes and the rest (15) had mtDNA haplotypes from Western Europe (Table S10). The PCoA placed these within and/or between the clusters of pure individuals (Fig. S9).

Table 1 Parameter estimates for best-fitting cline models for genetic and phenotypic clines using *HZAR* (Derryberry *et al*. 2014). Parameter *c* indicates the estimated cline centre (distance from sampling location VE in Tuscany) and *w* indicates the cline width (1/maximum slope). The parameters *pmin* and *pmax* indicate the allele frequencies at the ends of the cline for genetic markers and the corresponding values for phenotypic markers (transformed values to the second decimal point), and δ and τ are exponential decay curve parameters for the left and right tails. Two log-likelihood unit support limits are presented in parentheses. Note that the very high introgression of phenotypic characters makes the parameter estimates for the fit in the western part of the cline unreliable (see Fig. 4; Fig. S4)

Character	Sex	Best model	*c* (km)	*w* (km)	*pmin*	*pmax*	δL	τL	δR	τR
mtDNA		Model I	61.2 (56.0–68.8)	29.9 (18.3–50.3)	0	1	None	None	None	None
Hybrid Index		Model VII	100.5 (88.7–118.7)	15.2 (1.2–50.2)	0	1	None	None	2.37 (0.0–18.2)	0.131 (0.011–0.705)
Greenness	M	Model II	273.0 (254.3–278.0)	76.1 (56.2–93.6)	1.44	2.71	None	None	None	None
Greenness	F	Model II	228 (225.7–230.0)	5.1 (3.7–5.9)	0.07	1.82	None	None	None	None
Blackness	M	Model II	156.2 (124.3–279.8)	105.7 (20.1–309.3)	0.18	0.45	None	None	None	None
Blackness	F	Model VIII	226.6 (219.7–231.5)	22.1 (14.7–56.7)	0.06	0.23	None	None	292.4 (1.2–307.4)	0.743 (0.006–0.972)
Head length	M	Model I	213.2 (207.2–222.8)	14.8 (0.1–30.2)	−0.38	0.14	None	None	None	None
Head length	F	Model I	227.5 (215.2–238.9)	62.6 (25.4–125.7)	−0.72	0.08	None	None	None	None

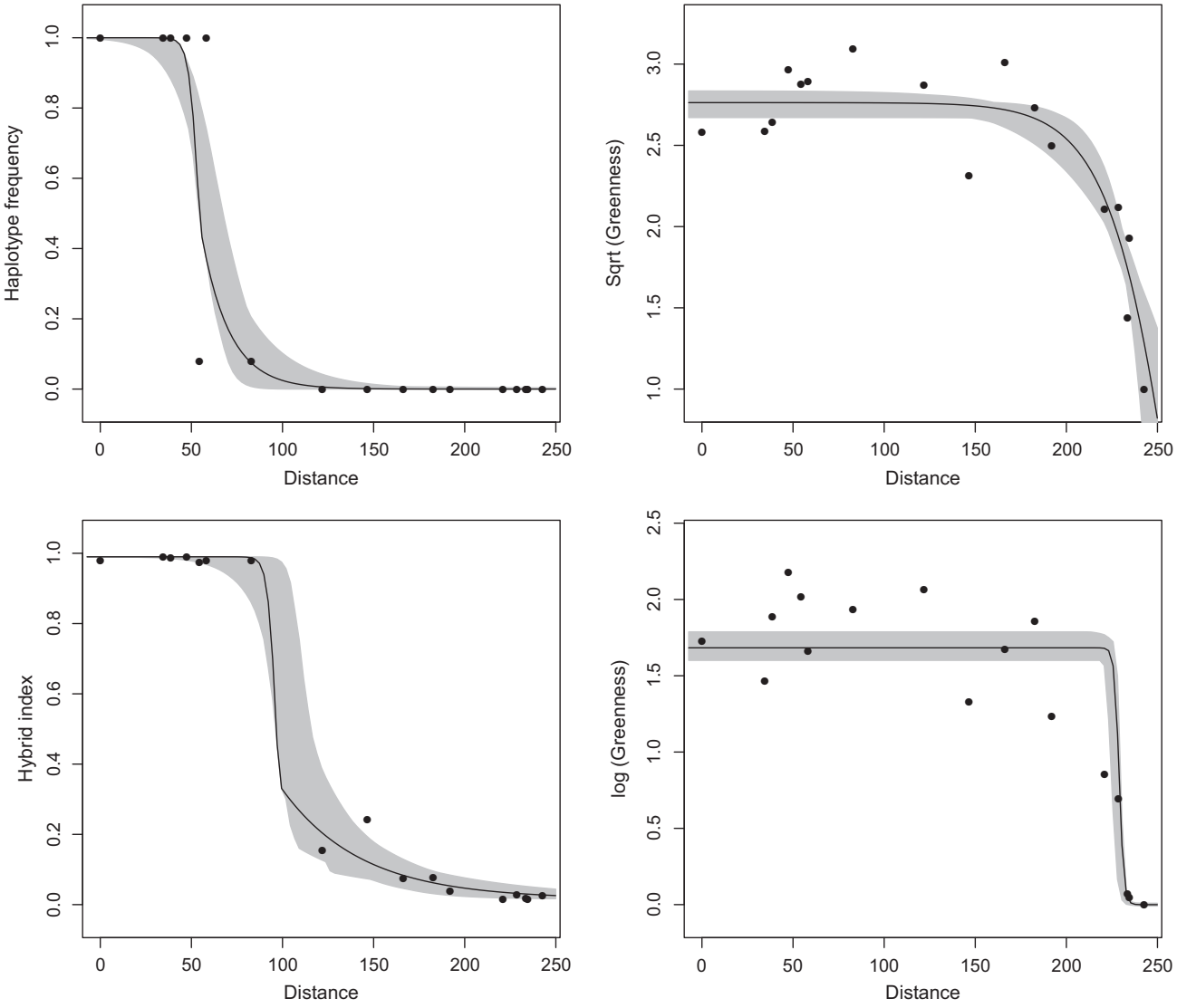

Figure 4 The maximum-likelihood cline and the 95% credible cline region for best-fitting models (Table 4) for mtDNA haplotype (top left), hybrid index (bottom left) and the lineage-characteristic dorsal colouration for males (top right) and females (bottom right). Greenness of dorsal colouration was scored on a scale of 1–10 and was transformed to improve fit to model assumptions (square root and logarithmic transformation for males and females respectively). Transect distance is the cumulative distance from the south-easternmost population Colle di Val D'Elsa in Tuscany with increasing distance westwards.

DISCUSSION

The evolutionary consequences of secondary contact should depend on the genetic and phenotypic divergence between lineages. Our results show that divergence in male competitive ability in allopatry causes asymmetric hybridisation and gene flow upon secondary contact in wall lizards. As a consequence, sexually selected introgression shapes phenotypic and genetic variation in both native and non-native populations.

Wall lizards in north-central Italy show exaggeration of characters that are under sexual selection in this (Sacchi *et al.* 2009) and other lizard species (Olsson & Madsen 1998). Our experiments show this is associated with an advantage in male–male competition for females, leading to an overall greater courtship, mating success and increased rates of hybridisation with females of the other lineage compared to males from the Western European lineage. These patterns are unlikely to be mediated by female choice as we have shown elsewhere that females do not discriminate between males of different lineages, whereas males prefer females from their own lineage (Heathcote 2013). Post-copulatory mechanisms also appear unlikely to explain patterns of paternity as there was no evidence for a competitive advantage for Italian males in sperm competition trials. Our results therefore suggest that male–male competition and male mate choice should drive patterns of genetic exchange in zones of secondary contact. This is in contrast with the majority of previously studied vertebrates, where female choice is believed to be both the primary barrier to hybridisation as well as the major cause of

asymmetric introgression of male characters (Wirtz 1999; Stein & Uy 2006; Pfennig 2007).

Even if small differences in viability or fertility remained undetected in our experimental crosses, the differences in male competitive ability should create asymmetric introgression; that is male-driven gene flow from the Italian lineage into the Western European lineage. Data from the native hybrid zone provide strong support for this prediction. Nuclear microsatellite markers revealed a westward shift in the position of the hybrid cline compared to mitochondrial markers. As a result virtually all hybrids exhibited Western European haplotypes. Furthermore, the phenotypic clines were shifted even further westwards, such that several populations that were genetically (i.e. based on both ncDNA and mtDNA) assigned to the Western European lineage were phenotypically indistinguishable from pure populations of the Italian lineage. Even if head size and dorsal and ventral colouration are not the direct targets of sexual selection, our enclosure experiments show that these characters are strong predictors of male dominance, a robust predictor of reproductive success. Thus, the stronger introgression of phenotypic characters compared to microsatellite markers imply that these characters not only bias the direction of hybridisation, but are also selectively favoured within the hybrid zone. However, analysis of selection at the leading front of the hybrid zone would be necessary to establish ongoing selection on male secondary sexual characters.

The results from the native hybrid zone were supported by genetic data from two locations where at least one of the lineages has been introduced. These patterns were weaker than those observed in the native zone, potentially because of strong founder effects that are likely to have occurred during establishment. Nevertheless, in both of the non-native populations the mitochondrial–nuclear discordance was consistent with hybridisation being primarily between males of the Italian lineage and females of the Western European lineage. Thus, the results from all three regions of secondary contact point towards asymmetric introgression and displacement of male characters of the less dominant lineage by intrasexual selection (Schulte *et al.* 2012b), providing evidence that introgression can be a source of secondary sexual characters.

Is male–male competition a general mechanism of directional introgression? Differences in male competitive ability are commonly invoked to explain displacement of one species by another in sympatry (Grether *et al.* 2013). This could promote asymmetric hybridisation by making males of one species rare relative to females (Hubbs 1955). This mechanism is supported by studies of interspecific competition over nest sites in flycatchers (Vallin *et al.* 2012), but introgression in this species is very limited due to low hybrid fitness (Veen *et al.* 2001). Species-specific male aggression is also consistent with the direction of introgression of plumage colour in manakins (McDonald *et al.* 2001), the movement of hybrid zones between hermit and Townsend's warblers (Peason & Rohwer 2000) and between two species' of house mice (Teeter *et al.* 2007). Nevertheless, the best evidence that sexual selection drives introgression still comes from studies of female choice (Parsons *et al.* 1993; Stein & Uy 2006; Baldassarre & Webster 2013; Baldassarre *et al.* 2014). This could partly be because of taxonomic bias. In lizards, male–male competition appears to be a stronger driver of variation in male reproductive success than female choice (Olsson & Madsen 1995). We therefore suggest that male–male competition often will be more important for the strength and direction of gene flow in lizard hybrid zones compared to, for example, bird hybrid zones.

The clines we observe in wall lizards are wider relative to the species dispersal ability than in other studies of sexually selected introgression (e.g. *Manacus* sp.; Uy & Stein 2007). In manakins, plumage introgression has been suggested to be limited by either habitat, which influences the conspicuousness of colour and geographically limits the benefit of golden plumage (Uy & Stein 2007), or by geographic barriers to dispersal (McDonald *et al.* 2001). In contrast, the habitat across the hybrid zone in the wall lizards typically consists of rocks and manmade structures (e.g. dry-stone walls) and geographic differences in the properties of this habitat are unlikely. Thus, there may be no limit to introgression along the coast in northwestern Italy and the geographic cline may be best viewed as a snapshot of an ongoing process of adaptive introgression that will eventually replace the phenotypes of the Western European lineage in this part of the species' distribution. In the introduced populations, we expect the formation of a hybrid swarm biased towards Italian characteristics, a process that evidently has already taken place in Holmsley.

Despite the close fit between our experimental data and the genetic and phenotypic clines, discordances between markers could also arise for a number of other reasons. We can refute most, if not all, of these for the native hybrid zone. First, the geographic scale of the discrepancy, compared to species dispersal distances, makes sex differences in dispersal highly unlikely as the cause of asymmetric introgression (Petit & Excoffier 2009). Lizard densities are also uniformly high across the zone. Second, we found no evidence that hybrid females are sterile, which rules out loss of fitness in female hybrids explaining differences in the mitochondrial and nuclear genetic clines (as expected from Haldane's rule; Haldane 1922). Third, environmental differences cannot explain the geographic pattern of phenotypic variation since the lineage differences persist in non-native populations and in captivity. Finally, the quantitative nature of the phenotypic characters means that it is unlikely that we are observing stochastic variation in introgression of loci across the genome, as could be the case for characters controlled by a single locus (e.g. colour polymorphisms; Mundy 2005).

In summary, we provide strong evidence that divergence in sexually selected traits in allopatry drives asymmetric hybridisation in wall lizards. This creates pronounced discordance between the phylogeography inferred from genetic markers and geographic patterns of phenotypic variation across multiple zones of secondary contact. These results suggest that, where post-reproductive isolation evolves slowly and female choice on male quantitative traits is absent or weak (as in lizards; Olsson & Madsen 1995), male–male competition may be an important cause of asymmetric introgression. This can lead to rapid introgression of potentially advantageous alleles and traits between species and ultimately promote novel genetic and phenotypic diversity in recipient populations.

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AUTHOR CONTRIBUTIONS

TU and GMW conceived of, designed, and organised the research. TU, GMW, SM, HEAM, NZ, PC, GPL, RS and MALZ conducted field work in Italy, France and England. JB conducted field work in Germany. RJPH and HEAM carried out the enclosure experiments, including assignment of parentage. TU, GMW, HEAM, TH and BF collected data from other experiments on captive animals. SM, NZ and HEAM generated the mtDNA and microsatellite data from native and UK populations. JB did the corresponding part for animals from the German populations designed and supervised by US, MV and AH. TU, GMW, RJPH, SM and JB analysed the phenotypic and genotypic data. TU and GMW wrote the paper with input from all authors.

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