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

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Abstract

Many populations are small and isolated with limited genetic variation and high 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 tease apart. Replicated introduction events in non-native species can offer insights into this problem because they allow us 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 to jeopardize long-term viability. However, there was no statistical correlation between population-level 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. We discuss these results in the context of human-mediated introductions and how the history of introductions can play a fundamental role in influencing individual and population fitness in non-native species.

Introduction

During the process of colonization, populations may experience dramatic changes in genetic diversity due to founder and bottleneck events (Sakai *et al.* 2001; Dlugosch & Parker 2008). Such reduction in genetic diversity can affect establishment success, population growth and adaptive potential (Nei *et al.* 1975; Lee 2002; Dlugosch *et al.* 2015). For example, a small population size increases the probability of inbreeding, which increases homozygosity and could lead to the expression of deleterious recessive mutations that reduce individual fitness (i.e., inbreeding depression) and population viability (Keller & Waller 2002; Charlesworth & Willis 2009). Establishing predictors of genetic diversity and its relationship to estimates of individual and population viability is therefore fundamental to our understanding of what promotes (or hinders) biological invasions and natural range expansion (Lee 2002; Keller & Taylor 2008; Excoffier *et al.* 2009; Bock *et al.* 2015; Dlugosch *et al.* 2015), insights that can ultimately assist in conservation management (Frankham *et al.* 2014).

Despite the importance of understanding the links between the demographic and ecological processes that reduce genetic diversity and lead to inbreeding depression, establishing these links empirically has proven surprisingly difficult. This is largely because the historical record is often poor and replication of colonization events limited, making it difficult to test for predictors of loss of genetic variation (Estoup & Guillemaud 2010; Uller & Leimu 2011). Generating good evidence for loss of fitness can also be problematic since inbred individuals may die at an early stage in development, making inbreeding depression cryptic or mistakenly classified as parental infertility (Hemmings *et al.* 2012). Indeed, some of the best examples that inbreeding depression (e.g. increased hatching failure) is associated with the severity of bottlenecks (Briskie & Mackintosh 2004; Heber & Briskie 2010) come from hole nesting passerines where early mortality or infertility can be determined with some accuracy (Bensch *et al.* 1994; Kempenaers *et al.* 1996; Spottiswoode & Moller 2004). Also, selection against inbred juveniles might reduce the evidence of inbreeding depression in adults (Keller & Waller 2002). Nevertheless, estimating inbreeding in natural populations is not trivial and data linking introduction history, loss of genetic diversity and inbreeding depression are therefore scarce in

other vertebrates. As a result, the extent to which loss of genetic variation and inbreeding depression negatively impact persistence of wild populations remains debatable (Bouzat 2010).

The common wall lizard, *Podarcis muralis* provides an opportunity to study how introduction history shapes genetic diversity and how well estimates of genetic diversity correlate with signs of 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.* 2012; Michaelides *et al.* 2013). In England, more than 30 extant populations were the result of escapees and deliberate release of captive animals and/or their offspring (Uller and While, unpublished; Lever 1977; Michaelides *et al.* 2013; Michaelides *et al.* 2015). A comprehensive analysis of the colonization history of 23 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 (i.e., the source was an already established population in England) and admixture (presence of mtDNA haplotypes of more than one lineage; Michaelides *et al.* 2013, Michaelides *et al.* 2015). Using 1546 native and non-native animals we test whether genetic diversity (measured using microsatellite markers) of non-native populations was 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). Furthermore, for 11 native and 13 non-native populations we also collected data on female fecundity, infertility and embryonic mortality to test if loss of genetic diversity and individual heterozygosity was associated with loss of fitness.

Material and methods

Sampling and molecular laboratory work

We used 1318 genotypes from Michaelides *et al.* (2015) and sampled 11 additional populations (228 individuals) from native locations in Italy and France (Figure 1, see also tables S1 and S2 in supplementary information). We extracted genomic DNA from tail tissue preserved in ethanol (70-90%) with DNeasy 96 plate kit (Qiagen, Valencia, CA) following the 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, from 10mM working stock) 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 labeled 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 (peaks with low relative fluorescence unit) were repeated (PCR and genotyping) 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 2012), allelic richness (A_R, corrected for sample size) using FSTAT (Goudet 1995, 2001) and genetic differentiation among populations (F_{ST}) and linearized F_{ST} ($F_{ST}/(1-F_{ST})$) in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010).

Genetic diversity in the native and non-native range

To determine how gene flow (or in the case of non-native populations, their introduction history) 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 (linearized 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. We further used Tukey's posthoc tests in R v.3.1.2 (R Development Core Team 2015) to identify significant pairwise comparisons between groups (native Italian, native French, non-native Italian, and non-native French).

Predictors of genetic diversity in the non-native range

We used a GLM with Gaussian distribution on transformed data to test if genetic origin (Italian vs. French) and introduction history explained variation in genetic diversity in non-native populations. We included the mode of introduction (primary vs. secondary), number of years since introduction (or first observed) and admixture (presence of mtDNA haplotypes of more than one lineage; yes vs. no) as our variables describing introduction history (Michaelides *et al.* 2015). 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 (supplementary table S1; see also Michaelides *et al.* 2013; Michaelides *et al.* 2015).

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 (see 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 the lack of egg shell (Olsson & Shine 1997). All other eggs had normal calcified egg shells. 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 did not attempt to score the exact developmental stage, but mortality typically happened before or soon after oviposition (based on the embryonic staging table in Dufaure & Hubert 1961).

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 a 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) and significant pairwise comparison between groups (native Italian, native French, non-native Italian, non-native French) was assessed using Tukey posthoc tests. In addition, for non-native populations we used a GLM with Gaussian distribution on transformed data (arcsine square root) to test if population 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-related traits (Reed & Frankham 2003). We assessed the relationship between expected heterozygosity and average clutch size (C_S),

194 infertility (I_N) and hatching failure (H_F) among non-native populations. Populations with fewer than 10
195 females with complete data on C_S, I_N and H_F were excluded from this analysis to minimize biased
196 estimates of averages.

197 At the individual level, heterozygosity-fitness correlations (HFC) are statistical associations
198 between individual multilocus heterozygosity and fitness traits. HFCs are expected to arise when there
199 is within population variation in inbreeding, heterozygosity and non-genetic component of trait
200 variance (Szulkin *et al.* 2010). Because spurious HFCs can arise when individuals are sampled from
201 different localities or geographic origins (e.g HFCs can be an artefact of between population variation,
202 Slate *et al.* 2004) and since some non-native populations have shown to share demographic history and
203 genetic composition (Michaelides *et al.* 2015) we used STRUCTURE (Pritchard *et al.* 2000) to assign
204 individuals (females) into demes (K), representing clusters of populations that share close genetic
205 relationships (e.g., because one was established through introduction of individuals from another;
206 Michaelides *et al.* 2015). We ran simulations with a burn-in of 10^5 iterations and a run length of 10^6
207 iterations from K = 1 to K=11 (for native females) or K=13 (for non-native females). Runs for each K
208 were replicated five times and the best K was determined according to the method described by
209 Evanno *et al* (2005) in the online software Structure Harvester (Earl & vonHoldt 2011). Multiple runs
210 were combined in CLUMPP (Jakobsson & Rosenberg 2007) and each female was assigned into a deme
211 when the proportion of membership (q) for a deme was ≥0.9. Structure results identified high posterior
212 probability at K=2 for native females (DemeNativeItalian and DemeNativeFrench) and K=4 for non-
213 native females (four demes with females belonging to populations of either Italian-only or French-only
214 populations; DemeIntroITA-A (BS, DL, PO, WS), DemeIntroITA-B (WW, SH), DemeIntroITA-C
215 (VT, VB, SW) and DemeIntroFRA (BU, CW, EP, WE). There was one deme that included females of
216 mixed ancestry (0.1<q<0.9); DemeIntroMix (BS, DL, SH, SW, VB, WE, WS); see Table S3 for list of
217 populations and their abbreviations). Therefore, for subsequent analyses we partitioned our data
218 accordingly to determine whether the presence and/or magnitude of HFC varied among the different
219 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 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 comparably low incidence of infertile eggs). 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 (Nakagawa & Cuthill 2007). 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 reverse 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 whether high offspring homozygosity was 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}. The 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

In the native range, there was a clear spatial genetic structure with the Italian region showing higher levels of genetic diversity (H_E and A_R) compared to the French (post hoc Tukey test $p < 0.05$, Figure 3). Across the whole data set most of the variation was found within populations with only 10-15% of variation between ranges and origins (Table 1). Significant isolation-by-distance patterns were observed within both the native and non-native populations (Mantel tests, $p < 0.05$, Figure 2).

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 only showed a weak loss of diversity compared to their native range (post-hoc Tukey tests between French native and French non-native being statistically significant only for A_R; Table 2 and Figure 3A and 3B). The number of years since introduction was the only statistically significant predictor of genetic diversity for H_E (this was not significant for A_R; Table 3), with older populations having lower genetic diversity. In 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).

Females from non-native populations had significantly larger clutches than females from native populations ($F_{1,411} = 6.17$, $p = 0.02$, Figure 3D). 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 (post-hoc Tukey test $p < 0.05$, Table 4, Figure 3C). Within the non-native range, none of the predictors (region of origin, admixture, mode of introduction and years since introduction) significantly affected population average hatching failure or fertility (Supplementary Table S4).

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 arise 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 statistically significant values only for the DemeIntroItalian-B ($g_2 = 0.067$, $p = 0.04$, see also Supplementary Table S5). Generalized Linear Mixed Models of HFCs indicated no significant association between female heterozygosity (F_{HL}) and fitness traits (H_F, C_S) in any of the data partitions (Supplementary Table S6). The overall average effect size on all demes combined was low ($\check{Z}r = 0.039$) and the 95% confidence interval included zero (Supplementary Table S6). Finally, within clutches, embryos that died before hatching where no more homozygous than their successfully hatched siblings ($\chi^2=0.01$, $p=0.91$; Supplementary Table S7).

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 have low genetic diversity and suffer from inbreeding depression. Our analyses of non-native wall lizard populations in England showed loss of genetic diversity and an increase in embryonic mortality compared to native populations. Despite this, we failed to establish individual-level correlations between heterozygosity and various measures of fitness.

During and following the colonisation of a new area, populations are expected to lose genetic variation and display increased differentiation amongst 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 compared to native populations. Interestingly, non-native populations from the native region with higher genetic diversity have lost proportionally more genetic variation. This could imply that bottlenecks may have been more severe for non-native Italian populations, but it may also reflect 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 lineages diverged from each other approximately 2-3 MYA (Gassert *et al.* 2013; Michaelides *et al.* 2013) and 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). Consequently, our study emphasizes how the phylogeographic structure in the native range may 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, despite that information regarding the number of founders was only available for seven populations, diversity increased significantly with the number of animals released. Older populations also harbored less genetic variation than more recently established populations. This may reflect a prolonged period of isolation and absence of gene flow. It is also possible that natural selection contributes to loss of diversity given the evidence that populations established several decades ago (approximately ten to forty generations) have adapted to the colder climate in the UK (While *et al.* 2015). In contrast there was no evidence for further reduction in diversity in secondary introductions. A loss of genetic variation is expected to be a characteristic of sequential founder events (Clegg *et al.* 2002), but our results are not unique for lizards. Successive colonization of *Hemidactylus*

mabouia in Florida (US), via human-mediated dispersal, did not result in further loss of genetic diversity (Short & Petren 2011). Secondary introductions from admixed populations may explain this pattern (e.g. Tonione *et al.* 2011) as genetic admixture is common in biological invasions and can increase genetic diversity (Kolbe *et al.* 2004; Genton *et al.* 2005; Kolbe *et al.* 2007; Facon *et al.* 2008) sometimes creating novel combinations of alleles in the new range (Ellstrand & Schierenbeck 2000). However, in our study there was no evidence that multiple introductions and admixture, occurring from genetically (and phenotypically) differentiated lineages in the native range, had higher overall nuclear genetic diversity. We can conclude that non-native wall lizard populations are less genetically diverse on average, but that populations have retained variation through secondary introductions and not gained much variation through admixture, at least with respect to neutral markers.

Small population size should result in mating between close relatives, which may cause 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 attributed 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 high hatching failure, reaching over 30% in some populations, compared to both their native counterparts (mean ca 7%) and non-native populations of French origin (10%). Because eggs were incubated at constant temperatures in the laboratory and hence environmental conditions were standardized across clutches, these effects are likely to be due to expression of deleterious recessives. A high hatching failure in non-native populations of Italian origin is consistent with the greater reduction in genetic diversity relative to the native range compared to French populations. This may suggest that populations of Italian origin have experienced stronger bottlenecks events (although the low sample size for French populations suggests the difference between lineages needs to be treated with caution). 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 sampling years and hence likely to reflect a significant genetic load in non-native populations.

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. In our study, only one of the non-native demes of shared ancestry showed statistically significant identity disequilibrium (ID, the correlation in heterozygosity and/or homozygosity across loci; Weir & Cockerham 1973; Szulkin *et al.* 2010). It is therefore perhaps not surprising that, despite a reasonable sample size relative to other published studies (Chapman et al. 2009), we did not find a statistically significant correlation between population genetic diversity and average clutch size or hatching failure among non-native populations. The average effect sizes across demes also suggested that the true effect size is close to zero. Also within clutches, we failed to detect any differences in heterozygosity between embryos that died early in development and their successfully hatched siblings. 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 high incidence of embryonic mortality.

It is unclear why the effect 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 (Asa *et al.* 2007), 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 only with close relatives could result in infertility if fertilization success is lower for genetically similar males, 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.

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? Although there are many known examples of individual multilocus heterozygosity and fitness correlations (reviewed in Chapman *et al.* 2009) effects are relatively weak and effect sizes generally small. If effects are strongest in males, 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 ~~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.* 2011). 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). Finally, our study was restricted to 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 neutral markers used might not be sufficient to capture HFCs adequately (Balloux *et al.* 2004; Miller & Coltman 2014), especially as g_2 values suggested a moderate level of inbreeding at most. Thus, our failure to detect ID and/or HFC's should not be taken as evidence that inbreeding depression is absent (Kardos *et al.* 2014). Using a large number of markers such as single nucleotide polymorphisms (e.g. Miller *et al.* 2014; Huisman *et al.* 2016) and/or analysis of functional genes such as genes of the Major-Histocompatibility Complex (e.g. Agudo *et al.* 2012) may be more appropriate when estimated genome-wide heterozygosity and the effect on fitness. The large number of independent introductions of wall lizards to England would provide a good study system to explore how consistent these measures of genetic variation correlate with introduction history and loss of fitness due to inbreeding.

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 greater 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 high in non-native populations of Italian origin. Although this is consistent with the greater loss of genetic diversity for Italian-origin populations, we found no evidence that heterozygosity across microsatellite markers is significantly correlated with inbreeding depression at the population or individual levels.

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Data accessibility

Sampling locations and genetic diversity data: Table S1 and S2 in supplementary information.
Population average fitness trait data: Table S3 in supplementary information.
Genotypes of individuals used in the genetics analyses are deposited in Dryad (XXX *to be completed upon acceptance*).

Author Contributions

SNM, GMW, and TU conceived of the project, collected data, and wrote the manuscript. SNM generated and analysed the genetic data with help of NZ, and MALZ, RS, BC and FA collected samples from native populations.

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	

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

H_E ~ Range * Origin			
Source of variation	***d.f.***	***F***	***P***
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.

H_E ~ origin + mode of introduction + admixture + years			
Variable	***d.f.***	***F***	***p***
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			
Variable	***d.f.***	***F***	***p***
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

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**			

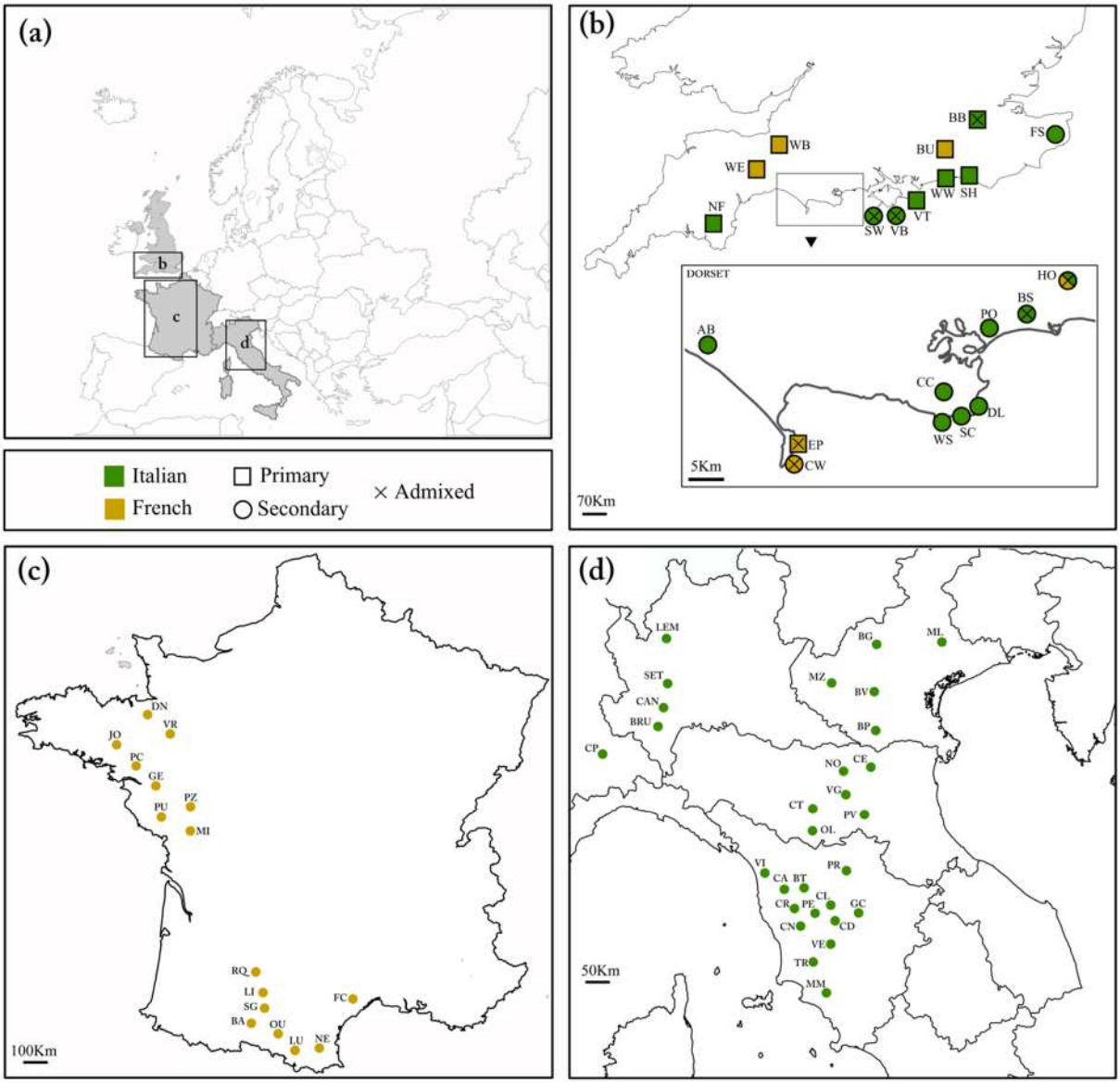

Figure 1 Distribution of sampling locations in the native and non-native range. Populations in England are coded based on their introduction history (Italian or French genetic origin, primary or secondary introduction and whether there was evidence of admixture; presence of mtDNA haplotypes from two or more lineages). Map modified from Michaelides *et al.* 2015.

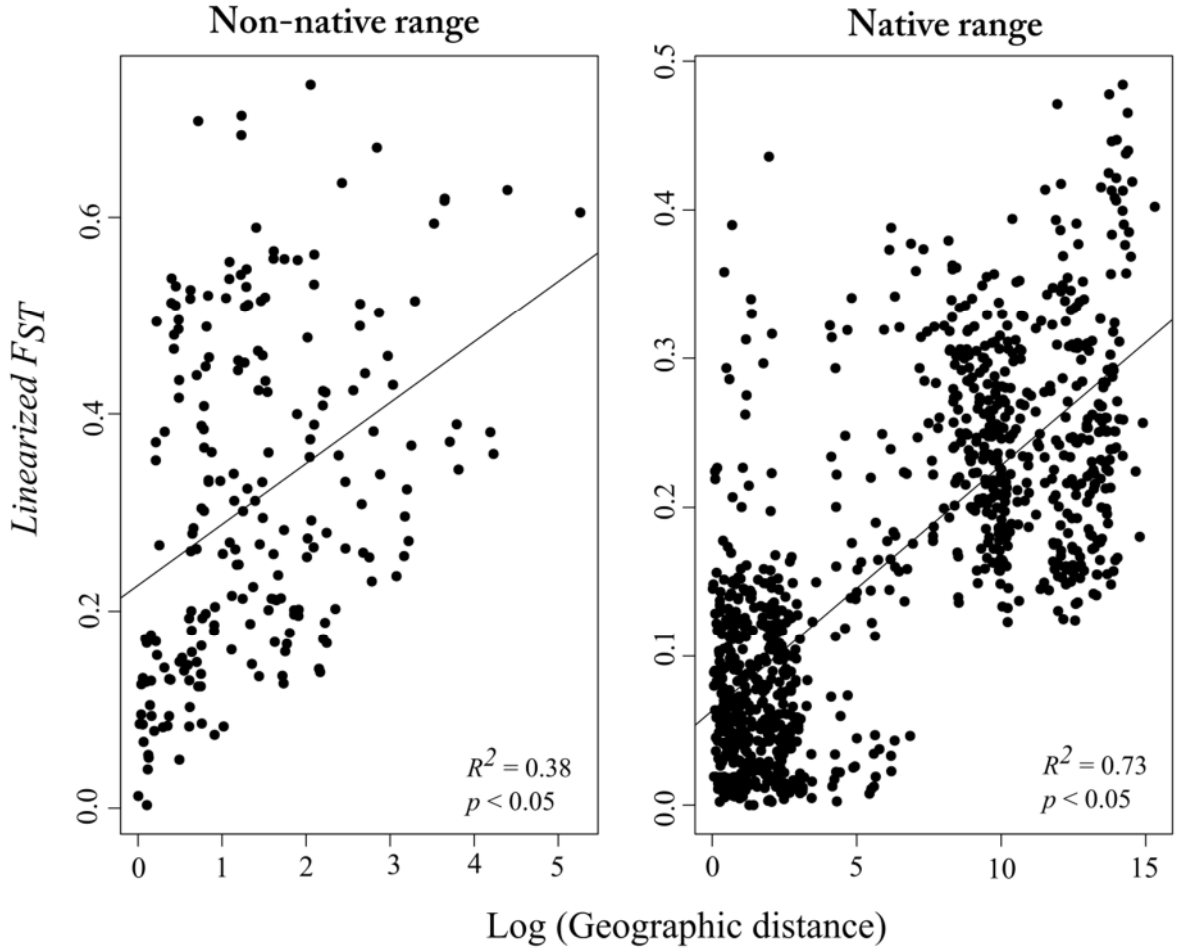

Figure 2 Correlation between genetic (Linearized F_{ST}) and geographic distance (log-transformed). There was evidence of isolation–by-distance in both the non-native and native range as assessed by Mantel tests (after 9999 permutations). Note different scales on the axes for the two plots.

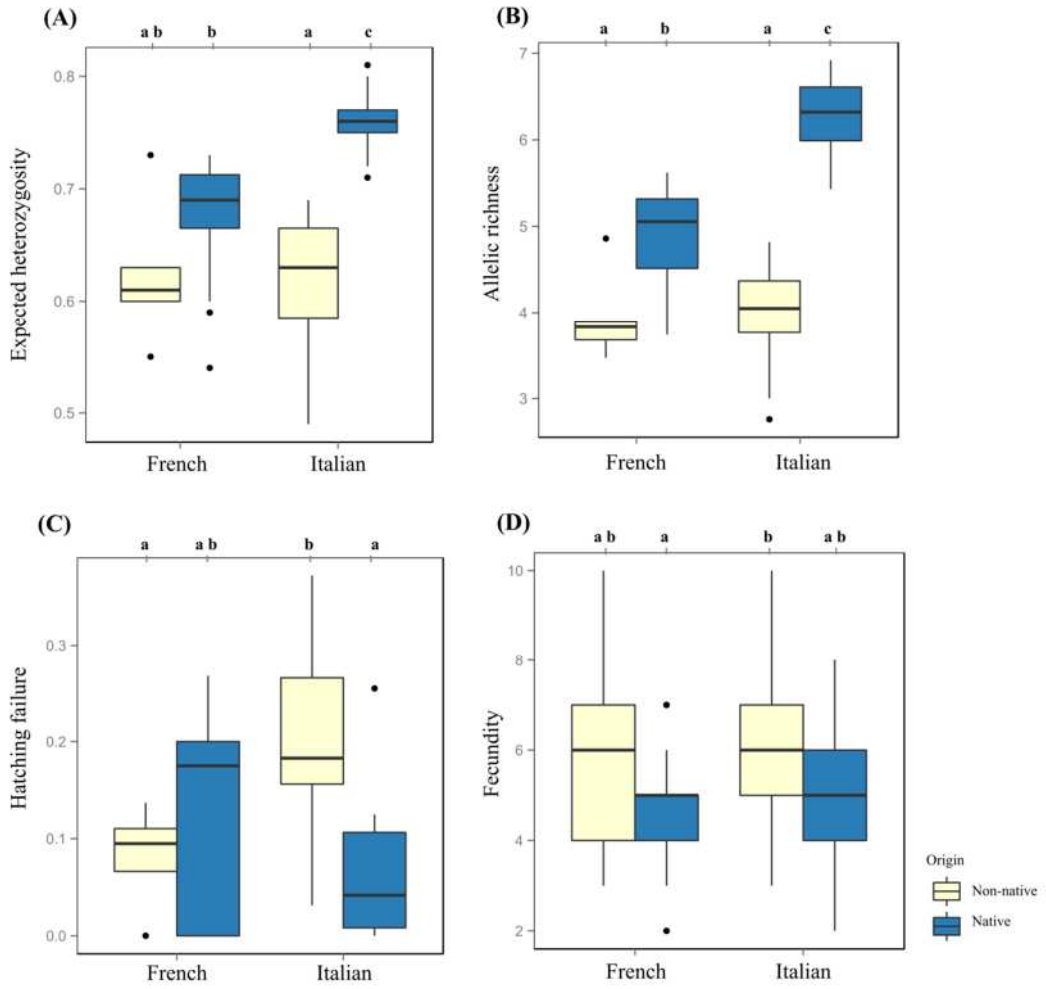

Figure 3 Genetic diversity and fitness related traits (hatching failure, fecundity (clutch size)) in native and non-native populations of French and Italian ancestry. (A) Expected heterozygosity; (B) Allelic richness; (C) Hatching failure; (D) Fecundity. Different letters above the plots indicate significantly different pairwise comparisons assessed by Tukey posthoc tests (groups sharing the same letter have non-significant differences).

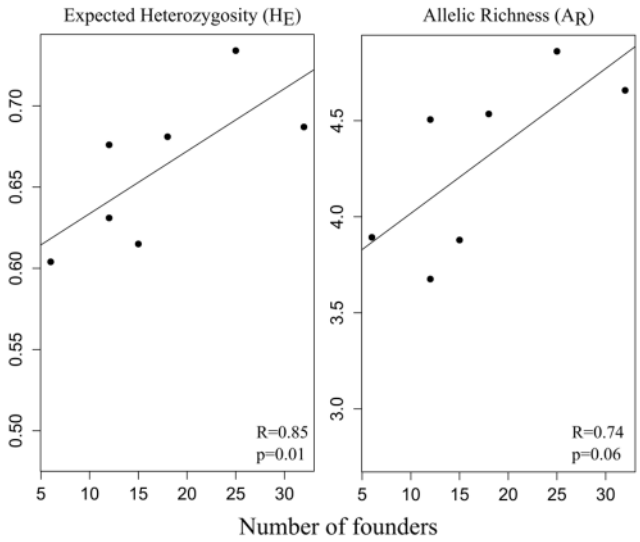

Figure 4 Correlation between number of founders and genetic diversity.