Increased individual homozygosity correlates with low fitness in a fragmented lizard population

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Running title: Homozygosity and fitness correlation in a fragmented lizard population.

Abstract

Isolation due to anthropogenic habitat fragmentation is expected to increase homozygosity of individuals, which may lower their fitness as a result of inbreeding depression. Using a fragmented population of the lizard *Psammodromus algirus* for which we had data about two correlates of fitness, we genotyped all individuals for 6 microsatellite loci that correctly capture genome-wide individual homozygosity of these lizards (as validated with an independent sample of lizards genotyped for both these microsatellites and > 70,000 SNPs). Our data revealed genetic structure at a very small geographic scale, which was compatible with restricted gene flow among populations disconnected in a matrix of inhospitable habitat. Lizards from the same fragment were genetically more related to one another than expected by chance, and individual homozygosity was greater in small than in large fragments. Within fragments, individual homozygosity was negatively associated with adult body size and clutch mass, revealing a link among reduced gene flow, increased homozygosity and lowered fitness that may deterministically reduce population viability. Our results contribute to mounting evidence of the impact of the loss of genetic diversity on fragmented wild populations.

Keywords: body size - breeding investment - genetic relatedness - habitat fragmentation - homozygosity - *Psammodromus algirus*

INTRODUCTION

Anthropogenic habitat fragmentation, which causes the decrease in size and spatial connectivity of habitat fragments, is a major driver of the ongoing biodiversity crisis (Fahrig, 2003; Foley *et al.*, 2005; Pimm *et al.*, 2014). Fragmentation not only involves the effective loss of habitat, but also hinders the movement of individuals among habitat fragments, which may compromise species persistence if reduced dispersal prevents the restoration of local extinction events or reduces gene flow among disconnected populations (Hanski, 1998; Nathan, Kanno, & Vokoun, 2017). Isolated populations may lose genetic diversity owing to genetic drift after population bottlenecks, an effect which can be aggravated if immigration from other habitat fragments is infrequent and this circumstance forces individuals to breed with close relatives (Young, Boyle, & Brown, 1996). In turn, genetic erosion may jeopardize population viability due to inbreeding depression, i.e. low fitness as a result of breeding of related individuals (Westemeier *et al.*, 1998; Crnokrak & Roff, 1999; Reed & Frankham, 2003), an effect which has long been believed to exacerbate extinction risks in fragmented habitat (Frankham, 2005; Wootton & Pfister, 2013). Inbreeding depression occurs when fixation rates of deleterious alleles are higher than rates of genetic rescue by migration or genetic purging by selection; the larger this difference, which is enhanced by the loss of connectivity between fragments, the larger the magnitude of the inbreeding depression caused by fragmentation (Hedrick & Garcia-Dorado, 2016).

Inbreeding depression has been found in a variety of wild populations (Kardos *et al.*, 2016), yet its effects on population dynamics have been historically debated because they may be masked or effectively overridden by environmental and demographic processes (Lande, 1988; Caro & Laurenson, 1994; Frankham, 2010; but see Wootton & Pfister, 2013). Ecologists and conservation biologists are therefore urged to determine

the true magnitude and fitness effects of inbreeding effectively caused by loss of genetic diversity in wild populations (Keller & Waller, 2002; Frankham, 2010), which is especially true when populations are threatened by habitat fragmentation. For example, restricted gene flow among disconnected population fragments is a well-documented phenomenon (Dixo *et al.*, 2009). In addition, reduced demographic growth of small populations inhabiting fragmented habitat has been attributed to negative genetic effects (Hanski & Saccheri, 2006), including a decrease in the ability of fragmented populations to adapt to environmental change (Yates & Fraser, 2014).

In fragmented populations, inbreeding may be common if subpopulations are small and dispersal among habitat fragments is rare. This circumstance would force individuals to mate within their habitat patch, which would increase genetic relatedness of individuals inhabiting the same patch and increase average homozygosity as a consequence. Increased individual homozygosity may contribute to inbreeding depression if homozygous individuals have problems to withstand trials of life such as parasite resistance (Coltman *et al.*, 1999) or competition for mates (Hoffman *et al.*, 2007), or show poor lifetime performance in terms of fecundity, longevity or offspring quality (Huisman *et al.*, 2016). Mounting evidence of negative homozygosity-fitness correlations (Hansson & Westerberg, 2002; Coltman & Slate, 2003; Reed & Frankham, 2003; Chapman *et al.*, 2009; Huisman *et al.*, 2016) supports the idea that such correlations can be used to estimate the impact of inbreeding in nature, which may be particularly true in fragmented populations where inbreeding is expected to be stronger than usual (Hansson & Westerberg, 2002; Szulkin, Bierne, & David, 2010).

We studied populations of the large psammodromus *Psammodromus algirus* in fragmented habitat in northern Spain (Santos *et al.*, 2008). In that region, this forest lizard is absent from many small habitat fragments that are otherwise suitable for

populations, according to known habitat preferences of the species (Díaz *et al.*, 2000). In addition, lizards show lower breeding success in small than in large habitat fragments (Díaz *et al.*, 2005). However, the question remains as to why habitat fragmentation may hamper breeding performance of these lizards. We analysed if low fitness of lizards in fragmented habitat is associated with loss of genetic diversity due to isolation in small habitat fragments. We explored four lines of evidence of the negative genetic effects of forest fragmentation, expecting to find (1) population structure among population fragments owing to reduced gene flow, (2) reduced out-crossing opportunities within habitat fragments, (3) increased individual homozygosity in small population fragments, and (4) negative correlations between individual homozygosity and fitness.

METHODS

During May-June in 2001 and 2002, we studied lizards in a fragmented forest located near Lerma, in northern Spain (42º06'N, 03º40'W), where suitable habitat fragments are separated by inhospitable cultivated land (Fig. 1). The fragmentation of continuous habitat to open cultivation fields in the past, which was especially intense after the Spanish Civil War (1936-1939), led to the formation of an archipelago of deciduous or evergreen woodlots. We captured lizards from three large habitat fragments (> 200 ha; CW, CE and Q) and from 14 small fragments (< 10 ha), which represent over 70% of the known species range in this locality. The 14 small forest fragments are separated by variable distances from the two large forest fragments CW and CE. The most distant site (Q, which is the largest habitat fragment; see Fig. 1) was also sampled because it had been considered to be a potential source of colonizers of the fragmented landscape in previous analyses of the regional distribution of lizards in this area (Díaz *et al.*, 2000; Santos *et al.*, 2008), and it also contributed data to the analysis of breeding investment

of lizards (Díaz *et al.*, 2005). To obtain adequate sample sizes, these habitat fragments were grouped into five different geographically defined habitat sectors (Fig. 1). These five groupings were defined on the basis of geographical proximity measured using the geometric centre of sites (between-site connectivity is inversely proportional to the distance between sites). To make the groupings objective, we conducted a cluster analysis of the matrix of among-site geographic distances, using Euclidean distances and complete linkage. Habitat fragments within the resulting clusters were separated by a maximum of 350 m of arable land (Fig. 1).

Overall, we captured 131 lizards (mean sample size ± 1 SE = 20.6 ± 4.8 individuals per habitat sector, range = 14 - 42). The individuals analysed had been included in an earlier study of the breeding performance of lizards (Díaz *et al.*, 2005), which allowed us to measure various traits associated with individual fitness (see below), thereby offering a unique oportunity to assess the correlations between fitness traits and individual homozygosity.

In the field, we recorded snout-to-vent length as a measure of lizard body size. We also collected tissue for DNA analyses by removing approximately 5 mm of lizards' tail tip. Tissue was stored in ethanol until DNA analysis. Four immature individuals (i.e., individuals not reaching the species' minimum body size at maturation) that were sampled for DNA analysis were excluded from the analysis of variation in body size. Captured gravid females (n = 48) were kept captive and monitored daily until they laid their clutches in the laboratory. We recorded clutch size and clutch mass at that time. All individuals were returned to site of capture after the study. A detailed description of field and husbandry methods can be found elsewhere (Díaz *et al.*, 2005).

In 2006, we used DNA extracted with a DNeasy Blood & Tissue Kit (Qiagen) to genotype all individuals for eight microsatellite loci specifically developed for *P.*

algirus as described elsewhere (Bloor & Dávila, 2008), only six of which were polymorphic in lizards from Lerma (one locus was monomorphic and another one was a microsatellite null allele). In 2015, we tried to recover next-generation sequencing data from the same samples, but the analysis was unfeasible because the samples had lost quality (not enough DNA of sufficient quality remaining).

Given the impossibility to gain next-generation sequencing information from our samples, but also considering the importance of genotyping individuals that had been scored for different fitness components in the laboratory, we decided to validate our measures of genetic diversity based on six microsatellite markers using a completely new sample of lizards for which we could obtain both microsatellite data and estimates of genome-wide diversity based on SNP calling. For that purpose, in 2015 we genotyped 17 lizards captured in 2008-2009 in the area of Lerma, using the same set of six microsatellites utilised in the analysis of homozygosity-fitness correlations with the sample of 131 lizards described above, and also SNPs. Thus, the microsatellites compared to the SNPs were the same as those in the original sample from all the populations, although alleles may be fewer (or different) in the latter, smaller sample. We obtained tissue samples from the tail tip of these lizards. We kept the samples in absolute ethanol at 4 ºC until DNA extraction. We purified DNA for library preparation using the Speedtools Tissue DNA Extraction kit (Biotools) with a cell lysis step of 24 hours and resuspension in DNase-free water at 60 ºC. The obvious alternative of taking a new sample of female lizards to the laboratory to obtain their fitness parameters and link them to GBS data was rejected for ethical reasons, inspired by the three R's principle (Replace, Reduce, Refine) in animal research (Russel & Burch, 1959). These lizards are not threatened in general, but in our study area they have obvious problems to thrive. Thus, the validation of our homozygosity estimates allowed us to replace the

use of lots of new animals with alternative techniques. This dramatic reduction in the use of animals came along with a clear refinement in terms of impact of our research on lizard populations. A repetition of the study (assuming equal sample sizes than our earlier dataset) would have required the capture of ca. 130 adult lizards, and it would have implied moving 75 females to the laboratory and removing ca. 350 juveniles from the metapopulation (since new regulations discourage the release of lab-bred juveniles back in the field).

SNPs were sequenced on an Illumina HiSeq2500 sequencer after GBS library preparation by using restriction enzyme *Pst1*. Filtering and SNP calling were performed using the UNEAK pipeline (Lu *et al.*, 2013), specifically designed for samples with no reference genome and implemented in TASSEL v.3.0 (Bradbury *et al.*, 2007). Sequence tags were aligned to each other to form 'networks' of tags, where each node is a single tag sequence, and each edge represents a single base pair difference between two tags. The networks were pruned to remove putative sequencing errors (low frequency alleles) using the error rate threshold parameter. The resulting dataset had 83,648 SNPs with a site depth of 5.96 ± 6.56 (mean ± sd) and a site missingness of 0.49 ± 0.33. We discarded loci with minor allele frequencies < 0.01 or that could be successfully sequenced in only one individual. We tested for Linkage Disequilibrium (LD) as a measure of correlation between loci (r^2) using the pruning criteria *--indep-pairwise* of plink software (Purcell *et al.*, 2007). The resulting dataset had 73,291 loci, with only one SNP per locus, and with a site depth of 6.60 ± 6.75 and a site missingness of 0.42 ± 0.31.

An analysis with GENEPOP (Rousset, 2008) of the six microsatellite markers did not detect significant deviations from Hardy-Weinberg equilibrium or pairwise linkage disequilibrium within habitat patches. Population differentiation and isolation-

by-distance effects were estimated using Arlequin 3.5 (Excoffier & Lischer, 2010), using R_{ST}-like genetic distances (Slatkin, 1995), although using standard F_{ST}-like genetic distances produced the same results. We computed maximum likelihood estimates of relatedness between pairs of individuals using the ML-relate software (Kalinowski, Wagner, & Taper, 2006). We used one-way ANOVA to test for variation in pairwise relatedness between individuals sampled in the same site or in different sites, but the significance of the analysis was computed as the probability of obtaining as large or greater F values than the observed one in 10,000 random matrices in which the site of origin of each individual was randomly assigned.

Homozygosity of individual lizards was measured using uncorrected homozygosity, internal relatedness, and homozygosity by loci, which were calculated using CERNICALIN (Aparicio, Ortego, & Cordero, 2006). Uncorrected homozygosity (H_O) was calculated as the proportion of loci at which an individual is homozygous. Internal relatedness (*IR*), which weighs on the basis of the frequency of the alleles, was calculated as $IR = (2H–\Sigma f_i)/(2N–\Sigma f_i)$, where H is the number of loci that are homozygous, N is the number of loci and f_i is the frequency of the ith allele contained in the genotype. Finally, homozygosity by loci (*HL*), was estimated to weigh the contribution of each locus to the homozygosity value depending on its allelic variability (Aparicio *et al.*, 2006). Homozygosity by loci is calculated as $HL = (\sum E_h)/ (\sum E_h + \sum E_j)$, where E_h and E_j are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively. These three estimates of homozygosity were highly correlated in our sample ($r > 0.97$, $n = 131$, $P < 0.0001$), and therefore we chose one of them (*HL*) for the analyses (using the other two estimates of homozygosity produced the same results).

We used a hierarchically nested variance components design to test for within-sector effects of patch size (small versus large habitat fragments) on lizard pairwise relatedness and homozygosity. In these analyses, sector was a random factor, and type of habitat fragment (small versus large) was a fixed factor. We used Satterthwaite's method of denominator synthesis to find the linear combinations of sources of random variation that serve as appropriate error terms for each effect. Thus, the degrees of freedom for the denominator mean square can be fractional rather than integer values, meaning that fractions of sources of variation were used in synthesizing error terms for significance testing (StaSoft Inc, 2016).

We used general linear mixed models to analyse homozygosity-fitness correlations using individual body size and clutch mass relative to female size as estimates of fitness. Body size indicates fitness in this population because larger lizards survive better and larger females lay more eggs (Díaz *et al.*, 2005). Clutch mass controlling for the effect of female body size combines the contributions of egg number (or fecundity) and egg size (or offspring quality) to total reproductive output, while removing the effect of female body size on realised breeding investment (Díaz *et al.*, 2005). In these models, site was included as a random factor nested within forest type, because lizards grow faster in deciduous than in evergreen fragments (Iraeta *et al.*, 2006), the former being more productive and of higher thermal quality than the latter (Santos *et al.*, 2008).

RESULTS

The correlation between microsatellite and genome-wide measures of heterozygosity was highly significant ($r = 0.730$, $p < 0.001$; Fig. 2), meaning that homozygosity estimates obtained with data of six microsatellite loci reliably measure genome-wide

homozygosity as scored using > 70k SNPs. However, with only 6 microsatellite loci there could be a chance for a single locus to be driving the effect. To exclude this possibility, we repeated the correlation six times, each time excluding one locus. Results remained significant in all cases (all $r > 0.627$ and all $p < 0.007$).

Based on microsatellite analyses, we found population structure among 17 forest fragments separated from one another by 140-12,800 m in a matrix of inhospitable habitat. In a hierarchical AMOVA, variation among five habitat sectors (groups of forest fragments separated from other similar groups; see Fig. 1) explained 5.6% of total genetic variance ($F_{CT} = 0.056$, $P < 0.0001$). However, populations from fragments within the same sector showed no sign of genetic isolation ($F_{SC} = 0$, $P = 0.830$). Isolation by distance could partly explain the pattern of among-site genetic differentiation within the study area, because the greatest forest fragment (Q) is distantly located from all other sites (Fig. 1) and shows the greatest average F_{ST} in pairwise population comparisons (comparisons with Q site $F_{ST} = 0.037$, mean ± SE of comparisons between other sites $F_{ST} = 0.013 \pm 0.003$). However, a Mantel test with 10,000 simulations of random distance matrices only found a weak and non-significant isolation-by-distance effect ($r = 0.178$, $P = 0.076$), which was clearly removed with the exclusion of fragment Q from the analysis ($r = 0.06$, $P = 0.29$), although population genetic structure among habitat sectors still explained a significant 4.3% of variance ($F_{CT} = 0.043$, $P = 0.013$).

An analysis of maximum likelihood estimates of pairwise genetic relatedness revealed that lizards from the same forest fragment were genetically more related to one another than expected by chance (difference in pairwise relatedness between lizards from the same site compared to lizards from different sites: observed F = 76.55, mean ± SE estimated from 10,000 simulations of random distributions of individuals among

sites: F = 1.02 ± 1.01; $P < 0.0001$; Fig. 3). We failed to find variation in pairwise relatedness between lizards from small or large forest fragments ($F_{1,14.62} = 0.02$, $P = 0.888$). However, lizards showed greater homozygosity in smaller than in larger forest fragments ($F_{1,14.43} = 16.04$, $P = 0.0012$; Fig. 4). More information on basic population statistics and sample sizes can be found in Table 1.

A general linear mixed model controlling for the effects of sex and site (nested within forest type) showed that homozygosity was negatively correlated with body size of adult lizards (Table 2: partial correlation = -0.236, $P = 0.016$). The same modelling approach showed that female homozygosity was negatively correlated with clutch mass while controlling for the effects of site (nested within forest type) and female body size (Table 2: partial correlation = -0.357, $P = 0.030$). Thus, homozygosity was negatively correlated with two important components of lizards' fitness. However, an alternative explanation of body size and fecundity patterns could be that they simply represent a case of reduced fitness due to resource limitation in small patches; although individual heterozygosity might also be reduced, this could be occurring in parallel with resource limitation impacts on fitness. In fact, the models uncovered variation among habitat patches, which was significant for lizard size and marginally non-significant for clutch size (Table 2). Therefore, we tried to separate the effects of patch size and (patch size-associated) homozygosity on fitness. For that purpose, we examined the correlation between patch size and residual lizard fitness estimated in the models. Because the appropriate sample unit for this analysis is habitat patch, residual fitness measures were computed for each site as the deviations of estimated patch means from the model grand mean. These analyses showed that variation in lizard fitness among habitat patches was not significantly correlated with patch size (body size: $r^2 = 0.023$, $F_{1,15} = 0.360$, $P = 0.558$; clutch mass: $r^2 = 0.088$, $F_{1,9} = 0.865$, $P = 0.377$).

DISCUSSION

Our results validated the estimates of individual homozygosity obtained with data of six microsatellite loci as reliable measures of genome-wide homozygosity in this lizard population, as scored using > 70k SNPs. We are aware that our analysis of population genetic structure and individual homozygosity based on microsatellite markers (the state-of-the art method to quantify heterozygosity when we initiated our study) is less powerful than the current high-throughput sequencing methods for genome-wide typing (Hoffman et al., 2014; Huisman et al., 2016). Nevertheless, the utility of microsatellites has been validated with large SNP datasets both for fitness-heterozygosity correlations and for population genetic differentiation and structure (Putman & Carbone, 2014). In addition, even a small number of microsatellite loci can provide much insight into the genetic features of individuals and populations (Forstmeier et al., 2012), as shown for example in an experiment with *Anolis* lizards that analysed population genetic structure and individual homozygosity using six microsatellite loci, which found persistent founder effects despite significant adaptive parallelism (Kolbe et al., 2012). Nevertheless, the inference of genome-wide properties based on limited microsatellite data remains challenging due to uncertainty about the mutational processes generating genetic diversity at different loci, dependence on genomic context (e.g. if mutation rates vary widely among genomic regions), or lack of power if the number of markers is too low (Putman & Carbone, 2014). In spite of these shortcomings, our validation step shows that the conclusions we can derive from few microsatellite markers are valid independently of the distribution of microsatellites along the genome, i.e. whether each locus is more or less linked to low-recombination regions of the genome (GBS libraries are based on restriction sites that are randomly distributed). Therefore, we are fully

confident that homozygosity estimates obtained with data of six microsatellite loci reliably measured genome-wide homozygosity in our original sample of lizards.

Our results provide empirical evidence of the links among reduced gene flow, increased individual homozygosity, and reduced fitness of individuals in fragmented habitat. Other studies conducted at the individual level have reported reduced fitness of inbred individuals in fragmented habitat (Johansson, Primmer, & Merilä, 2007; Reed *et al.*, 2011). In our study, habitat fragments preserve what is left of a lizard population that was geographically unstructured a few decades ago, but has recently been fragmented by agricultural practices. The rate at which this process has taken place may have depended on various unknown features of the system, including effective population sizes before the onset of the fragmentation process (which may have lasted over 30 lizard generations since its maximum in the 1930s but most likely started long before). In this context, genetic effects of habitat fragmentation (reduced gene flow and inbreeding, but not detectable isolation by distance, as expected from a rapid and intense fragmentation process) emerged with detrimental consequences for individuals. Thus, fragmentation-related homozygosity was negatively correlated with two important components of lizards' fitness: adult body size and reproductive investment. The observed negative correlation between homozygosity and fitness may reflect a lifelong impact of individual genetic variability on viability and reproductive performance of lizards (Johansson *et al.*, 2007; Huisman *et al.*, 2016). Reduced reproductive value of homozygous lizards in fragmented habitat helps to directly link population genetic erosion to low individual fitness of lizards in small habitat fragments (Díaz *et al.*, 2005). In a broader context, this kind of observation helps to interpret low population growth rates often observed in fragmented populations as a result of negative genetic effects (Hanski & Saccheri, 2006; Wootton & Pfister, 2013).

In our study, genetic structure could be viewed as the consequence of infrequent interchange of lizards among habitat fragments, an interpretation which was supported by genetic evidence that individuals had limited out-crossing opportunities. Habitat fragmentation theory predicts out-crossing opportunities to fall as population size decreases with decreasing fragment size. We did not find differences in mean relatedness of individuals between large and small habitat patches, a negative result that has also been found in other lizards (Lange et al. 2013). However, supporting our predictions, lizards from the same forest fragment were genetically more related to one another than expected by chance. More importantly, we found that homozygosity was higher in small than in large forest fragments. Therefore, fragmentation may reduce genetic variability of lizards by frequent inbreeding between fragment-locked individuals, combined with stochastic effects of bottlenecks and genetic drift, which may exacerbate the loss of genetic variation in the smallest habitat fragments (Young *et al.*, 1996). Evidence of inbreeding depression was provided by a negative correlation between individual homozygosity and two relevant components of lizards' fitness: adult body size, which directly depends on growth rate and age and determines survival, male attractiveness and female fecundity in this species (Martin & Forsman, 1999; Díaz *et al.*, 2005, 2007), and female breeding investment measured as clutch mass relative to female size (Sinervo & Licht, 1991).

Although the landscape configuration and reduced size of lizard populations forced us to use an unbalanced design with small per-site sample sizes, our results provide compelling evidence of genetic deterioration as a significant cause of reduced individual fitness in this fragmented lizard population (Díaz *et al.*, 2005). We lack data of population size in our study fragments because this species is extremely hard to census (Díaz *et al.*, 2000; Santos *et al.*, 2008). Therefore, we cannot provide direct

evidence of negative population trends associated with the genetic effects detected in our study. However, indirect evidence supports the view that the association between population fragmentation, individual homozygosity and low fitness is already having a negative demographic impact on this lizard population. In our study area, fragmentation increases westwards, and the westernmost part of the forest archipelago is separated from the eastern part by a motorway (Santos *et al.*, 2008). Earlier studies of lizards in the area failed to find lizards in large (> 10 ha) fragments of favourable habitat located west of the motorway, although the size and structural characteristics of these forest fragments predicted the presence of the species (Díaz *et al.*, 2000; Santos *et al.*, 2008). Given that the motorway was built only 25 years ago, when the highway A–1 (E–50 according to European nomenclature) substituted an unfenced, single carriageway road (N–1), we interpret the apparent absence of lizards on the western side of the road as a barrier effect of the motorway preventing restoration of locally extinct populations (Tellería *et al.*, 2011), which reinforces the view that gene flow is important for the long-term sustenance of lizard populations in this area. Further supporting this idea, reintroduced lizards thrive in fragments where populations apparently went extinct in the recent past: we translocated genetically unrelated lab-born juveniles to a 4.1-ha fragment free of lizards, and five years later the introduced population had grown as shown by the presence of numerous adults and juveniles (Santos et al., 2009).

Importantly, homozygosity was negatively correlated to lizard fitness in both large and small habitat fragments. Therefore, our results support the view that negative genetic effects may reduce population viability before population size becomes too small (Spielman, Brook, & Frankham, 2004), which may compromise the adaptability of populations in fragmented habitat (Yates & Fraser, 2014; Cheptou *et al.*, 2017). In our study area, populations are less likely found in evergreen than in deciduous forest

fragments of the same size, arguably because deciduous habitat has higher thermal quality and more abundant food (Iraeta *et al.*, 2006; Santos *et al.*, 2008). Therefore, lizards may have reduced viability and fecundity in poor habitat (Díaz *et al.*, 2005; Iraeta *et al.*, 2006), where extinction risk may therefore increase for populations with lowered genetic variability. Understanding the links among reduced gene flow, individual genetic condition and fitness in a broad range of taxa may be useful if we are to rescue populations on the verge of extinction in fragmented habitat (Hedrick & Garcia-Dorado, 2016). Such knowledge may also help policymakers to decide when management actions aimed at favouring out-breeding, such as translocation of unrelated individuals (Madsen *et al.*, 1999; Santos *et al.*, 2009), will help to enhance population viability.

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AUTHOR CONTRIBUTIONS

JPT, RC, JLT, TS & JAD initiated study; JPT, RC, JLT, TS & JAD undertook fieldwork; ALG & PB conducted molecular work; JPT & ALG analysed data, JPT & JAD wrote the first draft and all authors contributed substantially with revisions.

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Figure legends:

Figure 1. (a) Location of the fragmented forest of Lerma in the Iberian Peninsula (b) Aerial view (top) and simplified map (bottom) of the 17 habitat fragments where lizards were captured, which represent 71% of sites where the species is known to occur in the area (seven sites with lizards that were not sampled for this study are shown in black; note that other sites that were not intensely monitored could also house lizard populations). (c) Cluster tree displaying Euclidean linkage distances (the vertical bar shows the scale) used to define five habitat sectors (coded with the same colours as in b) based on proximity between sampling sites. The names follow published nomenclature (Santos *et al.* 2008).

Figure 2. Regression of genome-wide (> 70k loci) against microsatellite (6 loci) measures of heterozygosity (with 95% confidence bands).

Figure 3. Average pairwise relatedness (maximum likelihood relatedness estimates based on allele sharing) between lizards captured in different or in the same habitat fragments (means ± 95% confidence intervals).

Figure 4. Average homozygosity values (means ± SE) among fully grown lizards captured in large or small forest fragments.

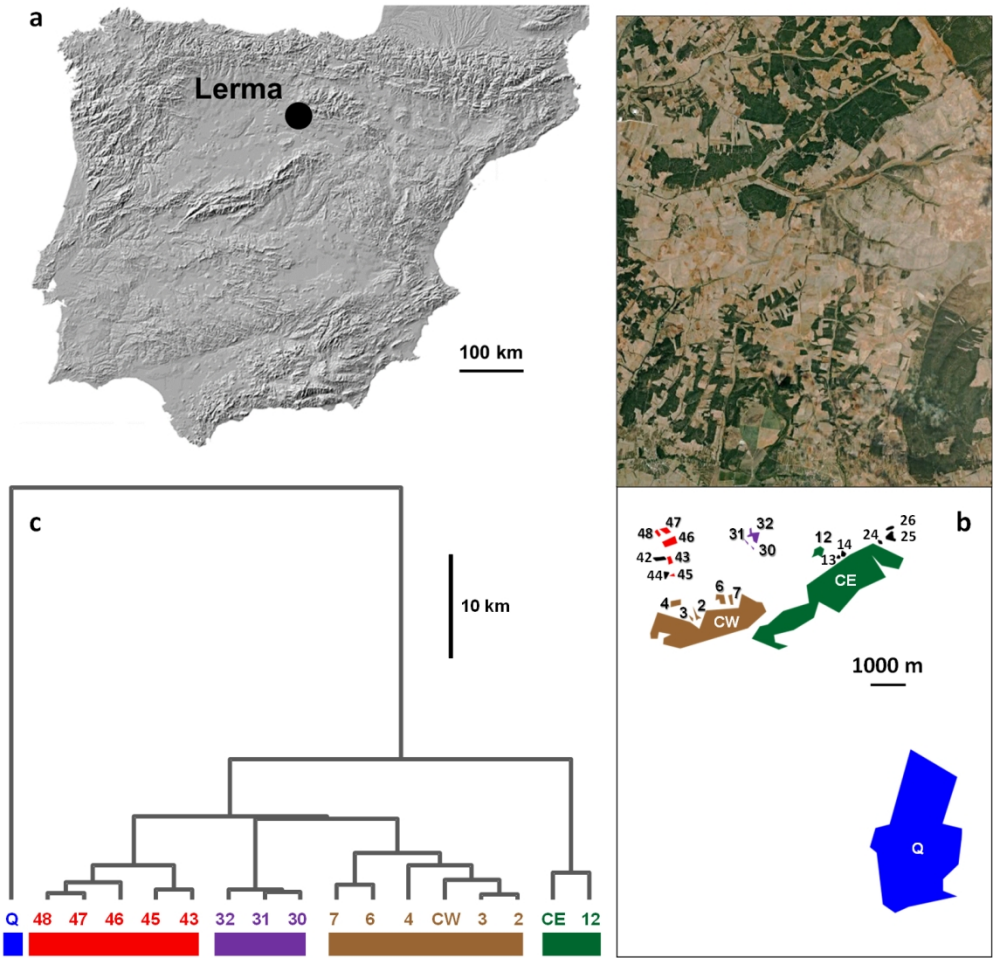
Tables:

Table 1. Basic population statistics for forest patches included in this study: site code (following published nomenclature, Santos *et al.* 2008), sector (see Fig. 1), forest type, patch size, sample size, average homozygosity (H0), average inbreeding coefficient (FIS; negative values indicate that individuals are less related than expected under a model of random mating), average allelic richness, and average relatedness.

Site	Sector	Forest type	Fragmentation	Area (ha)	Sample size (females, males, juveniles)	H0	FIS	Allelic richness	Average relatedness
Q	1	Evergreen	Forest	567	28 (17,11,0)	0.119	-0.032	1.854	0.091
43	2	Evergreen	Fragment	1.4	6 (2,4,0)	0.083	-0.115	1.831	0.151
45	2	Evergreen	Fragment	0.7	4 (3,1,0)	0.208	0.050	1.827	0.088
46	2	Evergreen	Fragment	6.5	7 (2,4,1)	0.214	0.073	1.842	0.088
47	2	Evergreen	Fragment	4.0	6 (4,2,0)	0.167	-0.024	1.816	0.069
48	2	Evergreen	Fragment	1.6	5 (2,1,2)	0.133	-0.020	1.852	0.040
30	3	Deciduous	Fragment	1.0	3 (1,2,0)	0.167	-0.017	1.822	0.028
31	3	Deciduous	Fragment	0.6	6 (4,2,0)	0.139	0.006	1.866	0.056
32	3	Deciduous	Fragment	5.2	10 (5,5,0)	0.200	0.051	1.840	0.057
CW	4	Deciduous	Forest	317	21 (13,7,1)	0.095	-0.081	1.838	0.079
2	4	Deciduous	Fragment	3.2	11 (6,5,0)	0.212	0.077	1.851	0.071
3	4	Deciduous	Fragment	0.6	2 (2,0,0)	0.000	-0.200	1.889	0.055
4	4	Deciduous	Fragment	2.6	1 (1,0,0)	0.167	----	1.833	----
6	4	Deciduous	Fragment	3.6	6 (3,3,0)	0.167	-0.031	1.811	0.091
7	4	Deciduous	Fragment	0.6	1 (1,0,0)	0.167	----	1.833	----
CE	5	Deciduous	Forest	227	7 (5,2,0)	0.095	-0.086	1.839	0.068
12	5	Deciduous	Fragment	6.8	7 (5,2,0)	0.238	0.057	1.804	0.104

Table 2. Homozygosity-fitness correlations based on estimates of homozygosity by loci (*HL*) of lizards. Effects of homozygosity on body size (measured as log snout-to-vent length) were estimated controlling for sex (females tended to have larger snout-to-vent length than males). Effects of homozygosity on clutch mass (log-transformed) were estimated controlling for body size (log snout-to-vent length). In both cases, site (nested within forest type, i.e. deciduous vs. evergreen forests) was included as a random factor to control for possible environmental differences among habitat fragments.

	body size				**clutch mass**		
	beta	F (d.f.)	*P*		beta	F (d.f.)	*P*
Homozygosity (HL)	-0.219	6.00 (1, 102)	0.016	Homozygosity (HL)	-0.248	5.10 (1, 35)	0.030
Sex		3.92 (1, 102)	0.050	Body size	0.852	62.83 (1, 35)	< 0.001
Site (nested within forest type)		1.90 (16, 102)	0.034	Site (nested within forest type)		1.79 (10, 35)	0.099



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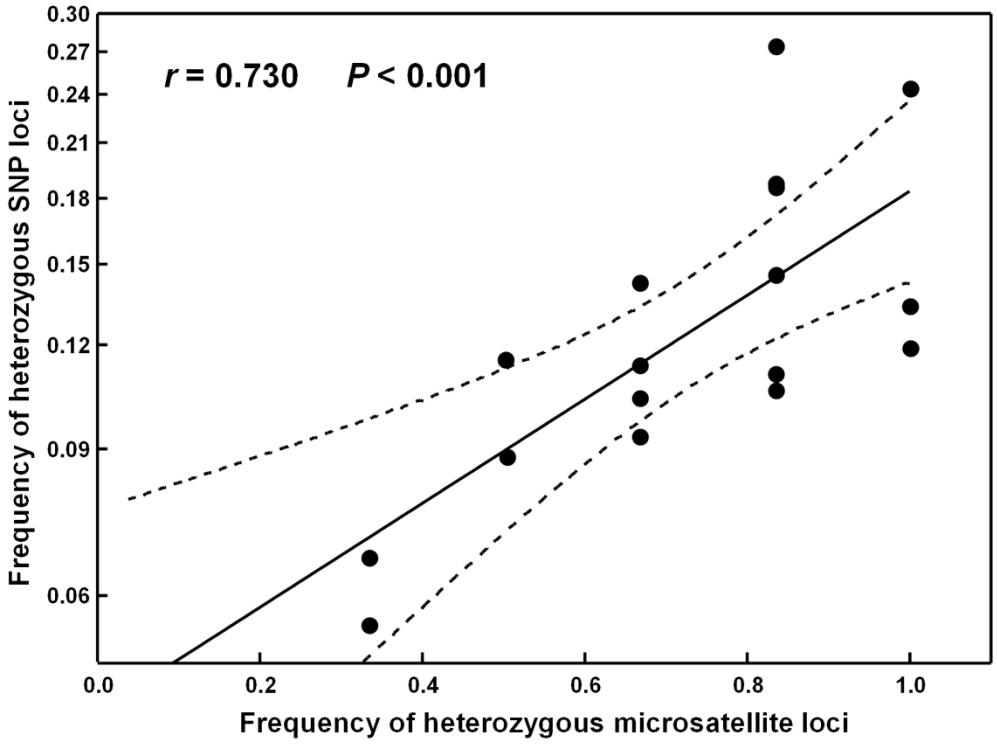

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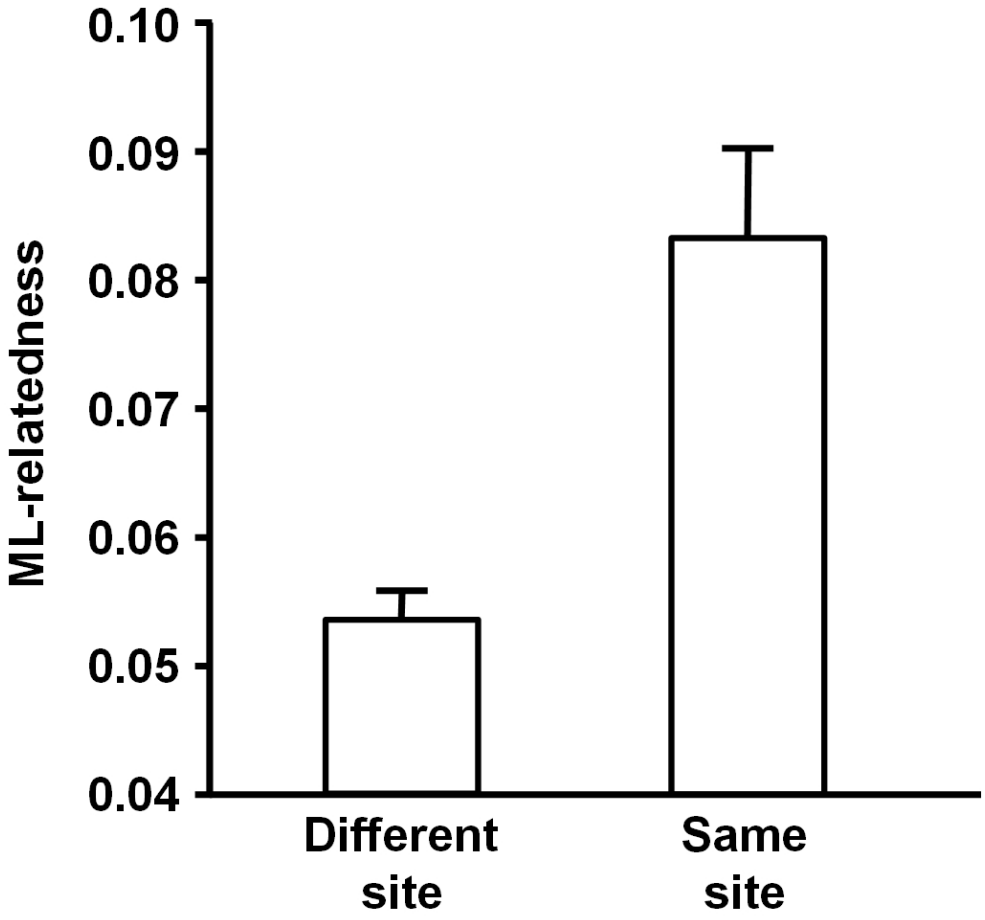

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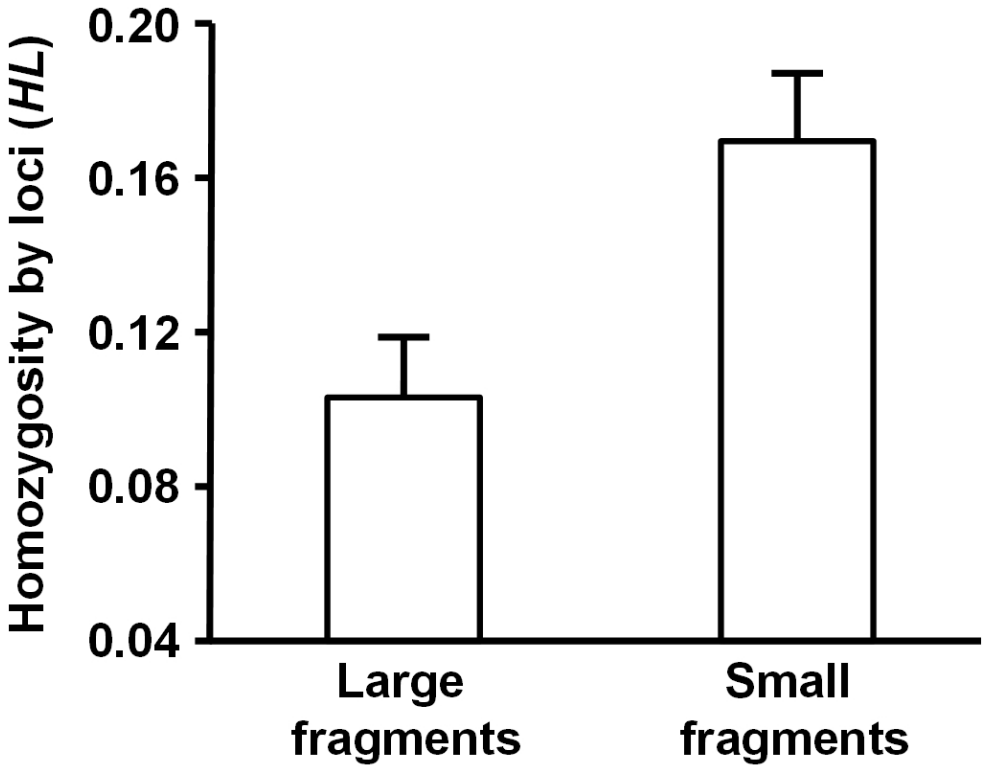

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176x144mm (150 x 150 DPI)