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Habitat fragmentation and genetic variability of tetrapod populations

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Abstract

In the last two centuries, the development of human civilization has transformed large natural areas into anthropogenic landscapes, making habitat fragmentation a pervasive feature of modern landscapes. In animal populations, habitat fragmentation may alter their genetic diversity and structure due to limited gene flow and dispersion and reduced effective population sizes, potentially leading to genetic drift in small habitat patches. We tested the hypothesis that habitat fragmentation affects genetic diversity of tetrapod populations through a meta-analysis. We also examined certain life history traits of species and particular external landscape factors that may determine the magnitude of genetic erosion observed in fragmented habitats. Our results showed that habitat fragmentation reduces overall genetic diversity of tetrapod populations. Stronger negative fragmentation effects were detected for amphibians, birds and mammals. Within each taxonomic group, species with large body size were more strongly affected by fragmentation. Particularly within mammals, we found that less vagile species with short generation times represent the most susceptible tetrapod group to lose genetic diversity in fragmented habitats. As external drivers, we found a nonsignificant trend of lower fragmentation effects in study systems of less than 50 years and stronger effects in older (>100 years) fragmented systems. As expected, the extent of habitat loss was also important in determining the magnitude of genetic erosion in tetrapods. Extreme habitat loss showed stronger negative effects on genetic diversity irrespective of taxonomic groups. The information gathered in this review also highlights research bias and gaps in the literature.

Introduction

Human activities have changed natural habitats into anthropogenic landscapes, resulting in habitat loss and fragmentation of originally continuous ecosystems. Such processes impose important changes in the structure and distribution of natural communities, which often results in the reduction of both the size and connectivity of plant and animal populations surviving in fragmented habitats (Saunders, Hobbs & Margules, 1991; Fahrig, 2003). Such rapid and drastic changes in land use across the globe represent the main driving forces behind current biodiversity loss and will continue to be so throughout the present century (Sala *et al*., 2000). Although not always properly acknowledged, genetic diversity represents one of the three forms of biodiversity. The amount of genetic diversity is crucial in determining the potential of populations to adapt and evolve in changing environments. Thus, it is important to assess the effects of habitat fragmentation on genetic diversity in order to help develop tools and strategies for the conservation of wild populations (Pertoldi, Bijlsma & Loeschcke, 2007).

After nearly three decades of research, considerable attention has been given to the effects of habitat fragmentation on population abundance and distribution of different taxonomic groups (e.g. Férnandez-Juricic, 2004). Within the last 15 years, however, there has been a growing interest in assessing the genetic consequences of habitat fragmentation (e.g. Cunningham & Moritz, 1998; Lindsay *et al*., 2008; Meyer, Kalko & Kerth, 2008). Changes in landscape configuration imposed by habitat fragmentation can affect the genetic characteristics of populations by limiting gene flow and dispersion, reducing the effective population sizes and increasing the effects of genetic drift in small habitat patches (Caizergues *et al*., 2003; Reed & Frankham, 2003), reducing genetic diversity and increasing mating between genetically

related individuals (inbreeding). As a result, the distribution of genetic diversity within and among populations (i.e. genetic structure) can change drastically. The immediate effects on genetic composition depend mainly on three factors: (1) the effective size of remaining populations; (2) the pattern of genetic diversity of the original population before fragmentation; (3) the rate of migration of individuals among patches (Bates, 2000; Meyer *et al.*, 2008).

Current evidence suggests that not all fragmentation scenarios result in genetic erosion of vertebrate populations. Landscape factors such as the extent of habitat fragmentation, the type and quality of matrix, the presence of physical barriers such as roads or fences, among others, will influence the magnitude of responses. On the other hand, life history and ecological features of animal species will also determine their ability to cope and maintain genetic variability in fragmented habitats (Cook *et al.*, 2002). For example, degree of vagility of vertebrate (tetrapod) species can be an important susceptibility trait. In this regard, amphibians and reptiles would be more likely to lose genetic diversity due to their low vagility and greater susceptibility to changes in the environment, compared with birds and mammals that may be able to move across matrices of unsuitable habitat (Moore *et al.*, 2008; Allentoft & O'Brien, 2010). Moreover, the size of mobile organisms determines the spatial scale of their habitat requirements. Tetrapod species with large body size require large foraging and reproductive areas and usually make use of different habitat types (Gurrutxaga & Lozano, 2006), which can be limited in fragmented habitats. Thus, within the same taxonomic group, large-body species may need more space, leading to lower population densities and thereby to smaller effective population sizes in fragments and consequently to a loss of genetic diversity. Furthermore, because genetic drift acts across successive generations, it is expected that species with short generation times, as some amphibians, birds and small mammals, would show signs of genetic erosion much faster than organisms with long generation time, as some birds, reptiles and large mammals (Schmeller, Schregel & Veith, 2007).

In addition to the potential susceptibility of particular life history traits of species, external drivers such as the time elapsed in fragmentation conditions and the extent of habitat fragmentation can determine the magnitude of fragmentation effects on genetic diversity of tetrapod populations. The time elapsed in fragmentation condition is an important factor to consider when evaluating genetic erosion. We may expect to observe stronger fragmentation effects on genetic variability of tetrapod populations subjected to longer periods of fragmentation conditions, where one or more generations have passed (Caizergues *et al.*, 2003; Aguilar *et al.*, 2008). Furthermore, because patch size tends to be correlated with genetic diversity (Frankham, 1995), we might expect that studies evaluating genetic consequences of fragmentation in tetrapod populations surviving in extremely fragmented habitats will show stronger effects than studies selecting less extreme or more moderately fragmented systems (Holmes *et al.*, 2013).

In this work, we conducted a quantitative review to evaluate the overall effects of habitat fragmentation on genetic diversity of tetrapod populations by testing some of the predictions of the conservation genetics paradigm. Specifically, we aim to determine: (1) the overall magnitude and direction of habitat fragmentation effects on genetic variability of tetrapod populations; (2) whether certain life history traits of species within the same taxonomic group, such as vagility, body size and generation times of species, determine the magnitude of fragmentation effects on genetic diversity; (3) whether external drivers such as the time elapsed in fragmentation conditions and the degree of habitat fragmentation also guide the magnitude of effects on genetic diversity.

Methods

Literature search

We conducted a systematic literature search comprising the period 1989–2013 through several databases such as Cambridge Scientific Abstracts, Science Citation Index, Searchable Ornithological Research Archive and databases of Biological Abstracts, and major publishers (Blackwell Science, Springer-Verlag and Elsevier) and scientific societies that group the most relevant journals in ecology, biology and conservation genetics. For this review, we concentrated on tetrapods (amphibians, reptiles, birds and mammals). We used a combination of the following keywords for conducting the literature search: (fragment* or 'habitat loss') and ('genetic diversity' or 'inbreeding') and ('vertebrate*' or 'amphibian*' or 'reptile*' or 'bird*' or 'mammal*'). We obtained 462 studies that were examined to determine whether they met the requirements for entry into the meta-analysis.

Because the process of anthropogenic habitat fragmentation produces habitat loss, reduces population size and increases isolation between populations, our review allowed the inclusion of studies analyzing any of these measures of fragmentation. We later evaluated the relative effects of each of these fragmentation parameters on genetic diversity. We only included studies that compared fragmented habitats with large extensions of continuous habitat. We excluded papers that analyzed correlations between population size and genetic variability with no explicit mentions to the effects of habitat fragmentation and also those studies assessing historically natural non-anthropogenic habitat fragmentation, which has taken place thousands of years ago.

In studies using codominant markers (i.e. microsatellites and allozymes), the measures of genetic variability considered were: expected heterozygosity (He), number of alleles (A) and inbreeding coefficient (F_{IS}). In studies using dominant markers [namely DNAmt sequences, random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphism (AFLPs)], we used molecular variance or gene diversity and these parameters were analyzed together with expected heterozygosity (Aguilar *et al.*, 2008). These genetic parameters were not necessarily evaluated

altogether within the same study, so the sample sizes for each of these genetic parameters in the meta-analyses were different. In studies that did not provide the inbreeding coefficient, it was calculated using the expected (*He*) and observed (*Ho*) heterozygosity ($F_{IS} = He - Ho/He$).

For each vertebrate species studied, we collected information on certain life history traits such as vagility, generation time and body sizes to compare their relative effect size within each taxonomic group. Information on these three traits for each species was searched within each study and in other literature sources (online databases) using the species name as the keyword search. We used continuous values for each of these three moderator variables (vagility, generation time and body size) to examine their potential relationship with effect sizes by means of meta-regressions (see below). In cases where range values were provided for any of these variables, we used an estimated mean to have a unique value. For some species, we were not able to find information on one or more of these traits, so analyses were conducted only with the species where such information was available (78–84% of species). Therefore, meta-analyses on these moderator variables differed in their sample size.

We further searched in each paper for information regarding the time elapsed in fragmentation conditions, which included rough estimates of the onset of fragmentation events given by the authors (estimated in few decades or centuries) and of time periods elapsed. With this information, we created three categories (under 50 years, between 50 and 100 years, and more than 100 years). Finally, because the studies varied in their extent or degree of habitat fragmentation, we created two broad categories (moderate and extreme habitat loss) to compare the magnitude of effect sizes. Following Winfree *et al.* (2009), we categorized as 'extreme habitat loss' studies in which the fragmented site was either of less than 5 ha in area, surrounded by less than 5% continuous original habitat or located at more than 5 km from the nearest continuous original habitat. 'Moderate habitat loss' category refers to study systems where these landscape parameters were less extreme.

Some authors assessed habitat fragmentation effects on genetic parameters in more than one species within the same paper and we included all these species in our meta-analysis. Because the magnitude and sometimes the direction of genetic responses to habitat fragmentation in each species within the same study were quite different, it is reasonable to assume that the effects are independent for each species (Gurevitch & Hedges, 2001; Aguilar *et al.*, 2008).

Data analysis

We used a categorical meta-analysis approach to assess population genetic parameters of tetrapods in two contrasting habitat conditions (fragmented habitats vs. continuous habitats), thus we obtained genetic parameters (*He*, *A* and F_{IS}) data from tetrapod populations living in fragmented and continuous habitat conditions. With these data, which were taken from the text, tables or graphs, we obtained mean values and standard deviations within each habitat condition. From each study, the magnitude of fragmentation effects on each genetic parameter was quantified by calculating Hedges' *d* (Gurevitch & Hedges, 2001). The effect size (*d*) can be interpreted as the difference between the genetic diversity of vertebrate populations in fragmented and continuous habitats measured in standard deviation units (Gurevitch & Hedges, 2001, see Aguilar *et al.*, 2008 for formula description).

We ran separate meta-analyses for each of the different genetic parameters assessed in each study. Negative values for the effect size (*d*) of *He* and *A* imply negative effects of habitat fragmentation on these parameters, while positive values of *d* imply positive effects of fragmentation. The interpretation of the direction of effect size for inbreeding coefficient (F_{IS}) is exactly the opposite; positive values of *d* imply negative effects of habitat fragmentation (high inbreeding), while negative values of *d* indicate positive effects of fragmentation (low inbreeding) (Aguilar *et al.*, 2008).

To analyze whether vagility, generation time and body size influence the magnitude of effects, we ran meta-regressions assessing the relationships between the effect size (Hedges' *d*) calculated for each species and the corresponding vagility, generation time or body size values. Previous to running meta-regressions, we log transformed these three parameters. To compare the relative effects of the two external drivers (time elapsed and extent of fragmentation), we used categorical comparisons using *Q* statistics (see below).

MetaWin software version 2.0 (Rosenberg, Adams & Gurevitch, 2000) was used to run the analyses and bootstrap resampling procedures as described in Adams, Gurevitch & Rosenberg (1997) and to calculate confidence intervals (CIs) of effect sizes. The effects of habitat fragmentation were considered significant if the 95% biased-corrected bootstrap CIs of the effect size (*d*) did not overlap zero (Rosenberg *et al.*, 2000). CIs based on resampling CI estimates are more conservative (Adams *et al.*, 1997). The data were analyzed with a random effects model, assuming that differences between studies are due to sampling errors and also to random variation (Raudenbush, 1994). The heterogeneity of effect sizes was evaluated with *Q* statistics (Gurevitch & Hedges, 2001). Specifically, we examined the *P*-values associated with Q_{between} statistics, which describe the variation in effect sizes attributed to differences between the categorical predictors (e.g. time elapsed in fragmentation conditions and extent of fragmentation).

Publication bias

Different methods were used to detect potential publication bias, first graphically (funnel plots and weighted histograms), and secondly by weighted calculation of the fail-safe numbers (Rosenberg *et al.*, 2000; Rosenberg, 2005). If the calculated fail-safe number is greater than $5n + 10$, where *n* is the number of studies, then publication bias can be ignored because the results are robust regardless of publication bias (Rosenberg, 2005).

Phylogenetic meta-analysis

In any meta-analysis involving multiple species, it is crucial to consider the phylogenetic relationships among them, because more closely related species may share similar response to the same factor (Chamberlain *et al.*, 2012). We used PhyloMeta software version 1.3 to conduct phylogenetically independent meta-analyses (Lajeunesse, 2011). Before running the analyses, we constructed a main phylogenetic tree for all tetrapod species included in this review (Supporting Information Appendix S1) using cytochrome b sequences for each species, retrieved from the GenBank database and aligned using the ClustalW algorithm (Thompson, Higgins & Gibson, 1994). We used 720 bp to estimate the length of the tree branches covering all species included in this study using PAUP 4 beta 10 (Swofford, 2003), and phylogenetic relationships were inferred under criterion of maximum likelihood (Felsenstein, 1981). The appropriate model of nucleotide substitution was selected with the Akaike information criterion using the software MrMTgui 1.0 (Nuin, 2008). The best model of nucleotide substitution for the analysis was the general time reversible model (GTR+1+G) (Lanave *et al.*, 1984).

The main tree was obtained using ultrametric length branches, adjusted to one (Sanderson, 2002) using R 2.9.2 (Paradist, Claude & Strimmer, 2004). Because we did not obtain the same kind of information for every tetrapod species included within the main phylogenetic tree (genetic parameters and moderator variables), we had to construct different sub-trees when running meta-analyses for each genetic parameter (A, He or F_{IS}) and when analyzing moderator variables (e.g. body size, time elapsed in fragmentation conditions). These sub-trees were obtained by trimming taxa off the main tree with the software Prunetree 5.0 (Lajeunesse lab synthesis and parasites, Tampa, FL, USA), so that the resulting sub-tree only contained the species used for a particular analysis (e.g. phylogenetically independent meta-analysis for A had 77 species whereas for He had 99 species, each of them with a particular phylogenetic sub-tree). Some of the tetrapod species were evaluated by more than one author (see Supporting Information Appendix S2). For the phylogenetic meta-analysis, we pooled these multiple effect sizes per species using a traditional meta-analysis with a fixed effects model (Koricheva, Gurevitch & Mengersen, 2013), so that we used one effect size per species. We used the model selection criteria (MSC) to compare model fit between the conventional meta-analysis and the phylogenetic-independent meta-analysis (Lajeunesse, 2011). The model with the smallest MSC was selected as the best fitting the data (Hedges & Olkin, 1985).

Results

Conventional and phylogenetic meta-analyses

The conventional meta-analysis provided a significantly better-fit model than the phylogenetically corrected meta-analysis (He: MSC = 296.23 vs. 335.21; A: MSC = 229.11 vs. 245.52; F_{IS}: MSC = 139.97 vs. 174.15), suggesting that phylogenetic structure is not influencing the variation among effects sizes and thus we only show the results from the conventional meta-analyses.

Sample of studies

We obtained a total of 94 scientific publications that evaluated the effect of habitat fragmentation on genetic diversity of tetrapod populations. These studies measured at least one genetic parameter in 92 species of vertebrates, of which 12.6% were amphibians, 20.0% were reptiles, 31.6% were birds and 35.8% were mammals. Some species were studied more than once by different authors, thus we obtained a total of 99 data points for the traditional meta-analysis for the expected heterozygosity (He), 77 for the number of alleles (A) and 49 for the inbreeding coefficient (F_{IS}). Most of the studies used microsatellites (75%) as genetic markers to assess the effect of habitat fragmentation on genetic variability, 11% used sequences, 9% used allozymes and 5% used RAPDs/AFLPs. Statistical comparisons of effect sizes obtained from different molecular markers showed no significant differences among them (He: $Q_{\text{between}} = 1.03$, $P = 0.411$; A: $Q_{\text{between}} = 0.18$, $P = 0.951$; F_{IS}: $Q_{\text{between}} = 0.75$, $P = 0.621$), implying that all markers used are comparable in detecting changes in genetic diversity in fragmented habitats.

The weighted histograms of He, A and F_{IS}, showed unimodal distributions with the highest frequency around zero, and the graph of effect size versus sample size showed a symmetric funnel shape, indicating no publication bias in our sample (figures not shown). Similarly, the fail-safe numbers calculated for each meta-analysis were always greater than $5n + 10$ (He: $4668.8 > (5 \times 99) + 10 = 505$; A: $4103.1 > (5 \times 77) + 10 = 395$; F_{IS}: $839.3 > (5 \times 49) + 10 = 255$), reinforcing the robustness of these results.

Overall, the average weighted effect sizes of habitat fragmentation on He and A were negative and significantly different from zero (Fig. 1). Although F_{IS} showed a trend towards increased inbreeding due to habitat fragmentation, this effect was not significantly different from zero (Fig. 1).

When looking separately at each vertebrate group, we found that fragmentation effects on He were significantly negative for amphibians, mammals and birds, whereas for reptiles overall mean effect was nonsignificant (Fig. 2). Overall effects on A were significantly negative for all four taxonomic groups (Fig. 2). Fragmentation effects on inbreeding coefficient (F_{IS}) were consistently nonsignificant for all vertebrate groups (Fig. 2). Amphibians and reptiles were the least represented groups and their overall effect estimations may be less precise than the other two groups.

The analysis of vagility within amphibians, birds and reptiles showed no significant relationships between fragmentation effects on any of the genetic parameters (He, A and F_{IS}; not shown). For mammals, however, we found significant meta-regressions for A ($Y_d = -1.87 + 0.605X_{(\text{vagility})}$; $r^2 = 0.645$, $P = 0.001$, $n = 24$; Fig. 3) and

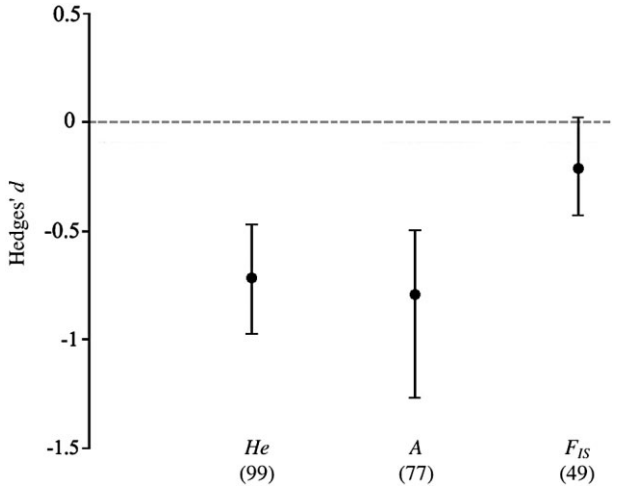

Figure 1 Overall weighted mean effect sizes and 95% bias-corrected confidence intervals (CIs) of habitat fragmentation on expected heterozygosity (*He*), number of alleles (*A*) and inbreeding coefficient (F_{IS}). Sample sizes for each meta-analysis are shown in parenthesis; dotted line indicates Hedges' $d = 0$.

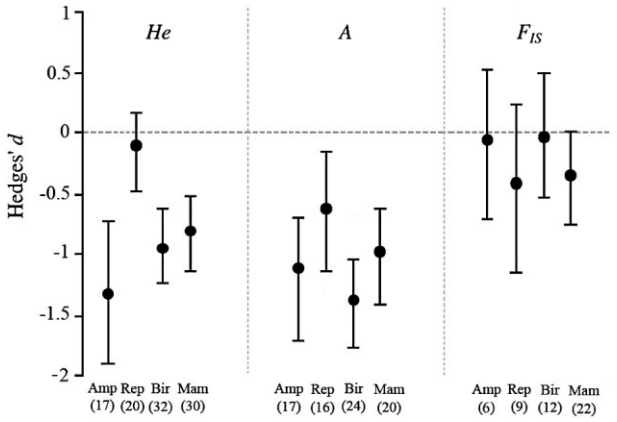

Figure 2 Weighted mean effect sizes and 95% bias-corrected confidence interval (CI) of habitat fragmentation effects on *He*, *A* and F_{IS} in different tetrapod groups (Amp = amphibians, Rep = reptiles, Bir = birds, Mam = mammals). Sample sizes for each group are given in parentheses; dotted line indicates Hedges' $d = 0$.

F_{IS} ($Y_d = 0.391–0.631X_{(\text{vagility})}$; $r^2 = 0.78$, $P = 0.002$, $n = 19$). Less vagile mammals showed stronger negative effects on *A*, and these effects decreased in species with increased vagility (Fig. 3). In the case of F_{IS}, stronger positive effects (higher inbreeding coefficients) were detected in less vagile species, and these effects decreased in more vagile species (Fig. 3). Both meta-regressions consistently indicate a higher susceptibility of genetic erosion with decreased vagility of mammal species.

Generation time of species within each tetrapod group did not drive significant differential susceptibility to losing genetic diversity in fragmented habitats. The only exception was within the mammal group, which showed a significant

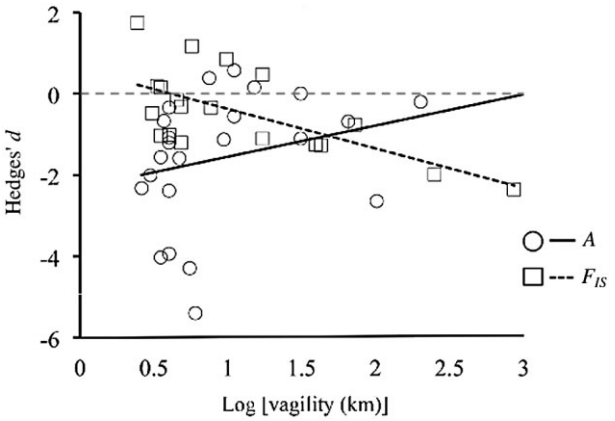

Figure 3 Relationships between the log-transformed values of vagility of mammals and the effect sizes of fragmentation on *A* ($r^2 = 0.645$, $P = 0.001$) and F_{IS} ($r^2 = 0.78$, $P = 0.002$).

relationship between effect sizes on F_{IS} and generation time of mammal species ($Y_d = 0.295–1.46_{(\text{generation time})}$; $r^2 = 0.42$; $P = 0.02$, $n = 21$). That is, species with shorter generation times showed stronger increases on inbreeding coefficients, while species with longer generation times showed weaker effects of fragmentation on F_{IS}.

The evaluation of body size within each tetrapod group revealed that fragmentation effects on *He* were negatively significantly related to body size of amphibians, birds and reptiles (amphibians: $Y_d = 0.724–2.441_{(\text{body size})}$; $r^2 = 0.123$, $P = 0.011$, $n = 16$; birds: $Y_d = 0.196–1.933_{(\text{body size})}$; $r^2 = 0.395$, $P = 0.001$, $n = 32$; reptiles: $Y_d = 1.568–1.445_{(\text{body size})}$; $r^2 = 0.248$, $P = 0.005$, $n = 21$; Fig. 4a). Thus, stronger negative fragmentation effects on *He* were observed in larger sized amphibians, birds and reptiles. When analyzing *A*, all tetrapod groups showed significant relationships between body size and the magnitude of fragmentation effects (amphibians: $Y_d = 3.821–5.232_{(\text{body size})}$; $r^2 = 0.255$, $P = 0.048$, $n = 14$; birds: $Y_d = 2.007–2.292_{(\text{body size})}$; $r^2 = 0.404$, $P = 0.006$, $n = 20$; reptiles: $Y_d = 2.091–2.179_{(\text{body size})}$; $r^2 = 0.308$, $P = 0.001$, $n = 17$; mammals: $Y_d = 0.195–1.273_{(\text{body size})}$; $r^2 = 0.126$, $P = 0.031$, $n = 27$; Fig. 4b). The response patterns remain as before, with stronger negative effect sizes as body size increases (Fig. 4b). In all cases, however, the proportion of variation explained by body size on fragmentation effects on *A* and *He* was moderate (r^2 range 0.123–0.404).

For the three genetic parameters evaluated, between 50 and 60% of the studies gave information about the time elapsed in fragmentation condition. While there was a trend of overall lower effect sizes in fragmented systems of less than 50 years for *He*, on average fragmentation effects did not statistically differ among the three time elapsed fragmentation categories (not shown). In contrast, when analyzing the extent of habitat fragmentation, we found that studies conducted in extremely fragmented habitats showed significantly stronger effects for *A* ($Q_{\text{between}} = 3.69$, $P = 0.007$). Although for *He* and F_{IS} there was a similar

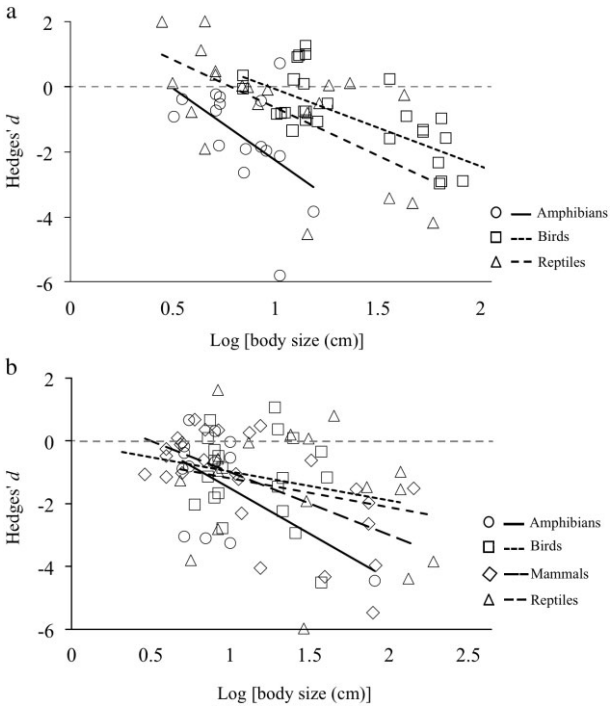

Figure 4 Relationships between the log-transformed values of body size of tetrapods and the effect sizes of fragmentation on (a) *He* [amphibians (r^2 = 0.123, P = 0.011); birds (r^2 = 0.395, P = 0.001); reptiles (r^2 = 0.248, P = 0.005)] and on (b) *A* [amphibians (r^2 = 0.255, P = 0.048); birds (r^2 = 0.404, P = 0.006); reptiles (r^2 = 0.308, P = 0.001); mammals (r^2 = 0.126, P = 0.031)].

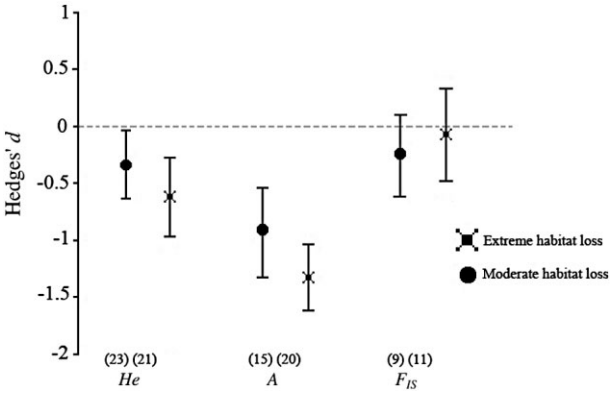

Figure 5 Weighted mean effect sizes and 95% bias-corrected confidence interval (CI) of habitat fragmentation effects on *He*, *A* and F_{IS} of tetrapod populations subjected to different extent of habitat fragmentation (extreme and moderate habitat loss). Sample sizes for each group are given in parentheses; dotted line indicates Hedges' d = 0.

trend of weaker effects in less extremely fragmented habitats, these effects were nonsignificant (*He*: $Q_{between} = 2.364$, $P = 0.501$; F_{IS}: $Q_{between} = 0.268$, $P = 0.634$) (Fig. 5).

Discussion

In this study, we showed that habitat fragmentation reduces overall genetic diversity of tetrapod populations. The four groups of tetrapods showed similar negative fragmentation effects in allelic richness. Although relatively smaller effect sizes were calculated for amphibians and reptiles, we still detected lower genetic diversity in fragmented habitats. Decreases in allelic richness are usually the immediate result of sudden population reductions due to habitat loss and fragmentation, generating bottlenecks on genetic variation. The impact of bottlenecks in genetic variation depends primarily on two factors: the effective size of the population when the bottleneck started and the time during which the population is kept small. Drastic reduction in the effective size of populations caused by habitat fragmentation reduces the genetic variation of remaining populations. If no gene flow is maintained among them, these remaining populations will keep losing genetic variation in the following generations through genetic drift (Hoelzel, 1999). We also observed negative fragmentation effects on the expected heterozygosity in amphibians, birds and mammals, but not in reptiles. Reduced expected heterozygosity in fragmented populations can be the result of genetic drift. When populations remain small and isolated for some generations, reductions in genetic variability occur by random elimination of rare alleles, affecting the number and frequencies of alleles (Caizergues *et al*., 2003; Reed & Frankham, 2003).

In contrast to the genetic diversity parameters, we did not observe significant changes in the inbreeding coefficients in fragmented habitats. In the vast majority of the studies included here, the inbreeding coefficients were estimated based on adult genotypes (as stated by the authors), not on progeny, thus mostly reflecting mating patterns of long-lived adult individuals, which may not yet show signs of inbreeding. Therefore, it would be particularly interesting in future studies to determine inbreeding exclusively on progeny generated in fragmented habitats. This way we may be able to detect changes in mating patterns towards increased biparental inbreeding as a result of new habitat configurations imposed by habitat fragmentation (Aguilar *et al*., 2008).

We observed that amphibian populations surviving in fragmented conditions showed a stronger decrease in genetic diversity, especially in expected heterozygosity. Because of their inherent low vagility, amphibian populations can be especially affected by decreased connectivity in fragmented habitats, strongly limiting gene flow between populations (Saunders *et al*., 1991; Allentoft & O'Brien, 2010). Moreover, amphibians are comparatively shorter lived, thus individuals living in fragments would undergo stronger genetic drift affecting their expected heterozygosity more strongly than the rest of the tetrapods (Cushman, 2006). Furthermore, lower genetic diversity in fragments may be due to other specific life history traits, such as their need to spend the first part of their life cycle in water, which considerably constraints their vagility capacity as adults, making them more vulnerable to fragmentation of their

habitats (Beebee & Griffiths, 2005). The loss of genetic diversity in amphibian populations has been little recognized as a potential factor in their worldwide decline. Our results suggest that genetic erosion imposed by habitat fragmentation may play an important role in the rate of species loss of amphibians (e.g. Allentoft & O'Brien, 2010).

In reptiles, we only observed fragmentation effects in allelic richness. The lack of a significant decrease in expected heterozygosity of fragmented reptile populations may be due to their relatively long generation times and high population densities even in fragmented landscapes (McCoy *et al*., 2010). In fact, by taking a close look at the 20 species included in this review, the reptile group showed the longest average generation time compared with the other three groups. In addition, only three of the studies in this group assessed fragmented systems of more than 100 years. Thus, the combination of these two variables within our sample of reptile studies may have influenced the results observed. Another potential reason may be due to taxonomic bias of the studied species within reptiles. Most of the species belong to the suborder *saurians* (lizards), which have higher mobility compared with the suborder ophidians (snakes) that have been less well studied (e.g. Cunningham & Moritz, 1998; Moore *et al*., 2008; Marsack & Swanson, 2009; McCoy *et al*., 2010).

The observed negative effects of habitat fragmentation on the genetic diversity of birds are surprising, given that this group is considered highly vagile and presumably able to cross large areas of unsuitable habitat compared with the other tetrapod groups (Avise, 1996; Wang & Schreiber, 2001). Most of the studies until now have been conducted in bird species of the orders Passeriformes and Galliformes. Within the Passeriformes, there is high incidence of bird species with restricted flight capacity and specific habitat requirements (Avise, 1996; Kurtis, Fahrig & Merriam, 1999). Therefore, for this particular taxonomic group, habitat fragmentation may reduce gene flow between remnant populations, thereby increasing genetic drift (e.g. Bates, 2000; Mercival *et al*., 2007; Lindsay *et al*., 2008; MacDougall-Shackleton *et al*., 2011). However, habitat fragmentation may also affect birds that have greater vagility as is the case of Galliformes. This group of birds has specific reproductive habitat requirements, and only fly short distances (not more than 80 m) at low altitudes (up to 2 m) to move among different habitats. Thus, habitat fragmentation can similarly impede movements and erode genetic diversity. Members of this group also tend to have low effective population sizes as a result of both historical hunting pressures and recent habitat loss (e.g. Caizergues *et al*., 2003; Bech *et al*., 2009).

Like amphibians and birds, mammals had lower genetic diversity in fragmented environments. The majority (65%) of species studied are small mammals that are particularly sensitive to environmental perturbations. Such biological characteristics make them particularly vulnerable because isolated populations of small mammals are less capable of dispersing across the inhospitable matrix, restricting gene flow, increasing genetic drift and inbreeding, thereby leading to loss of genetic variability (e.g. Lada, Nally & Taylor, 2008; Meyer *et al*., 2008; Olivieri *et al*., 2008; Pacioni, Wayne & Spencer, 2011).

Contrary to expectation, vagility and generation time of tetrapods did not drive differential susceptibility to losing genetic diversity in fragmented habitats. Mammals were the only tetrapod group showing significant relationships in the hypothesized direction of these two traits with the magnitude of fragmentation effects on genetic variability. A probable explanation for such results may lay in the relative range scale of vagility and generation time within the mammal species included in our review. By far, mammals showed the greatest range of variation in all life history traits assessed, as they included species of very small rodents as well as bears, gorillas and horses (Supporting Information Appendix S1). In contrast to the other tetrapod groups, vagility and generation time were relatively more homogeneous among species. Therefore, vagility and generation time are detectable susceptibility traits to lose genetic diversity in fragmented systems only within mammal species.

In accordance with our expectation, genetic variability of species with large body size within each tetrapod group was more strongly affected by habitat fragmentation than that of small-bodied species. Body size is positively related to the range of distribution, as larger species require more habitats for feeding and breeding. Furthermore, large-sized species usually occur in low densities. Therefore, larger spatial requirements together with lower population densities may make large-sized species particularly susceptible to genetic erosion in fragmented habitats (Bergl *et al*., 2008). In addition, bird and mammal species of large body size in particular have reproductive traits such as low number of offspring per reproductive event and longer time to reach sexual maturity, which can also increase genetic erosion susceptibility. However, the large-sized species typically present longer generation times than smaller species, which should lead to a delayed manifestation of fragmentation effects and may become evident in the future (Frankham, 1995; Prugh *et al*., 2008).

Finally, we observed that the time elapsed in fragmentation conditions and the extent of habitat fragmentation are important factors determining the magnitude of effects observed on genetic diversity of tetrapod populations. However, because we were only able to gather information about these factors in a subset of the studies within this review, the analyses were limited in their power. Future research should systematically incorporate information about the fragmented systems they study, which will help make more robust conclusions about potential fragmentation thresholds of spatial scales and time frames.

Conservation implications

The controversy about whether ecological and demographic factors are more important than genetic factors for the decline and extinction of populations or even species has been recently evaluated (Frankham, Ballou & Briscoe, 2003;

Spielman, Brook & Frankham, 2004). Most taxa are not driven to extinction before genetic factors have been negatively affected (Spielman *et al*., 2004). Currently, the main causes triggering increased extinction risks of animal species are anthropogenic, either through land use changes or indiscriminate hunting. In our review, we found that tetrapod species surviving in fragmented habitats are, overall, likely to suffer genetic erosion, compared with populations living in continuous habitats. Therefore, it is crucial to identify susceptible tetrapod groups of species that may experience lower evolutionary potential due to specialized ecological requirements and life history traits.

Here we observed that habitat fragmentation reduces allelic richness of all tetrapod groups evaluated, and also the genetic diversity expressed as expected heterozygosity of amphibian, bird and mammal populations. Our results indicate that less vagile mammal species with short generation times represent the most susceptible tetrapod group to genetic erosion in fragmented habitats. Moreover, large-bodied species living in highly fragmented systems are particularly prone to suffer strong genetic erosion, regardless of their taxonomic identity. The information gathered in this quantitative review should help identify and determine the extinction risk of wild populations and to prioritize conservation efforts (Aguilar *et al*., 2008).

Despite these unequivocal signs of fragmentation effects on genetic variability, there is a clear gap in the literature of population genetics of tetrapods that prevents additional generalizations. Most data come from adults, and their genetic makeup may differ from that of their progeny that have been subjected to fragmentation conditions. We call upon an increase in studies assessing genetic effects on tetrapod progeny, which will allow us to estimate mating and gene flow patterns in fragmented conditions, and to assess how changes in mating patterns may affect the genetic diversity of future generations of tetrapod populations.

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References

Adams, D.C., Gurevitch, J. & Rosenberg, M.S. (1997). Resampling tests for meta-analysis of ecological data. *Ecology* **78**, 1277–1283.

Aguilar, R., Quesada, M., Ashworth, L., Herrerías-Diego, Y. & Lobo, J. (2008). Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Mol. Ecol.* **17**, 5177–5188.

Allentoft, M.E. & O'Brien, J. (2010). Global amphibian declines, loss of genetic diversity and fitness: a review. *Divers. Distrib.* **2**, 47–71.

Avise, J.C. (1996). Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States. In *Conservation genetics: case histories from nature*: 431–470. Avise, J.C. & Hamrick, J.L. (Eds). New York: Chapman & Hall.

Bates, J.M. (2000). Allozymic genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. *Condor* **102**, 770–783.

Bech, N., Boissier, J., Drovetski, S. & Novoa, C. (2009). Population genetic structure of rock ptarmigan in the 'sky islands' of French Pyrenees: implications for conservation. *Anim. Conserv.* **12**, 138–146.

Beebee, J.C. & Griffiths, R.A. (2005). The amphibian decline crisis: a watershed for conservation biology? *Biol. Conserv.* **125**, 271–285.

Bergl, R.A., Bradley, B.J., Nsubuga, A. & Vigilant, L. (2008). Effects of habitat fragmentation, population size and demographic history on genetic diversity: the cross-river gorilla in a comparative context. *Am. J. Primatol.* **70**, 848–859.

Caizergues, A., Rätti, O., Helle, P., Rotelli, L., Ellison, L. & Rasplus, J.Y. (2003). Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented versus continuous landscapes. *Mol. Ecol.* **12**, 2297–2305.

Chamberlain, S.A., Hovick, S.M., Dibble, C.J., Rasmussen, N.L., Van Allen, B.G., Maitner, B.S., Ahern, J.R., Bell-Dereske, L.P., Roy, C.L., Meza-Lopez, M., Carrillo, J., Siemann, E., Lajeunesse, M.J. & Whitney, K.D. (2012). Does phylogeny matter? Assessing the impact of phylogenetic information in ecological meta-analysis. *Ecol. Lett.* **15**, 627–636.

Cook, W.M., Lane, K.T., Foster, B.L. & Holt, R.D. (2002). Island theory, matrix effects and species richness in habitat fragments. *Ecol. Lett.* **5**, 619–623.

Cunningham, M. & Moritz, C. (1998). Genetic effects of forest fragmentation on a rainforest restricted lizard (Scincidae, *Gnypetoscincus queenslandiae*). *Biol. Conserv.* **83**, 19–30.

Cushman, S.A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biol. Conserv.* **128**, 231–240.

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. Syst.* **34**, 487–515.

Férnandez-Juricic, E. (2004). Spatial and temporal analysis of the distribution of forest specialists in an urban-fragmented landscape (Madrid, Spain): implications for local and regional bird conservation. *Landsc. Urban Plan.* **69**, 17–32.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genet. Res.* **66**, 95–107.

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2003). *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Gurevitch, J. & Hedges, L.V. (2001). Meta-analysis: combining the results of independent experiments. In *Design and analysis of ecological experiments*: 347–369. Scheiner, S.M. & Gurevitch, J. (Eds). Oxford: Oxford University Press.

Gurrutxaga, M.V. & Lozano, V.P. (2006). Efectos de la fragmentación del habitat y pérdida de la conectividad ecológica dentro de la dinamica territorial. *Pol. Rev. Geog.* **16**, 35–54.

Hedges, L.V. & Olkin, I. (1985). *Statistical methods for meta-analysis*. Orlando: Academic Press.

Hoelzel, A.R. (1999). Impact of population bottlenecks on genetic variation and the importance of life-history: a case study of the northern elephant seal. *Biol. J. Linn. Soc.* **68**, 23–39.

Holmes, S.M., Baden, L.A., Brenneman, R.A., Engberg, S.E., Louis, E.E. Jr. & Johson, E.S. (2013). Patch size and isolation influence genetic patterns in black-and-white ruffed lemur (*Varecia variegate*) populations. *Conserv. Genet.* **14**, 615–624.

Koricheva, J., Gurevitch, J. & Mengersen, K. (2013). *Handbook of meta-analysis in ecology and evolution*. Princeton & Oxford: Princeton University Press.

Kurtis, T.M., Fahrig, L. & Merriam, G. (1999). Independent effects of forest cover and fragmentation on the distribution of forest breeding birds. *Ecol. Appl.* **9**, 586–593.

Lada, H., Nally, R.M. & Taylor, A.C. (2008). Responses of a carnivorous marsupial (*Antechinus flavipes*) to local habitat factors in two forest types. *J. Mammal.* **89**, 398–407.

Lajeunesse, M.J. (2011). PhyloMeta: a program for phylogenetic comparative analyses with meta-analysis. *Bioinformatics (Ofx)* **27**, 2603–2604.

Lanave, C., Preparata, G., Saccone, C. & Serio, G. (1984). A new method for calculating evolutionary substitution rates. *J. Mol. Evol.* **20**, 86–93.

Lindsay, D.L., Barr, K.R., Lance, R.F., Tweddale, S.A., Hayden, T.J. & Leberg, P.L. (2008). Habitat fragmentation and genetic diversity of an endangered, migratory songbird, the golden-cheeked warbler (*Dendroica chrysoparia*). *Mol. Ecol.* **17**, 2122–2133.

MacDougall-Shackleton, E.A., Clinchy, M., Zanette, L. & Neff, B.D. (2011). Songbird genetic diversity is lower in anthropogenically versus naturally fragmented landscapes. *Conserv. Genet.* **12**, 1195–1203.

Marsack, K. & Swanson, B.J. (2009). A genetic analysis of the impact of generation time and road-based habitat fragmentation on eastern box turtles (*Terrapene c. carolina*). *Copeia* **4**, 647–652.

McCoy, E.D., Mushinsky, H.R., Shockley, W.J. & Alvarez, M.R. (2010). Skeletochronology of the threatened Florida sand skink, Plestiodon (Neoseps) reynoldsi. *Copeia* **2010**, 38–40.

Mercival, R.F., Giggs, H.L., Galetti, M., Lunardi, V. & Galetti, P.M. Jr. (2007). Genetic structure in a tropical lek-breeding bird, the blue manakin (*Chiroxiphia caudata*) in the Brazilian Atlantic Forest. *Mol. Ecol.* **16**, 4908–4918.

Meyer, F.J., Kalko, K.V. & Kerth, G. (2008). Small-scale fragmentation effects on local genetic diversity in two phyllostomid bats with different dispersal abilities in Panama. *Biotropica* **69**, 17–32.

Moore, S.K., Mantua, N.J., Kellogg, J.P. & Newton, J.A. (2008). Local and large-scale climate forcing of Puget Sound oceanographic properties on seasonal to interdecadal timescales. *Limnol. Oceanogr.* **53**, 1746–1758.

Nuin, P. (2008). MrMTgui: cross-interface for ModelTest and Mr Modeltest. URL http://www.genedrift.org/software/mrmtgui.html (accessed 15/02/2013).

Olivieri, G.L., Sousa, V., Chikhi, L. & Radespiel, Y.U. (2008). From genetic diversity and structure to conservation: genetic signature of recent population declines in three mouse lemur species (*Microcebus spp.*). *Biol. Conserv.* **141**, 1257–1271.

Pacioni, C., Wayne, A.F. & Spencer, P.B.S. (2011). Effects of habitat fragmentation on population structure and long distance gene flow in an endangered marsupial: the woylie. *J. Zool. (Lond.)* **283**, 98–107.

Paradist, E., Claude, J. & Strimmer, K. (2004). APE: analysis of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290.

Pertoldi, C., Bijlsma, R. & Loeschcke, V. (2007). Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. *Biodivers. Conserv.* **16**, 4147–4163.

Prugh, L.R., Hodges, K.E., Sinclair, A.R.E. & Brashares, J.S. (2008). Effect of habitat area and isolation on fragmented animal populations. *Proc. Natl Acad. Sci. U.S.A.* **105**, 20770–20775.

Raudenbush, S.W. (1994). Random effects models. In *The handbook of research synthesis*: 301–321. Cooper, H.C. & Hedges, L.V. (Eds). New York: Russell Sage Foundation.

Reed, D.H. & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237.

Rosenberg, M.S. (2005). The file drawer problem revisited: a general weighted method for calculating fail safe numbers in meta-analysis. *Evolution* **59**, 464–468.

Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (2000). *MetaWin statistical software for meta-analysis. Version 2*. Sunderland: Sinauer Associates.

Sala, O.E., Chapin, F.S. III, Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000). Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774.

Sanderson, M.J. (2002). Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* **19**, 101–109.

Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991). Biological consequences of ecosystem fragmentation: a review. *Conserv. Biol.* **5**, 18–32.

Schmeller, D.S., Schregel, J. & Veith, M. (2007). The importance of heterozygosity in a frog's life. *Naturwissenschaften* **94**, 360–366.

Spielman, D., Brook, B.W. & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proc. Natl. Acad. Sci. USA* **101**, 1526–15264.

Swofford, D.L. (2003). *PAUP. Phylogenetic analysis using parsimony. Version 4 beta 10*. Sunderland: Sinauer Associates.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Wang, M. & Schreiber, A. (2001). The impact of habitat fragmentation and social structure on the population genetics of roe deer (*Capreolus capreolus L.*) in Central Europe. *Heredity* **86**, 703–715.

Winfree, R., Aguilar, R., Vázquez, D.P., LeBuhn, G. & Aizen, M.A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* **90**, 2068–2076.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. List of publications and tetrapod species used for the meta-analyses.

Appendix S2. Phylogenetic tree of the 92 unique tetrapods species used to performing phylogenetically independent meta-analyses in PhyloMeta.