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NEWS AND VIEWS

COMMENT

Species–genetic diversity correlations in habitat fragmentation can be biased by small sample sizes

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Abstract

Predicted parallel impacts of habitat fragmentation on genes and species lie at the core of conservation biology, yet tests of this rule are rare. In a recent article in *Ecology Letters*, Struebig *et al.* (2011) report that declining genetic diversity accompanies declining species diversity in tropical forest fragments. However, this study estimates diversity in many populations through extrapolation from very small sample sizes. Using the data of this recent work, we show that results estimated from the smallest sample sizes drive the species–genetic diversity correlation (SGDC), owing to a false-positive association between habitat fragmentation and loss of genetic diversity. Small sample sizes are a persistent problem in habitat fragmentation studies, the results of which often do not fit simple theoretical models. It is essential, therefore, that data assessing the proposed SGDC are sufficient in order that conclusions be robust.

Keywords: genetic diversity, population genetics, rarefaction method, sampling strategy, small sample size

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Understanding the impacts of habitat fragmentation on genes and species is fundamentally important to conservation biology and landscape ecology. However, there is a broad consensus that the genetic consequences of habitat fragmentation are more complex than those predicted by simple theoretical models (Ewers & Didham 2006; Feeley & Terborgh 2008; Bacles & Jump 2011). Because of the dependence of responses on a species' ecology and evolutionary history, it has been difficult to quantitatively summarize results reported to date in order to reach sound conclusions about the effects of habitat fragmentation on population genetics.

Methodological limitations of habitat fragmentation surveys can also weaken our ability to understand ecological mechanisms underlying forest fragmentation effects. For example, sample size, replicate independence and uncontrolled variation in confounding variables remain critical problems in fragmentation studies (McGarigal & Cushman 2002). Consequently, understanding the impacts of habitat fragmentation on genes and species and the relationship between the two—the species–genetic diversity correlation (SGDC)—poses a major challenge in data collection if reliable conclusions are to be reached. To illustrate this point, we explore the impact of small sample sizes in a recent test of the SGDC in forest fragments by Struebig *et al.* (2011).

In an overall excellent study, Struebig *et al.* (2011) compare parallel impacts of habitat fragmentation on species and genetic diversity of insectivorous bats. The authors attempted to synthesize the SGDC across species with contrasting ecological traits using richness-based measures. Taken at face value, their results indicate that an area-dependent decrease in species richness is mirrored by a parallel decrease in genetic diversity.

However, Struebig *et al.* (2011) report a negative impact of fragmentation on the genetic diversity of populations of just one (*Kerivoula papillosa*) of three bat species studied. The authors suggest that the inability to detect a correlation between fragment size and the genetic diversity of the other two species studied is heavily dependent on their ecological traits. Although this explanation may well be true, it is equally important to point out that results for this species have been estimated via rarefaction methods based on very small sample sizes and extrapolated to a common sample size of 15 (whilst 63% of the population samples include <15 individuals, 19% of samples include five individuals or less). Results estimated from the smallest sample sizes are likely to be highly error prone and may, therefore, produce false positives in the final analysis. Indeed, small sample sizes are *per se* the greatest problem in studies that aim to detect genetic consequences of habitat fragmentation, and there is no adequate current method to correct for low sampling intensity (Belkhir *et al.* 2006).

Whilst Struebig *et al.* (2011) deserve credit for an elegant study, their methods highlight intrinsic problems in analyses that are substantially based on small sample sizes. The problem arises from the fact that the authors used hypervariable loci (e.g. microsatellite markers) from very small samples. Such loci are acknowledged as ineffective in the analysis of small sample sizes, when they provide very little information about the presence of rare alleles (Leberg 2002; Belkhir *et al.* 2006).

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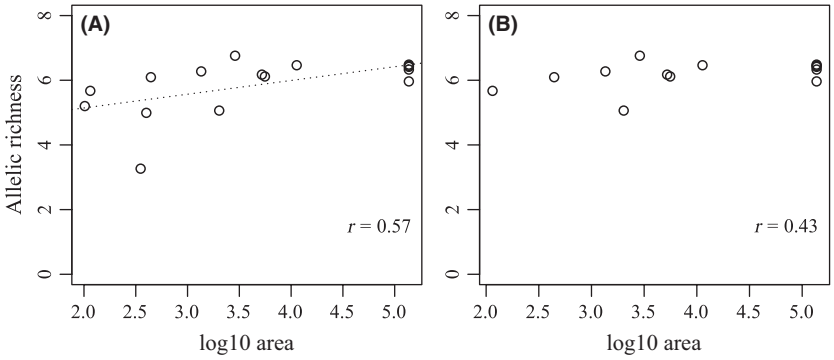

Fig. 1 Effects of small sample size on relationship between allelic richness and $\log_{10}$ area of *Kerivoula papillosa* populations: (A) negative impact of forest fragmentation on the genetic diversity with sample sizes larger than three individuals ($P = 0.023$); (B) no impact of forest fragmentation with sample sizes larger than five individuals ($P = 0.146$).

Allelic richness is highly impacted by population reduction because of the rapid elimination of rare alleles (Cornuet & Luikart 1996). Consequently, it is unclear how Struebig *et al.* (2011) can detect a loss of rare alleles from populations based on data estimated from only a few individuals. For instance, the *Kp30* locus used by the authors in the genetic analysis of *K. papillosa* shows 19 alleles across a sample of 22 individuals (Struebig *et al.* 2008). In this case, the apparent reduction in allelic richness observed for *K. papillosa* by Struebig *et al.* (2011) cannot reliably be attributed to forest fragmentation because the sample size analysed was not adequate to detect rare alleles and was, therefore, insufficient to assess their loss. Critically, although the rarefaction method they employ returns a reassuringly narrow confidence interval on allelic richness estimated from very small population samples, those samples are likely to be a very poor representation of the population from which they are derived (Leberg 2002). With issues as complex as habitat fragmentation and the SGDC, no comparable study to Struebig *et al.*'s (2011) study has used such limited samples (Vellend 2004; Cleary *et al.* 2006; He *et al.* 2008; Evanno *et al.* 2009).

To explore the effect of small sample size on the results reported for *K. papillosa* by Struebig *et al.* (2011), we used the Pearson correlation coefficient between allelic richness and $\log_{10}$ area from data presented by Struebig *et al.* (2011), $\log_{10}$ transforming area due to reduced heteroscedasticity. Like Struebig *et al.* (2011), we find a significant correlation between variables ($N = 16$, Pearson $r = 0.57$, $P = 0.023$, see Fig. 1A). However, when samples with <6 individuals are excluded from the analysis, there is no significant correlation between habitat area and the allelic richness (fragments and continuous forest samples: $N = 13$, Pearson $r = 0.43$, $P = 0.146$, see Fig. 1B; fragments only: $N = 8$, Pearson $r = 0.39$, $P = 0.341$). Furthermore, the relationship between mean number of alleles per locus and sample size becomes stronger when small samples are excluded (rising from $r = 0.64$ with all samples to $r = 0.84$ with sample sizes ≥10; both significant at $P = 0.05$), suggesting overall that the results observed by Struebig *et al.* (2011) for *K. papillosa* are significantly biased by the very small sample sizes included in this study. In effect, the positive SGDC reported appears to be driven by only three small population samples in a single species.

As reduction in population sizes will become more common because of habitat fragmentation and degradation, it is paramount that we understand the impact of habitat reduction on diversity both within and between species. However, it is equally important that data assessing the relationship between these measures are sufficient in order that conclusions be robust. Whilst the work by Struebig *et al.* (2011) remains highly valuable overall in its contribution to conservation biology, it does not lend strong support to the proposed SGDC. Further work based on greater sample sizes and additional measures, such as the reduction in genetically effective population size (N_e), is required to adequately assess this rule.

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