

INVITED REVIEW

Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches

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Abstract

Conservation of genetic diversity, one of the three main forms of biodiversity, is a fundamental concern in conservation biology as it provides the raw material for evolutionary change and thus the potential to adapt to changing environments. By means of meta-analyses, we tested the generality of the hypotheses that habitat fragmentation affects genetic diversity of plant populations and that certain life history and ecological traits of plants can determine differential susceptibility to genetic erosion in fragmented habitats. Additionally, we assessed whether certain methodological approaches used by authors influence the ability to detect fragmentation effects on plant genetic diversity. We found overall large and negative effects of fragmentation on genetic diversity and outcrossing rates but no effects on inbreeding coefficients. Significant increases in inbreeding coefficient in fragmented habitats were only observed in studies analyzing progenies. The mating system and the rarity status of plants explained the highest proportion of variation in the effect sizes among species. The age of the fragment was also decisive in explaining variability among effect sizes: the larger the number of generations elapsed in fragmentation conditions, the larger the negative magnitude of effect sizes on heterozygosity. Our results also suggest that fragmentation is shifting mating patterns towards increased selfing. We conclude that current conservation efforts in fragmented habitats should be focused on common or recently rare species and mainly outcrossing species and outline important issues that need to be addressed in future research on this area.

Keywords: conservation genetics, habitat fragmentation, mating systems, meta-analysis, plant genetic diversity, rarity status

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Introduction

The development of human civilization throughout the last two centuries has resulted in the transformation of vast natural areas into anthropogenic landscapes, resulting in a process of habitat fragmentation that alters the structure, distribution, and functioning of natural ecosystems

(Saunders *et al.* 1991). Immediate consequences of this process include habitat loss, the formation of remnant habitat patches of varied forms and sizes, a reduction of population sizes, and an increase in the degree of isolation of the remaining populations immersed in an anthropogenic matrix (McGarigal & Cushman 2002; Fahrig 2003). These persistent phenomena are well recognized as the main current driving forces of biodiversity loss in terrestrial ecosystems across the planet (Sala *et al.* 2000).

Studies of fragmentation effects in plants have largely concentrated on population demographic processes,

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especially evaluating plant reproductive dynamics in fragmented habitats (Hobbs & Yates 2003; Ghazoul 2005; Honnay *et al.* 2005; Aguilar *et al.* 2006). However, within the last decade there has been an increased interest in assessing the genetic consequences of habitat fragmentation in plants as stated by initial and recent reviews on this subject (Young *et al.* 1996; Lowe *et al.* 2005; Ouborg *et al.* 2006; Honnay & Jacquemyn 2007). The expected genetic consequences of fragmentation, which creates small, discrete, and isolated populations, are based on traditional island biogeography and metapopulation theories (MacArthur & Wilson 1967; Levins 1969). Thus, habitat fragmentation is expected to erode genetic variability and to increase inter-population genetic divergence of plant populations due to increased random genetic drift and inbreeding, and reductions in gene flow (e.g. Young *et al.* 1996; Sork *et al.* 1999; Lowe *et al.* 2005).

The most immediate effects of fragmentation on the genetic composition of plant populations depend on two factors: the effective population size within fragments and the patterns of genetic variability of the original populations previous to fragmentation (Nason *et al.* 1997; Hamrick 2004). Once a continuous forest is cleared and subdivided into small patches, from a metapopulation viewpoint, the distribution of genetic variability within and between the remaining populations in the landscape will depend on the spatial scale of fragmentation relative to the spatial scale of the pre-existent breeding neighbourhood (Nason *et al.* 1997; Hamrick 2004). Some hypotheses have been proposed to address the effects of habitat fragmentation on plant population genetics. As an immediate result, the genetic variation of populations is reduced due to genetic bottlenecks; specifically, a lower proportion of polymorphic loci and a reduction in the number of alleles per locus are expected within the fragments (Nei *et al.* 1975; Ellstrand & Elam 1993; Young *et al.* 1996). If fragmentation conditions persist over successive generations, decreased heterozygosity due to random drift and increased inbreeding are expected resulting in the accumulation of deleterious recessive alleles, lowering the fecundity of individuals, increasing seed/seedling mortality, and reducing the growth rate of individuals, eventually driving populations to extinction (e.g. Lande 1988; Young *et al.* 1996). The loss of genetic variation may reduce a population's ability to respond to future environmental change, such that the probability of extinction is increased or, at best, opportunities for evolution are limited (Caro & Laurenson 1994; Young *et al.* 1996; Nason *et al.* 1997; Booy *et al.* 2000).

The hypotheses concerning the negative impact of fragmentation on genetic diversity are the basis for the conservation genetic paradigm (Ouborg *et al.* 2006). The field of conservation genetics is relatively recent, and one of its main concerns is to develop basic and applied knowledge to create tools and strategies for conserving the

genetic resources and the evolutionary potential of species (Amos & Balmford 2001; Ouborg *et al.* 2006; Pertoldi *et al.* 2007). In order to develop such tools for effective conservation efforts, it is crucial to arrive to generalizations of plant genetic response patterns of plant species to habitat fragmentation.

Nevertheless, the empirical evidence from the literature provides inconsistent results to support these hypotheses, implying that not all fragmentation episodes necessarily result in genetic erosion of plant populations (e.g. Young *et al.* 1996; Collevatti *et al.* 2001; Lowe *et al.* 2005; Kettle *et al.* 2007). In this regard, quantitative statistical approaches are especially useful tools to integrate and synthesize the body of evidence from published literature (Arnqvist & Wooster 1995). Quantitative reviews such as meta-analysis allow us to reach general conclusions about a domain of research despite the apparent contradictory response patterns of individual studies (Gurevitch & Hedges 2001). This is accomplished by treating individual published results as if they were subjected to sampling uncertainty; thus, we are able to obtain not only the magnitude and direction of each effect (regardless of their *P* values), but also the variability of effects among individual studies (Hedges & Olkin 1985; Arnqvist & Wooster 1995; Gurevitch & Hedges 2001). Consequently, we can estimate the average magnitude of the effect across all studies, test whether the effect is significantly different from zero, and examine potentially causative differences in the effects among studies (Gurevitch & Hedges 2001).

Particular life-history traits of plants may confer different vulnerability to fragmentation effects. Because genetic erosion in fragmented habitats should be more pronounced after several generations, it is expected to find stronger negative effects on the adult generation of short-lived species compared to long-lived species (Young *et al.* 1996); or more precisely, in any plant population subjected to fragmentation conditions for several generations. Similarly, the ability of plants to reproduce clonally, via vegetative spread, may also buffer the genetic effects of fragmentation as a result of delaying the time between generations (Honnay & Bossuyt 2005). Also, the ploidy level of plants may influence the effects on genetic diversity due to fragmentation; as theory predicts, autotetraploids are less subject to the loss of genetic diversity by genetic drift than diploids (Bever & Felber 1992; Moody *et al.* 1993). Finally, the mating system of plants determines the spatial distribution of genetic variation within and among populations (Loveless & Hamrick 1984). Outcrossing plants typically show higher genetic variation within populations, whereas in selfing plants most of the genetic variation is found among populations (Loveless & Hamrick 1984; Hamrick & Godt 1989). Sudden decreases in effective population sizes due to habitat fragmentation would then have stronger negative effects on within-population genetic diversity of outcrossing

species. The fewer individuals remaining after fragmentation, the more severe the genetic bottleneck, which will have particularly large effects on the maintenance of rare alleles (Nei *et al.* 1975).

Likewise, some ecological processes, especially pollination and seed dispersal of plants, can shape the level of demographic and genetic connectivity among populations in fragmented habitats (Nason *et al.* 1997; Nathan & Muller-Landau 2000; Tewksbury *et al.* 2002; Hamrick 2004). The ability of vectors to move pollen and seeds through the fragmented landscape will determine the potential of plant species to offset the effects of genetic drift. In animal-pollinated or animal seed-dispersed plants, the level of genetic connectivity among fragments will depend on vector distribution, abundance, composition, and behaviour (Nason *et al.* 1997), attributes of pollinators and seed dispersers that are usually affected by habitat fragmentation (Didham *et al.* 1996; Graham 2001; Aizen & Feinsinger 2003; Griscom *et al.* 2007). Therefore, animal-pollinated and animal seed-dispersed plant species are expected to show decreased genetic connectivity due to habitat fragmentation compared to abiotically pollinated and abiotically seed-dispersed plants (Nathan & Muller-Landau 2000; García *et al.* 2007). The rarity of species can also determine susceptibility to genetic erosion. Naturally rare species, defined by their narrow geographical range, restricted habitat specificity or small local population sizes (*sensu* Rabinowitz 1981) are usually genetically less diverse than more widespread or common species (Karron 1987; Hamrick & Godt 1989; Ellstrand & Elam 1993). Then, common species may be more susceptible to lose genetic variation due to habitat fragmentation compared to rare species. Including rarity in models is problematic because authors do not uniformly assess rarity; species categorized as rare are not always *naturally* rare, but rather *recently* rare as a consequence of anthropogenic disturbance and habitat fragmentation (Gitzendanner & Soltis 2000). Thus, this categorization usually overlaps with the conservation status of the species (i.e. recently rare species are typically threatened or endangered). Once common and now rare species are expected to show stronger effects on genetic diversity than naturally rare species, as the former have suffered recent (i.e. in *non-evolutionary time*) decreases in regional or local abundance of populations (Huenneke 1991; Gitzendanner & Soltis 2000).

Certain characteristics of published studies may also influence the sensibility to find fragmentation effects. The ability to find fragmentation effects on genetic parameters may be different when using allozymes vs. DNA-based genetic markers. Specifically, because DNA-based genetic markers such as microsatellites have higher mutation rates (and consequently higher levels of variation), they may have higher resolution to detect changes in inter- and intra-population genetic variation compared to allozymes. Also,

fragmentation effects on genetic erosion may not be detected on adult individuals of long-lived woody species, but may be detected in their progeny. Thus, in species of long generational time, the type of tissue used by authors to measure genetic diversity (either from adult or progeny) may determine the magnitude of fragmentation effects. Finally, the time elapsed since fragmentation occurred should be an important factor to assess genetic erosion in plants. Effects are expected to be stronger in plant populations subjected to fragmentation conditions for larger periods of time, where a few or several generations have passed.

In this paper, we conduct a quantitative review to assess the overall effects of habitat fragmentation on plant population genetic parameters and test the predictions of the conservation genetic paradigm. Specifically, we determined (i) the overall magnitude and direction of fragmentation effects on the genetic variability of plant populations; (ii) whether longevity, ploidy level, mating system, clonal growth, type of pollen and seed dispersal vector, and rarity status of plants determine differential susceptibility to genetic erosion in fragmented habitats; (iii) whether different methodological approaches used by the authors determine the ability to find fragmentation effects; and (iv) whether there is a relationship between time elapsed in fragmentation conditions and the magnitude of fragmentation effects in genetic parameters.

Methods

Literature search

We surveyed the literature through different databases using a combination of 'fragment*' AND 'genet*' AND 'plant' as keywords. Searches were conducted in the Science Citation Index and Biological Abstracts databases and also in the main editorials (Blackwell Science, Springer-Verlag, and Elsevier) and scientific societies that group the most relevant indexed journals of ecological genetics and conservation biology. We obtained a large number of papers that were examined for suitability in the meta-analyses. Considering that habitat fragmentation produces three main outcomes in the landscape (namely habitat loss, decreased population sizes, and increased isolation among populations), we included studies using any of these measures of fragmentation, which were statistically compared to assess whether any one of them had particularly stronger effects on genetic parameters. Thus, we included studies conducted in real habitat fragments, in natural plant populations of different sizes and/or degrees of isolation. We also considered a few studies evaluating the effects of fragmentation due to selective logging on genetic parameters. This type of disturbance introduces changes in population density of adult trees, a measure of population size (Kunin 1997; Lowe

et al. 2005), without necessarily creating habitat fragments. We excluded articles that exclusively analysed correlations among population size and genetic variability without any explicit mention to the effects of habitat fragmentation (see Leimu *et al.* 2006). We included only studies that correlated genetic variability with population size as an indirect assessment of habitat fragmentation effects.

As measures of genetic variability, we considered expected heterozygosity (H_E), percent polymorphic loci (P), number of alleles (A), and inbreeding coefficient (F_{IS}). In cases where heterozygosity was not given (typically in studies using random amplified polymorphic DNA or amplified fragment length polymorphism), we used molecular variance or gene diversity and analysed these parameters together with expected heterozygosity. These four genetic parameters were not necessarily evaluated all together in each study, thus sample sizes for each meta-analysis differed. In several studies, we were able to calculate inbreeding coefficients from observed and expected heterozygosity values ($F_{IS} = H_E - H_O / H_E$). Whenever available, we also included measures of outcrossing rate (OR) in fragmented habitats.

For each plant species studied, we gathered information on several life-history traits and ecological aspects as well as on the methodology used by the authors of each study as potential predictors of the genetic responses to habitat fragmentation. We determined: (i) the longevity associated to the different life forms (woody long lived, herbaceous perennial or herbaceous short lived); (ii) whether vegetative reproduction occurred; (iii) the ploidy level (polyploid or diploid); (iv) the mating system, whether a species was mainly outcrossing (which included strictly self-incompatible species, as well as self-compatible species with a predominant outcrossing mating system) or selfing (including species with predominant selfing mating system and some self-compatible species with mixed mating system with clear capability of selfing) as explicitly declared by the authors; (v) pollen dispersal vector (biotic or wind); (vi) seed dispersal vector (biotic or abiotic); (vii) rarity (common, naturally rare or recently rare). We also evaluated the type of genetic marker (allozyme or DNA based) and the plant tissue used for each study (either from adult individuals or progenies). We further searched in each paper for information regarding the time elapsed in fragmentation conditions; this included rough estimates given by authors (expressed as *a few decades or centuries, more than or between* certain amount of time) and also more precise dates or time periods elapsed. With this information, we created three categories (less than 50 years, between 50 and 100 years, and more than 100 years) to compare the magnitude of effect sizes. Furthermore, within the group of publications where authors gave a more precise date of when fragmentation started, we searched for the approximate lifespan of each species. We found information on lifespans in the same or different

publications for 35 out of 47 species. For some species, we used genus-level lifespan information. For the remaining 12 species, we conservatively assigned a tabulated lifespan for woody and non-woody perennials following Ehrlén & Lehtilä (2002). For these species, we calculated the number of generations under fragmentation conditions by dividing the time period of fragmentation by the lifespan of the species, and ran correlation analyses between the number of generations and the effect sizes for H_E and F_{IS} . Based on theoretical grounds, these two genetic parameters are expected to be correlated with the number of generations under fragmentation conditions, showing stronger negative effects as more generations pass by. All the species' information was obtained from the same article, from other publications on the same species, or by contacting the authors. However, not every species' characteristic was available, thus predictor variables within a meta-analysis do not necessarily share the same sample size.

Seven articles evaluated the effects of fragmentation on genetic parameters in two species simultaneously and we included each of these species in the same analysis. Because the magnitude and sometimes direction of the responses of each species to habitat fragmentation within the same study were quite different, it is reasonable to assume that the effects are independent for each species (Gurevitch & Hedges 2001).

Data analysis

We used a categorical meta-analytical approach due to the large majority of studies evaluating population genetic parameters of plants in contrasting conditions (i.e. fragmented vs. non-fragmented). We obtained the mean value (\bar{X}) and standard deviations (SD) of each genetic parameter (H_E , A , P , OR , and F_{IS}) from plant populations (n) in each of the two conditions (fragmented and continuous habitats) in each published study. These data were taken either from text, tables or graphs (data from graphs were scanned using Datathief II software available online).¹ For each study, the magnitude of the effect of fragmentation on each of the genetic parameters (d_i) was estimated as the unbiased standardized mean difference (Hedge's d) between the mean value of the genetic parameter in fragmented and continuous habitats:

$$d_i = \frac{\bar{x}^F - \bar{x}^C}{SD^{FC}} J$$

where \bar{X}^F is the mean value of a given genetic parameter in fragmented habitats, \bar{X}^C is the mean value of the same genetic parameter in continuous habitats, SD^{FC} is the pooled standard deviation, of both groups and J is a term

¹ <http://www.nikhef.nl/~keeshu/datathief/>

that corrects for bias due to small sample size (see Gurevitch & Hedges 2001). The effect size d can be interpreted as the difference between the genetic diversity of plants in fragmented habitats and continuous conditions, measured in units of standard deviations. Thus, large differences and low variability generate the largest effect sizes (Gurevitch & Hedges 2001). For each genetic parameter, the overall weighted mean effect size estimate (d_{++}^*) was calculated as:

$$d_{++}^* = \frac{\sum_{i=1}^n w_i d_i}{\sum_{i=1}^n w_i}$$

where d_i is the effect size of the i^{th} study and w_i is the weight (reciprocal of the sampling variance) of the i^{th} study.

Positive values of the effect size (d) for H_E , A , P , and OR , imply positive effects of habitat fragmentation on these parameters whereas negative d values imply negative effects of fragmentation on these parameters. The interpretation of effect sizes for inbreeding coefficients is exactly the opposite: positive values of d imply negative effects of habitat fragmentation (i.e. higher inbreeding) whereas negative d values imply positive effects of fragmentation (i.e. lower inbreeding). For studies using correlational approaches to evaluate fragmentation effects (typically using population size as the independent variable), we calculated the mean value, standard deviation and sample size by pooling the data points for the lower-half (used as fragmented condition values) and higher-half values (used as non-fragmented condition values) of the continuous independent variable.

We used MetaWin 2.0 (Rosenberg *et al.* 2000) to run the analyses and bootstrap re-sampling procedures as described in Adams *et al.* (1997) to calculate confidence intervals of effect sizes. An effect of habitat fragmentation was considered significant if the 95% biased-corrected bootstrap confidence intervals (CI) of the effect size (d) did not overlap zero (Rosenberg *et al.* 2000). Confidence intervals based on bootstrapping methods are generally wider than standard CI, which implies that re-sampling estimates of CI are more conservative (Adams *et al.* 1997). Data were analysed using random-effect models (Raudenbush 1994). This model assumes that differences among studies are due to both sampling error and random variation, which is usually the rule in ecological data (Gurevitch & Hedges 2001). The heterogeneity among effect sizes was assessed with Q statistics. Specifically, we examined the P values associated with Q_{between} statistics, which describe the variation in effect sizes that can be ascribed to differences between the categories of each predictor variable (i.e. species' life history and ecological traits, and studies' methodologies). We also used these statistics to compare the effect sizes between studies that used different factors of

analyses (e.g. fragment size, logging, population size) to evaluate habitat fragmentation. Within the species used for the meta-analyses, we found few congeneric species (Table S1, Supporting information). We re-ran analyses using data pooled by congeneric species and found no difference in magnitude or direction of effects compared to the analyses performed using all the species as independent data points. We also tested for potential interactions among predictor variables by measuring their pairwise level of dependence with chi-squared tests.

Quantitative reviews of published studies have the intrinsic problem of potential publication bias. That is, studies showing significant results may have a greater probability of publication than those showing non-significant results. We explored this possibility graphically (weighted histograms and funnel plots), and by calculating weighted fail-safe numbers. If the fail-safe number is larger than $5n + 10$, where n is the number of studies, then publication bias may be safely ignored (i.e. results are robust regardless of publication bias; Rosenberg 2005).

Results

Sample of studies

From the literature search, we obtained 101 publications from 28 international indexed journals throughout the period of 1989–2008 that evaluated the effects of habitat fragmentation on plant population genetic parameters (Appendix S1, Supporting information). These studies measured at least one parameter of genetic variability on 102 unique plant species to conduct the meta-analyses, which yielded 101 data points for expected heterozygosity (H_E), 77 data points for number of alleles (A), 57 data points for percent polymorphic loci (P), 18 data points for outcrossing rate (OR), and 62 data points for inbreeding coefficients (F_{IS}). Although the species included in this review comprise a wide sample of plants with different biological and ecological attributes, there is some bias in these characteristics and also in the approaches used by authors to study genetic consequences of habitat fragmentation. Herbaceous perennial and woody long lived (shrubs and trees) represent 53% and 40% of the studied species, respectively, while herbaceous short-lived species comprised only 7% of the studied species. There are larger proportions of insect pollinated species (72%) and abiotically seed-dispersed species (77%). Diploid species are also a majority (84%). Although self-compatible (54%) and self-incompatible (46%) plants are approximately equally represented in the sample, within self-compatible plants there is a high number of mainly outcrossing plants as declared by the authors. Hence, there is a higher proportion of mainly outcrossing species (75%) compared with selfing plants (25%). Species without the capability of vegetative reproduction are slightly

more represented (61%). Common species represent 48%, whereas naturally and recently rare species represent 25% and 27% of the sample, respectively. We found no significant pairwise associations among any of these predictor variables (not shown), which indicate they can be considered statistically independent. Most of the studies evaluate the effects of habitat fragmentation on the genetic variability of adult individuals (72%), using mainly allozymes (60%) as genetic markers.

A comparison of the different factors of analysis used by the authors as measures of habitat fragmentation showed no significant differences in the effect sizes for H_E ($Q_{\text{between}} = 1.35$; $P = 0.493$), A ($Q_{\text{between}} = 1.68$; $P = 0.441$), P ($Q_{\text{between}} = 2.05$; $P = 0.162$), and F_{IS} ($Q_{\text{between}} = 0.76$; $P = 0.652$). That is, fragmentation effects on each of these genetic parameters are comparable whether considering fragment size, degree of isolation, habitat loss, population size, or density of conspecifics (i.e. logging) as factors of analysis.

Weighted histograms showed unimodal distributions with the highest frequency around zero (not shown) and funnel plots of effect sizes vs. sample sizes showed no skewness (not shown), which indicates no bias in reporting results (cf. Aguilar *et al.* 2006 for details of interpretation). Similarly, the calculated weighted fail-safe numbers for each meta-analysis were larger than $5n + 10$ [H_E : $2249.4 > (5 * 101) + 10 = 515$; A : $2805.4 > (5 * 78) + 10 = 400$; P : $1444.2 > (5 * 57) + 10 = 295$; OR : $168.3 > (5 * 18) + 10 = 100$; F_{IS} : $1306.6 > (5 * 62) + 10 = 320$], reinforcing the robustness of these results.

Habitat fragmentation and genetic variability

Overall weighted-mean effect sizes of habitat fragmentation on H_E , A , and P were negative and significantly different from zero (Fig. 1). The OR , which was only consistently assessed in 18 studies, also showed an overall negative weighted-mean effect size (Fig. 1). Habitat fragmentation showed non-significant overall effects on F_{IS} (Fig. 1), despite the small positive value implying a slight trend of increasing inbreeding due to habitat fragmentation.

From the evaluation of all predictor variables associated with the species' life history and ecological attributes for each of the genetic parameters, we present only the results that showed significant ($P < 0.05$) values of Q_{between} statistics in text and figures. We found that fragmentation effects were significantly different for H_E between common, naturally rare and recently rare species ($Q_{\text{between}} = 23.18$; $P < 0.001$). On average, common and recently rare species showed strong, negative and significant effects of fragmentation on H_E , whereas naturally rare species showed non-significant effects on H_E (Fig. 2a). The same trend was found for A and P , although the heterogeneity among effect sizes was only marginally significant (for A : Q_{between}

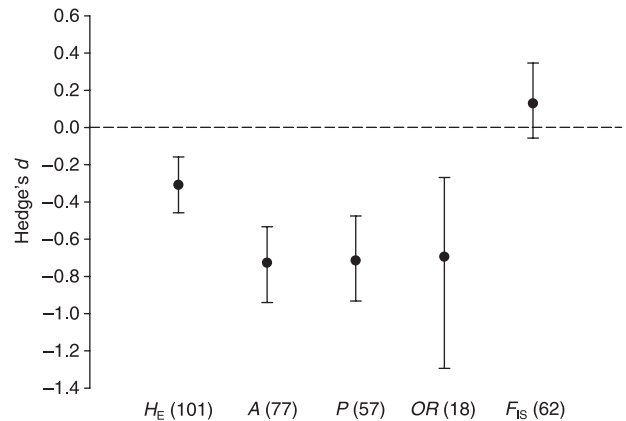


Fig. 1 Overall weighted-mean effect sizes and 95% bias-corrected confidence intervals of habitat fragmentation on expected heterozygosity (H_E), number of alleles (A), percent polymorphic loci (P), outcrossing rate (OR), and inbreeding coefficient (F_{IS}). Sample sizes for each meta-analysis are shown in parenthesis; dotted line indicates Hedge's $d = 0$.

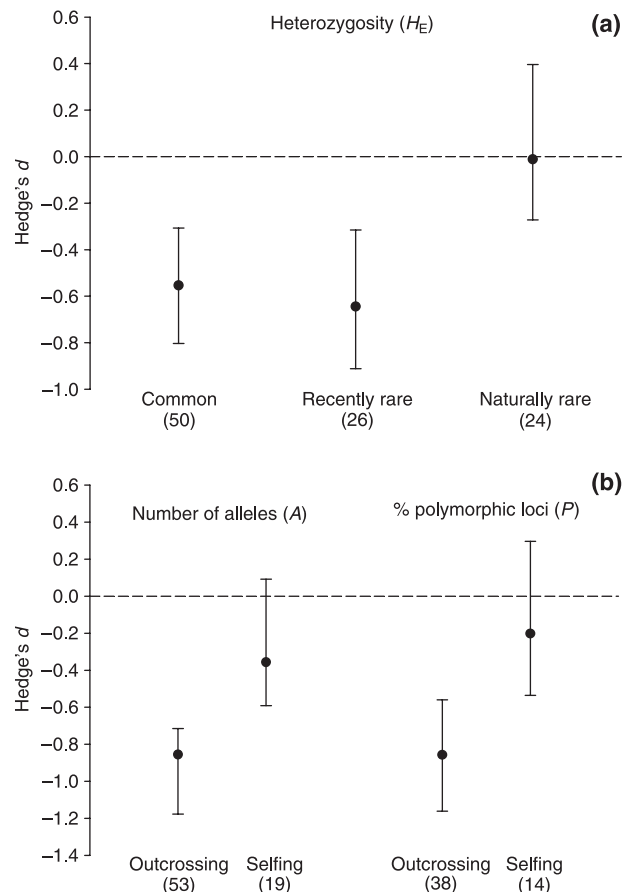


Fig. 2 Weighted-mean effect sizes and 95% bias-corrected confidence intervals of habitat fragmentation on (a) H_E of plant species with different categories of rarity (common, recently rare, and naturally rare) and on (b) A and P of plants with different mating systems (outcrossing and non-outcrossing) showing statistically significant heterogeneity (Q_{between}). Sample sizes of each category are given in parentheses. Dotted line shows Hedge's $d = 0$.

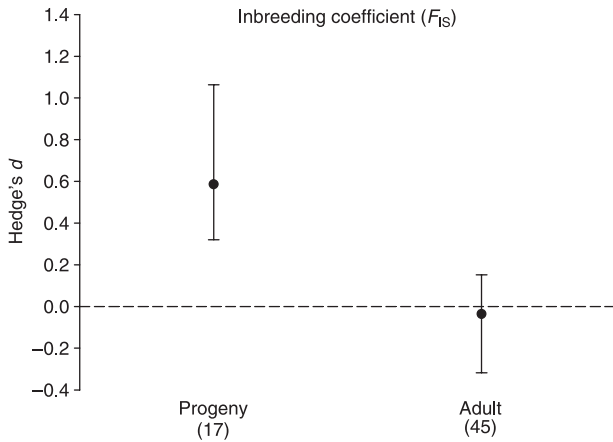


Fig. 3 Weighted-mean effect sizes and 95% bias-corrected confidence intervals of habitat fragmentation on inbreeding coefficient (F_{IS}) of studies evaluating adult and progeny tissues. Sample sizes of each category are given in parentheses. Dotted line shows Hedge's $d = 0$.

= 4.72; $P = 0.094$; for P : $Q_{\text{between}} = 5.01$; $P = 0.081$). Furthermore, for A and P , there were significant differences in mean effect sizes between outcrossing and selfing species ($Q_{\text{between}} = 14.96$; $P = 0.028$ and $Q_{\text{between}} = 9.17$; $P = 0.05$ for A and P , respectively). Outcrossing species showed stronger negative effects of fragmentation on A and P compared to selfing species (Fig. 2b). A similar but marginally significant trend was observed for H_E , [$d_{\text{outcrossing}} (n=69) = -0.57$, $d_{\text{selfing}} (n=24) = -0.22$; $Q_{\text{between}} = 3.24$; $P = 0.071$]. In the case of inbreeding coefficients, none of these predictor variables showed significant heterogeneity, implying that fragmentation not only does not have an overall effect on inbreeding, but also no particular life-history trait is showing susceptibility to fragmentation (not shown). Surprisingly, fragmentation effects on inbreeding were only studied in one short-lived species, which precluded the formal comparison between short- and long-lived species. None of the other life history (life form, vegetative growth capability, and ploidy level) and ecological traits (pollination and seed dispersal vector types) evaluated as predictor variables showed significant heterogeneity in effect sizes of fragmentation on these genetic parameters (not shown).

The use of different genetic markers (allozymes vs. DNA based) did not significantly alter the magnitude of effect sizes for each of the genetic parameters evaluated in fragmented habitats (not shown). Furthermore, effect sizes of fragmentation on H_E , A , and P were also homogeneous among studies sampling adult or progeny tissues (not shown). For inbreeding coefficients, on the contrary, there was a significant difference in mean effect sizes between studies evaluating adult and progeny tissues ($Q_{\text{between}} = 16.80$; $P = 0.012$; Fig. 3): progenies showed a significant positive overall mean effect size value while adults showed a non-significant mean effect size (Fig. 3). This result implies

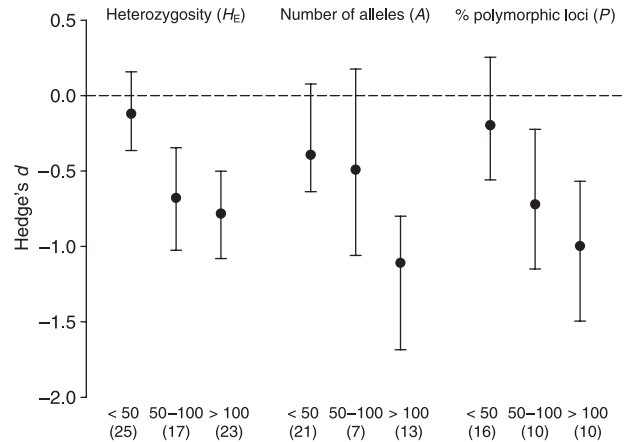


Fig. 4 Weighted-mean effect sizes and 95% bias-corrected confidence intervals of habitat fragmentation on H_E , A , and P of plant populations subjected to different time periods in fragmentation conditions: less than 50 years (< 50), between 50–100 years (50–100), and more than 100 years (> 100). Sample sizes of each category are given in parentheses. Dotted line shows Hedge's $d = 0$.

that progenies generated in fragmented habitats (which comprised mostly non-established seeds) presented higher inbreeding coefficients than progenies produced in continuous habitats; whereas for adult individuals no difference in mean F_{IS} values were observed between fragmented and continuous habitats. Depending on the parameter evaluated, between 53% and 64% of the studies gave at least rough information on the time elapsed in fragmentation condition. Overall, species subjected for more than 100 years in fragmentation conditions had significantly stronger effects on H_E ($Q_{\text{between}} = 17.72$; $P = 0.009$), A ($Q_{\text{between}} = 6.68$; $P = 0.05$), and P ($Q_{\text{between}} = 15.57$; $P = 0.018$; Fig. 4) compared to species evaluated in fragmented systems of less than 50 years, which showed non-significant mean effect sizes on these three genetic parameters (i.e. CI's overlapping zero value; Fig. 4).

Finally, we were able to estimate the number of generations elapsed in fragmentation conditions for 47 and 35 case studies evaluating H_E and F_{IS} , respectively. We log-transformed the number of generations and ran correlations with the effect sizes of fragmentation on these two parameters. We found a significant negative correlation between the number of generations elapsed and the species' effect sizes for H_E ($r = -0.36$, $P = 0.012$, Fig. 5). That is, the more generations elapsed in fragmentation conditions for any given plant population, the stronger negative magnitude of effect sizes on H_E . In the case of fragmentation effects on inbreeding coefficient, we found a non-significant positive correlation with the number of generations ($r = 0.29$, $P = 0.102$, $n = 35$), suggesting a trend of higher inbreeding as more generations pass by in fragmentation conditions.

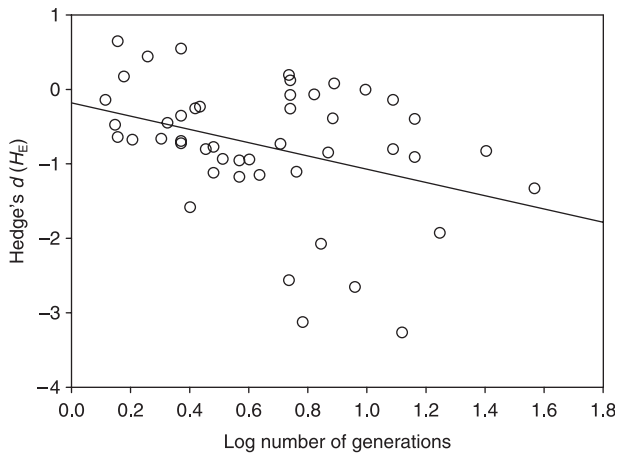


Fig. 5 Correlation between the log-transformed number of generations of plant populations in fragmented habitats and the effect sizes of fragmentation on H_E for 47 plant species. Correlation coefficient $r = -0.36$, $P = 0.012$.

Discussion

Anthropogenic habitat fragmentation is a recent phenomenon in evolutionary time but a pervasive feature of modern landscapes (Fahrig 2003). Plant populations that remain in habitat fragments are confronted with modified environments of reduced area, increased isolation, and new ecological boundaries, potentially affecting their biotic and abiotic interactions (e.g. Fahrig 2003; Ewers & Didham 2006). The genetic consequences of fragmentation on plant populations have been studied for over two decades and no clear response patterns have emerged from the literature. Recently, two reviews have focused on the relationship between genetic diversity and population size (Leimu *et al.* 2006; Honnay & Jacquemyn 2007), one of the immediate possible demographic consequences of habitat fragmentation. Nevertheless, fragmentation is a complex process that involves several different factors simultaneously (McGarigal & Cushman 2002; Fahrig 2003; Ezard & Travis 2006; Leblois *et al.* 2006; Ouborg *et al.* 2006); thus analyzing solely reductions of population size may not fully reflect what is happening in real fragmented scenarios. Population size per se may not be very important for animal pollinators and seed dispersers, whereas the degree of population isolation or the matrix characteristics surrounding the fragments may have more influence on their foraging behaviour (Kunin 1997; Ricketts 2001), affecting their ability to maintain gene flow among fragmented populations. These different factors, which often interact in diverse ways, are difficult to separate in observational or non-experimental designs, the rule in fragmentation studies. Authors tend to focus on one factor and do not usually control for the others (Leblois *et al.* 2006;

Ouborg *et al.* 2006; but see e.g. Prober & Brown 1994; Honnay *et al.* 2007). Thus, the cause of reduced genetic diversity in fragmented habitats should not be adjudicated to one single factor, but rather to the interacting effects of, at least, population size, degree of isolation and matrix characteristics (Ezard & Travis 2006).

In this review, we explicitly focused on fragmentation studies and arrived at a conclusive generalization: habitat fragmentation decreases the genetic diversity of plant populations. The vast majority of studies were conducted on adult populations of long-lived species in relatively recently fragmented systems, which indicates the effects observed on genetic diversity, especially on A and P , are probably mainly the result of genetic bottlenecks, the most immediate consequence of fragmentation (e.g. Young *et al.* 1996; Nason *et al.* 1997; Oostermeijer *et al.* 2003; Lowe *et al.* 2005). These species and studies' characteristics may also be the reason for the absence of overall significant effects on inbreeding coefficients (i.e. most sampled adults have probably established before fragmentation took place) and the comparatively smaller mean effect size observed on H_E , which may be mostly due to the overall reduction in number and frequency of alleles (Barret & Kohn 1991; Nason *et al.* 1997). In a closer examination of the subset of studies that provided dates of fragmentation events, it was clearly observed that time and, more precisely, the number of generations elapsed under fragmentation conditions, are crucial in determining stronger genetic diversity reductions in plant populations, especially in heterozygosity, which may take a number of generations to become apparent (Young *et al.* 1996; Lowe *et al.* 2004, 2005). Studies conducted in more than 100-year-old fragmented systems presented significantly stronger negative effects on genetic diversity (Fig. 4). This notion was more specifically supported by the significant correlation between the estimated number of generations for a subset of species and the magnitude of negative fragmentation effects on H_E (Fig. 5), primarily as a result of random genetic drift (e.g. Young *et al.* 1996; Young & Clarke 2000; Lowe *et al.* 2004).

Gene flow and mating patterns in fragmented habitats

The amount of gene flow among remnant populations is a key element that will ultimately determine the genetic consequences of habitat fragmentation (Sork *et al.* 1999; Frankham *et al.* 2002; Hamrick 2004; Sork & Smouse 2006). Moderate or even relatively low levels of gene flow via pollen or seeds between fragmented populations can significantly alleviate the loss of genetic diversity by preventing the effects of genetic drift (e.g. Sork *et al.* 1999; Couvet 2002). In this regard, we found no evidence of any particular pollinator or seed dispersal vector type (either biotic or abiotic) to confer differential susceptibility to the loss of genetic diversity. Although this result does not give

us information about the patterns of gene flow per se in fragmented habitats, it does indicate there is no obvious type of vector able to conduct consistently extensive gene flow between fragments within the studies analysed.

In the present review, we were not able to evaluate contemporary gene flow due to the current paucity of this kind of study and the difficulty of generating effect size measures from gene flow parameters. However, the results observed for outcrossing rates and inbreeding coefficients may indirectly support the possibility of disrupted gene flow and/or changes in mating patterns of fragmented plant populations. Outcrossing rates in fragmented populations showed a significant overall decrease compared to populations in continuous habitats (Fig. 1), suggesting that fragmented plant populations are suffering changes in mating patterns towards increased selfing. Moreover, inbreeding coefficients will increase immediately in the first generation of progenies if mating patterns are biased towards higher selfing or mating among related individuals (e.g. Young *et al.* 1996; Lowe *et al.* 2005; Kettle *et al.* 2007). Precisely, we found that progenies in fragmented habitats presented significant mean higher inbreeding coefficients than progenies in non-fragmented habitats (Fig. 3), indicating adult individuals in fragmented populations are mating more frequently among related individuals and/or through autogamous pollination.

In addition to reduced heterozygosity due to random genetic drift in populations that remain fragmented for several generations, heterozygosity erosion is more severe when inbreeding accompanies fragmentation (e.g. Young *et al.* 1996; Nason *et al.* 1997; Young & Clarke 2000). The few species subjected to fragmentation conditions for many generations presented quite strong negative effect sizes on H_E (Fig. 5), probably as a result of both drift and increased inbreeding. In the hypothetical scenario of anthropogenic fragmentation ceasing and landscapes remaining as they are today, the effects on genetic diversity of plants will still be much stronger in the future than we have estimated here if mating patterns continue shifting towards selfing.

Mating systems

Self-incompatible (SI) and mainly outcrossing self-compatible species, which contain most of their genetic variability within populations, suffered greater losses of alleles and polymorphic loci than non-outcrossing self-compatible and selfing species. For self-incompatible species in particular, this may result in the loss of low-frequency self-incompatibility alleles (S) (Wright 1965; Nei *et al.* 1975). In genetically controlled self-incompatibility systems, sharing of even a single S allele can prevent mating between individuals (De Nettancourt 2001). Thus, SI plants surviving in small, isolated populations may experience mate limitation due to reduced S allele diversity

so that the effective population size is further reduced (Byers & Meagher 1992; Glémin *et al.* 2008). Such synergism between genetic and demographic processes has great potential to influence population viability of these species (Young & Clarke 2000; Glémin *et al.* 2008). In fact, animal-pollinated SI species are also strongly negatively affected in terms of effective pollination service and seed production by habitat fragmentation (Aguilar *et al.* 2006), thus these species are exceptionally vulnerable to fragmentation as a consequence of both, ecological and genetic mechanisms. These results represent a clear example of how genetic erosion can have short-term impacts on individual fitness and population viability (e.g. Cascante *et al.* 2002; Fuchs *et al.* 2003).

Rarity status

Our results support the initial hypothesis regarding the rarity status of plants: because common species have comparatively higher levels of genetic variability than naturally rare species, they are expected to lose more diversity due to recent fragmentation processes. Whether rarity is a cause or a consequence of evolutionary and ecological processes is still an open question (Rabinowitz 1981; Gitzendanner & Soltis 2000). In this regard, naturally and recently rare species may represent different timescales and origins of disturbance, which affect the genetic characteristics they possess in the present (Karron 1987; Ellstrand & Elam 1993; Gitzendanner & Soltis 2000; Oostermeijer *et al.* 2003). If this is true, it would be important to distinguish in the system under study whether habitat fragmentation is a consequence of natural phenomena, and thus occurred through evolutionary time, or whether is the result of anthropogenic activity, occurring in recent ecological times. While evolutionary fragmentation may be a more gradual and slower process that may also 'have an end', ongoing ecological fragmentation is a much faster increasing, non-random process (Saunders *et al.* 1991; McGarigal & Cushman 2002; Fahrig 2003). Given the ubiquitous nature of anthropogenic habitat fragmentation in today's landscapes, the results presented here are important and of interest to conservation biology as they situate common species in potential risk of genetic erosion, which is counterintuitive to current conservation principles that almost exclusively emphasize efforts on rare or threatened species (Honnay & Jacquemyn 2007; Gaston & Fuller 2008).

Conservation implications and future directions

Conservation of genetic diversity within populations has direct implications not only for ecosystem functioning but also for providing resilience in the face of environmental change (Luck *et al.* 2003; Reusch & Hughes 2006). The

controversy about whether ecological and demographic factors are more important than genetic factors in driving species to extinction (Lande 1988; Frankham *et al.* 2002) has been recently quantitatively assessed: most taxa are not driven to extinction before genetic factors affect them adversely (Spielman *et al.* 2004), an assertion also supported by further research on plants in fragmented habitats (e.g. Endels *et al.* 2007). Thus, revealing which plant traits are more susceptible to suffer genetic erosion in fragmented habitats is crucial to detect lowered evolutionary potential, compromised reproductive fitness, and elevated extinction risks of wild populations, which should help generate criteria to prioritize conservation efforts (Young *et al.* 1996; Young & Clarke 2000; Amos & Balmford 2001; Lowe *et al.* 2005). Our results indicate that such efforts should be directed to common or recently rare species and mainly outcrossing species. Strictly self-incompatible, animal-pollinated species are at even greater risk due to their additional reproductive impairment in fragmented habitats (Aguilar *et al.* 2006).

Despite these unequivocal signals of susceptibility in plants, there is a clear gap in the literature of plant population genetics in fragmented habitats that precluded us making further generalizations. Such is the case of the poor representation of short-lived species as study targets and the dearth of studies evaluating contemporary gene flow via pollen and seeds on plant species with different life forms coupled with ecological information on the biotic dispersal vectors. Also, special attention should be given to the study of established progenies (seedlings and saplings) in fragmented habitats. Most of the progeny tissue evaluated up to now comes from non-established seeds (personal observation) and their genetic composition may differ markedly from that of the progeny that is actually being recruited in fragmentation conditions if they are subjected to selective pressures shaped by seed predators and herbivores (e.g. Cascante *et al.* 2002) and/or if they come from seed banks of previous reproductive episodes (Mandák *et al.* 2006; Honnay *et al.* 2008). Increasing these types of studies may allow us to determine whether gene flow mediated by animals is in fact changing and how changes in mating patterns will affect the genetic diversity of future generations of plant populations. Including precise measures and information on the history and characteristics of fragmented systems is particularly important, not only to determine timescales of fragmentation but also to test for possible fragmentation thresholds below which genetic variation is lost (e.g. Prober & Brown 1994; Ezard & Travis 2006). These approaches imply the merging of population genetics, plant–animal interaction ecology, and landscape ecology, a multidisciplinary endeavor that will provide knowledge-based tools for conserving the evolutionary potential of species and for managing ongoing anthropogenic modified landscapes.

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The authors share research interests in the ecology, evolution and genetics of plant reproductive dynamics. They have been evaluating the effects of forest fragmentation and other anthropogenic disturbances on pollinators, sexual reproduction and the genetic structure of flowering plants. They are interested in generating basic and applied knowledge in ecology and conservation biology of plant-animal interactions and their role in preserving the genetic diversity of plant populations in human-altered landscapes. Other research interests involve the study of hybridization processes between genetically modified plants with native relatives, the role of pollinators as ecological service providers for human well-being, and the conservation of tropical and subtropical dry forests.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 List of 102 unique plant species included in the meta-analyses. For each species, we provide the botanical family, the genetic parameters evaluated in the study, the molecular marker used, the compatibility system, mating system, pollination vector, seed dispersal vector, ploidy level, vegetative growth capability, life form, type of rarity, studied tissue, time elapsed in fragmentation conditions and the source publication where information was obtained.

Appendix S1 List of complete references of the studies included in the analyses.

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