

Life-history variation following habitat degradation associated with differing fine-scale spatial genetic structure in a rainforest cycad

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Abstract Habitat degradation can result in drastic environmental changes potentially affecting the life-history of populations and aspects of the reproductive biology and the genetic structure within and among populations. Here, we explore how life-history differences between subpopulations from contrasting habitats may affect mating availability, which in turn will indirectly affect the strength of spatial genetic structure within populations of a tropical rainforest cycad (*Zamia fairchildiana*). Subpopulations exposed to higher light availability in degraded-forest habitats had male individuals that grew faster, reproduced earlier, and invested more in reproduction than in native-forest habitat subpopulations. These differences in life history resulted in degraded-habitat subpopulations showing a higher proportion of reproductive adults and greater mate availability in a reproductive season. Subpopulations in the degraded habitat showed weaker SGS, i.e., a smaller slope in the linear regression of genetic relatedness on linear distance. Environmentally induced changes in life history and subsequent changes in the strength of the SGS after habitat degradation may have important consequences for population viability and should be of concern in conservation.

Keywords Gene dispersal · Genetic relatedness · Mating availability · *Zamia fairchildiana*

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Introduction

Habitat loss and degradation are major threats to species persistence worldwide (Fischer and Lindenmayer 2007). In the case of tropical rainforests, habitat fragmentation and extractive activities result in forests that differ drastically from the original habitat in forest structure and species composition (Noble and Dirzo 1997; Tabarelli et al. 2004). Habitat degradation can affect plant survival and reproductive rates because of general detrimental effects of environmental changes, and by increasing genetic drift and inbreeding or altering interactions with pollinators and dispersers (reviewed in Nason and Hamrick 1997; Ghazoul 2005; Honnay et al. 2005). In addition to direct effects of habitat modifications on the demographic viability of populations, the patterns of genetic exchange and the distribution of genetic variation between plant populations can be altered as a result of anthropogenic activities (Young et al. 1996; Sih et al. 2000; Honnay et al. 2005). If this is the case, then information on the spatial genetic structure (SGS) of populations is necessary to delineate appropriate significant units for conservation (Moritz 1994; Fraser and Bernatchez 2001), and for restoration programs that seek to minimize negative effects of movement of individuals between populations, e.g., because of outbreeding depression (Frankham 1999; Hufford and Mazer 2003). Consequently, studies focused on determining the scale of genetic structure between populations are common in conservation biology (e.g., Jacquemyn et al. 2003; Murren 2003; Hoofman et al. 2004; Galeuchet et al. 2005). One issue that has received less attention is the potential effect of habitat modifications on the distribution of genetic variation at a fine-scale level or at the within-population level.

Fine-scale SGS, i.e., the non-random distribution of genotypes in space, is a widespread phenomenon in plant

populations and in populations of other sedentary organisms where the distance of propagule dispersal is small compared to the area covered by a population. SGS in plant populations usually results from limited seed dispersal across geographical distance (Hamrick et al. 1993; Ennos 1994; Vekemans and Hardy 2004), but it can also result from demographic events affecting the spatial aggregation of related individuals within a population, like random mortality of sibs aggregated in patches or differential selection of individuals across space (Ennos 2001; Kalisz et al. 2001; Jones and Hubbell 2006). In addition, population density could affect the patterns of SGS within populations, through its indirect effects on the levels of genetic drift and inbreeding (Hamrick et al. 1993; Vekemans and Hardy 2004). SGS has important consequences for population biology, as the distribution of genetic variation within populations might in turn affect effective population sizes and patterns of viability selection (Hamrick and Nason 1996). Drastic changes in habitat conditions have been shown to affect the strength of the fine-scale SGS of several plant species (Epperson and Chung 2001; England et al. 2003; van Rossum and Triest 2006; Chung and Nason 2007; Yamagishi et al. 2007). Nevertheless, the mechanisms behind differences in the fine-scale SGS between populations from different habitats are poorly understood, but seed dispersal patterns and post-dispersal demographic and selection events are likely to be involved (Kalisz et al. 2001; Jones et al. 2006).

Several studies suggest that changes in dispersal and recruitment patterns in degraded-habitat populations are the main factors underlying differences in the SGS between populations (England et al. 2003; van Rossum and Triest 2006; Yamagishi et al. 2007). However, other factors besides patterns of seed dispersal, pollen movement, and the post-dispersal mortality of juveniles could affect the strength of the fine-scale SGS of populations (Schnabel et al. 1998; Hardy et al. 2004; Torimaru et al. 2007). For example, lower mate availability and smaller effective population sizes, that could be common in degraded-habitat populations, might result in higher inbreeding and higher average relatedness among seeds in individual fruits (i.e., correlated mating in sibships) and affect the SGS within populations (Wells and Young 2002; Hardy et al. 2004). Particularly in dioecious species, where the average relatedness among sibships (proportion of full-sibs and half-sibs) depends on the amount of potential pollen donors, mate availability could influence the strength of SGS within populations by affecting the degree of relatedness among seeds produced by females and dispersed around them. Strong SGS has been found in dioecious species with kin-structured dispersal, i.e., seeds from the same mother that disperse together, because of the clustering of sibs around the parental plant that does not breakdown even

with high pollen movement (Ingvarsson and Giles 1999; Torimaru et al. 2007).

Few studies have explored the patterns of fine-scale SGS in long-lived plant species and how the SGS could be altered after habitat degradation (but see Young and Merriam 1994; Cloutier et al. 2007). *Zamia fairchildiana* (Zamiaceae) is a long-lived cycad (Gymnospermae) typical of the understory of tropical rainforests in Costa Rica and Panama (Norstog and Nicholls 1997). In part of its distribution range, populations of *Z. fairchildiana* persist in forests affected by fragmentation and other anthropogenic activities (i.e., degraded habitats), where environmental conditions differ considerably from their native habitats. This cycad species, like most understory plants in tropical forests, is particularly affected by changes in light availability (Clark and Clark 1987), as light is the most limited resource in these habitats (Chazdon et al. 1996; Brienen and Zuidema 2006). In this paper, we describe how differences in canopy cover between native and degraded habitats are associated with significant differences in life-history traits like growth rate and allocation to fecundity, which in turn could affect the proportion of reproductive adults in a reproductive season and the availability of mates within the populations. We compared growth and fecundity rates among populations in their native habitat and degraded forests, and predicted increased fecundity allocation and mate availability in degraded-habitat populations resulting from increased light availability in this habitat, as has been suggested for other rainforest *Zamia* species (Clark and Clark 1987). Furthermore, we describe the SGS for two subpopulations of *Z. fairchildiana* in native- and degraded-forest habitats, to explore the potential effects of differing life-history traits on the distribution of genetic variation within subpopulations, particularly regarding the effects of considerable variation in mate availability observed in this species between subpopulations in native and degraded habitats.

Materials and methods

Study species and sampled subpopulations

Zamia fairchildiana (Zamiaceae) is a shade-tolerant cycad that inhabits the understory of lowland and mountain wet-forest between 0 and 1,500 m asl in SW Costa Rica and W Panama (Gomez 1982). Populations of *Z. fairchildiana* appear in large tracts of mature, relatively unaltered forest and also in degraded habitats throughout its geographical range. In the study site, *Z. fairchildiana* is an arborescent cycad with a stem up to 2 m high that bears 5–20 compound leaves. The number of leaflets increases progressively with age, from 4 to 6 leaflets in the seedlings to

about 50–60 in the adults. Growth occurs in annual episodes, where leaves are produced in a flush. Cycads are dioecious, i.e., male and female cones are produced in separate individuals, but the mechanism of sex determination is unknown (Norstog and Nicholls 1997). Females produce 1 cone with 50–200 seeds, and males produce 1–3 cones with 150–600 sporophylls (parts of the cone containing pollen sacs). Reproductive events are annual and synchronous, but every year only a small percentage of individuals of the population produce reproductive organs (Norstog and Nicholls 1997). Pollination is carried out by small Coleopterans (Erotylidae and Belidae). Seed development lasts for several months, and most seeds are dispersed locally by gravity when the cone parts rot away. Secondary dispersal after dispersal by gravity has not been observed for *Zamia* species in rainforest, but studies on seed dispersal are scarce.

Individuals of *Z. fairchildiana* grow in discrete patches in the understory, and isolated individuals growing in between these patches are extremely rare. We chose six patches (hereby called subpopulations, see below) of individuals for monitoring growth and fecundity rates in the Osa Peninsula region in SW Costa Rica. Three subpopulations were located in old-growth, undisturbed forest within Corcovado National Park (Sirena Station, 8°32'25"N, 83°23'50"W). The other three subpopulations were located in degraded forests within the Golfo Dulce Forest Reserve (El Tigre station, 8°28'46"N, 83°35'11"W), in an area affected by deforestation, logging, hunting, and mining for the last 5–6 decades. Subpopulations consist of a few hundred individuals, and are separated at least 1 km from each other within habitats. The two study sites (native vs degraded habitat) are separated by a linear aerial distance of 20 km. Subpopulations were located in sites with similar topography, soil types, and vegetation type, in an attempt to remove potential site effects that could be confounded with the habitat effect, although completely removing site effects is difficult in a study of this scale with a long-lived tree-like species like *Z. fairchildiana*.

Estimation of growth, fecundity, and mate availability

In three subpopulations/habitat, we sampled all individuals present in a 100 × 20 m plot in the native-habitat subpopulations or a 50 × 10 m plot in the degraded-habitat subpopulations (for a total of three plots per habitat). Plots were smaller in the degraded habitat to keep similar samples sizes across subpopulations (samples sizes varied from 10 to 45 juveniles, and 35 to 154 adults per subpopulation). To estimate the average growth rate in each subpopulation, we counted the number of new leaves in the leaf flush/plant produced in the growing seasons of 2005 or 2006. In cycad populations, not all individuals produce a leaf flush in a

given growth season, therefore we sampled two growing seasons to obtain values for leaf production of as many individuals as possible within each subpopulation. Leaf production of individuals in *Zamia* species depend on plant size and may be affected by light availability (Clark et al. 1992). To estimate the effect of plant size on leaf production, we estimated individual size as total leaf area, calculated as the product of the total number of leaflets in all leaves by the average leaflet area of an individual. Leaflet size is similar across leaves in an individual, thus leaflet area was calculated using digital photos and an imaging software for four leaflets randomly chosen from the middle part of a mature leaf. To explore the effect of light availability on leaf production, we estimated the percentage of canopy openness around each measured plant, by counting the proportion of points without canopy cover in a spherical densiometer. To compare average growth rate (or leaf production) between habitats, we used a linear mixed model (GLMM) with habitat as a fixed factor, subpopulation nested within habitat as a random factor, and total leaf area and canopy openness as covariates. Comparisons of average growth rates across subpopulations were done separately for juveniles (smaller than the observed minimum size for reproduction) and adult individuals.

In the reproductive seasons of 2004 and 2005, we registered all individuals in the subpopulations that produced cones (for samples sizes, see Fig. 3). The total number of reproductive adults in both reproductive seasons was relatively low, and in preliminary analyses, subpopulation did not have an effect on the levels of fecundity in plants; thus, we pooled data for all subpopulations and both years within each habitat to compare fecundity allocation across degraded- and native-forest habitats. Allocation to fecundity was estimated as the slope of the regression between fecundity and plant size (Aarssen and Taylor 1992) of all reproductive plants in each habitat. Plant size was estimated as total leaf area, as described before. Fecundity was measured as the product of the number of cones (only 1 in females) and cone size, i.e., the number of sporophylls (cone parts bearing seeds or pollen sacs). We performed ANCOVA analyses to test for differences in fecundity allocation between habitats, i.e., to compare the slope of the relationship between total leaf area and fecundity.

In 2004, all adult individuals in subpopulations were counted to estimate total census size for each subpopulation. Using the number of females and males that reproduced in 2004 and 2005, we estimated the proportion of reproductive females and males in relation to the total number of adults in each subpopulation for both reproductive seasons. To estimate the proportion of reproductive females or males, we assumed that the total number of adults in a subpopulation was composed of 50% of females

and 50% of males. Adult sex ratios are mostly unknown for cycad populations, but the only two studies that have estimated them, found them to be close to a 1:1 sex ratio (Ornduff 1987, 1996). Nevertheless, in many cycad species, reproduction is more costly for females and they reproduce less often; therefore, in a given reproductive season, there is usually a sex bias towards more males (Clark and Clark 1987; Tang 1990). We estimated mate availability for females in each subpopulation, i.e., the number of males available for a female as pollen donors, as the average number of males/female in a given reproductive season. Statistical analyses for all the linear models were performed in SPSS version 13.0 (SPSS 2004).

Estimation of the fine-scale spatial genetic structure

For the estimation of genetic parameters, we developed microsatellite loci following the protocol by Hamilton et al. (1999) using DNA extracted from one individual of *Z. fairchildiana* from the Fairchild Tropical Garden collections (accession FTG93-812). Six microsatellite loci, highly polymorphic, were used for genetic analyses (see Table 1 for information on the microsatellite loci). To genotype individuals, we collected leaf samples of 200 and 250 randomly chosen individuals in one subpopulation in the native and the degraded habitat, respectively (for a total of 450 sampled individuals). Loci were unlinked, but most loci showed deviations from Hardy–Weinberg equilibrium (HWE in Table 1) in tests carried out with the software Arlequin (Schneider et al. 2000). Nevertheless, inferences

on the SGS within populations should not be affected by this deviation from HWE, as the relatedness coefficients used to estimate the SGS do not assume Hardy–Weinberg equilibrium (Hardy and Vekemans 2002). Finally, we estimated the neutral genetic differentiation between the subpopulations across habitats using an R_{ST} coefficient (Rousset 1996) implemented by the software SpaGeDi (Hardy and Vekemans 2002). The genetic differentiation among subpopulations was extremely low [$R_{ST} = 0.011$, standard error (SE) = 0.005], suggesting that some gene flow could happen between subpopulations and that our sample units cannot be considered as independent populations in different habitats.

To quantify the strength of SGS, i.e., the strength of the correlation of genetic relatedness and spatial linear distance, we used the Sp statistic (Vekemans and Hardy 2004). The Sp statistic allows making direct quantitative comparisons of the magnitude of SGS among populations. The Sp statistic combines information on the slope of the regression of pairwise relatedness on the logarithm of distance between individuals (b_F), and the average relatedness between neighbor plants (F_1), according to the formula $Sp = -b_F/(1 - F_1)$. We estimated pairwise relatedness coefficients among all individuals within patches using two kinship coefficients (Loiselle et al. 1995; Ritland 1996) that calculate the probability of identity of alleles of two homologous genes sampled randomly within each of a pair of individuals. These two coefficients have been shown to perform well in estimations of SGS with highly polymorphic loci (Vekemans and Hardy 2004). To

Table 1 Primer sequences (R: reverse primer, F: forward primer) and Genbank accession numbers (Genbank) for 6 microsatellite loci for the cycad *Zamia fairchildiana*

Locus name	Primer sequence	Genbank	Native-forest habitat subpopulation				Degraded-forest habitat subpopulation			
			N_a	H_{obs}	H_{exp}	N	N_a	H_{obs}	H_{exp}	N
Zfairchildiana	R: AGGACGATCAGAAATGGAAG	EU179839	15	0.83	0.87*	190	14	0.76	0.81	204
Clone Zf-05	F: GTGGCAAGTGTCCTGTTG									
Zfairchildiana	R: GGCCACCCTGGATTCTAA	EU179840	7	0.96	0.64*	146	7	0.93	0.62*	220
Clone Zf-06	F: AAGTCCTGGCATTGCACCT									
Zfairchildiana	R: AGCATTCAAAGGTGGCAAGT	EU179841	17	0.83	0.89	192	17	0.73	0.83*	212
Clone Zf-08	F: GGACGATCAGAAATGGAAGC									
Zfairchildiana	R: GGTGGAATAACTAATGGGTCAAA	EU179842	28	0.75	0.94*	155	27	0.80	0.92*	185
Clone Zf-10	F: CCCTAAAGGTCCCTTTGCTT									
Zfairchildiana	R: CCCTAAAGGTCCCTTTGCTT	EU179843	18	0.54	0.62*	179	16	0.35	0.39*	232
Clone Zf-11	F: TGGGTCAAAATATGTTATGCTTT									
Zfairchildiana	R: TGACCTTGGATGTGGAAAAGA	EU179844	16	0.90	0.79*	142	14	0.81	0.76*	162
Clone Zf-18	F: AGAGCACTTAAACCCAGGACA									

Number of alleles (N_a), levels of observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}), and number of individuals successfully genotyped (N) are presented for each subpopulation

* $P < 0.01$ in Hardy–Weinberg equilibrium tests

estimate the magnitude of SGS using the Sp statistic, we used all juvenile and adult individuals in the sampled plots, as separate analysis for juveniles and adults were not possible given the small number of juveniles in the sample. Average relatedness among individuals was calculated for distance classes of 5 m, ranging from neighboring individuals (less than 5 m from each other) to individuals separated by more than 100 m. Geographical distance between individuals was calculated as the Euclidian distance from two-dimensional spatial coordinates obtained for plants within each subpopulation used for genetic analyses. We performed the estimation of the parameters for the calculation of the Sp statistic and its SE using the software SPAGeDi (Hardy and Vekemans 2002). Jackknifing over loci was used to obtain multilocus averages and SE for all parameters, and we performed 1,000 permutations of the location of individuals within populations to test the hypothesis that the slope (b_F) was larger than zero.

Results

Growth, fecundity, and mate availability

Average canopy openness around *Z. fairchildiana* individuals was significantly higher in the degraded habitat (GLM $F = 55.64$, $P < 0.001$; Fig. 1). Leaf production in individuals was associated to total leaf area and to canopy openness around the individual plant, except that canopy openness did not have an effect on leaf production of juveniles (Table 2). Juveniles had similar levels of leaf production (0.9 ± 0.3 leaves in a leaf flush) across subpopulations in native- and degraded-forest habitats (Table 2). In contrast, adults in the degraded-habitat subpopulations had a higher leaf production (2.1 ± 0.4 leaves

in a leaf flush) than adults in the native-forest habitat (1.9 ± 0.5 leaves in a leaf flush) (Table 2). Total leaf area was strongly associated to fecundity in males and females (Fig. 2). Fecundity allocation in males was higher in the degraded-habitat subpopulations (ANCOVA $F = 4.31$, $P = 0.041$; Fig. 2a), and the minimum size to reproduction in males was smaller in the degraded-habitat subpopulations, as evidenced by the smaller intercept in the fecundity allocation curve (Fig. 2a). Finally, females had similar fecundity allocation and minimum size to reproduction in native- and degraded-forest habitats (ANCOVA $F = 1.03$, $P = 0.313$, Fig. 2b).

The total number of adults (or the census population size, estimated by counting all individuals in the subpopulation) across subpopulations tended to be larger in the native-forest habitat (ANOVA $F = 4.489$, $P = 0.101$, Power = 0.368; Fig. 3). However, population density of adults was higher in the degraded-habitat subpopulations (0.052 ± 0.008 plants/m²) when compared to adult density in the native-habitat subpopulations (0.018 ± 0.002 plants/m²). In both habitats, no more than 8% of the total number of females, or 18% of the total number of males produced cones in a single reproductive season (Fig. 3). The proportion of reproductive females and males tended to be higher in the degraded-forest habitat in both reproductive seasons (Fig. 3), although in general we did not have enough statistic power to detect significant differences between habitats. Similarly, the number of males per female in both reproductive seasons tended to be higher in the degraded-forest habitat (average \pm SE = 2.52 ± 0.10 in 2004 and 3.21 ± 0.90 in 2005) than in the native-forest habitat (average \pm SE = 2.14 ± 0.17 in 2004 and

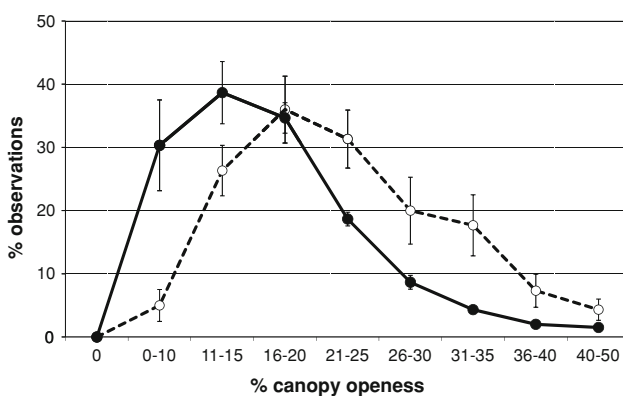


Fig. 1 Distribution of canopy openness values around *Zamia fairchildiana* individuals in subpopulations from native-forest (closed circles, solid line) and degraded-forest (open circles, dashed line) habitats

Table 2 Effect of habitat and subpopulation (nested within habitat) on leaf production for juveniles and adults in subpopulations of *Z. fairchildiana*

Effect	SS	df	df den	F	P	Power
Juveniles						
Habitat	0.124	1	140.08	1.783	0.184	0.264
Subpopulation (habitat)	0.231	2	145	1.683	0.189	0.350
Leaf area	0.704	1	145	10.266	0.002	0.889
Canopy openness	0.169	1	145	2.464	0.119	0.344
Adults						
Habitat	3.708	1	149.58	4.897	0.028	0.594
Subpopulation (habitat)	4.139	2	554	2.930	0.054	0.571
Leaf area	50.801	1	554	71.928	<0.001	0.999
Canopy openness	9.020	1	554	12.771	<0.001	0.946

A GLMM was used with habitat as a fixed factor, subpopulation as a random factor, and leaf area and canopy openness as covariates

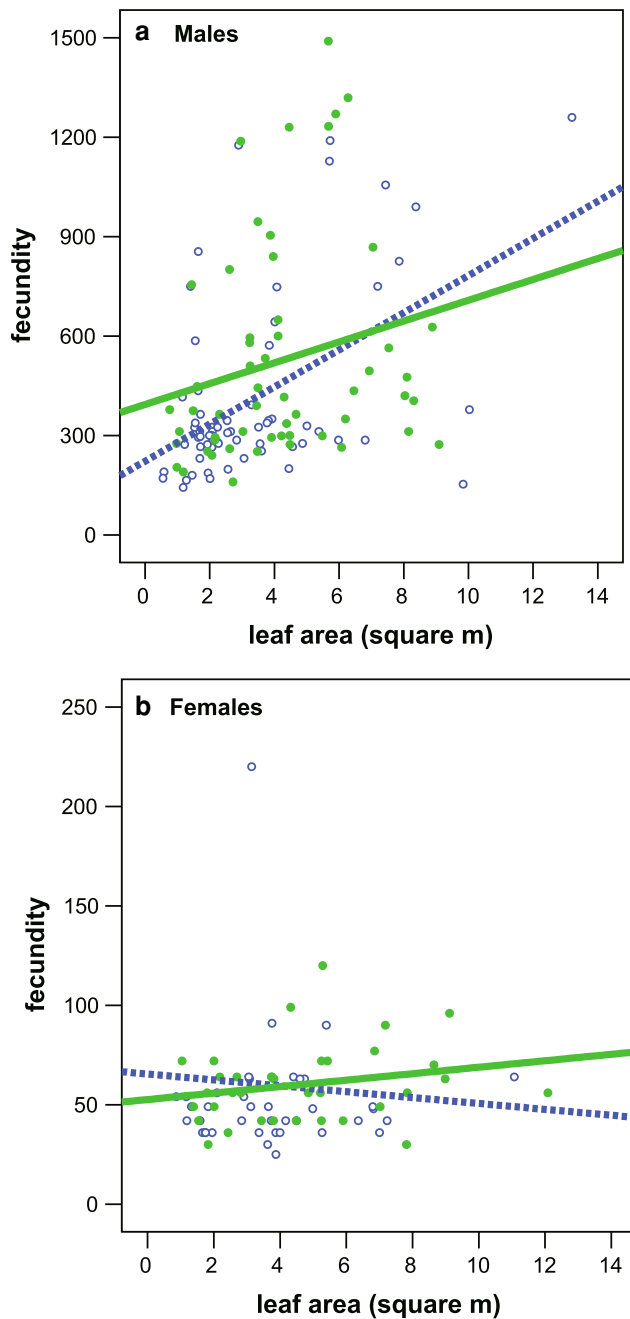


Fig. 2 Fecundity allocation for males (**a**) and females (**b**) in subpopulations of *Z. fairchildiana* from native-forest (closed circles, solid line) and degraded-forest (open circles, dashed line) habitats, as estimated by the slope of the relationship between total leaf area and fecundity in individuals

2.05 ± 0.18 in 2005), suggesting higher mate availability for females in a given reproductive season in that habitat.

The spatial genetic structure within populations

Both relatedness coefficients showed that average relatedness is lower in the degraded-habitat subpopulation for the

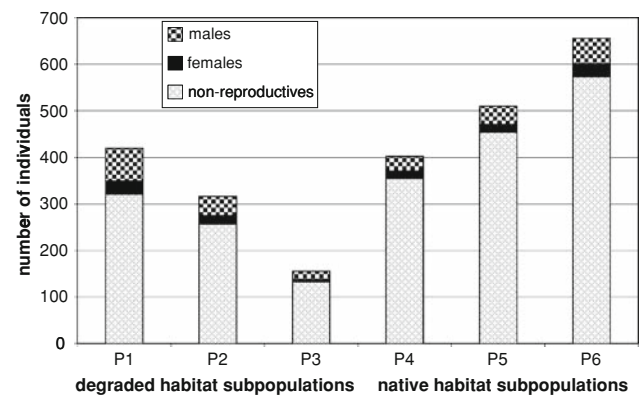


Fig. 3 Total number of non-reproductive adults, female and male individuals in subpopulations of *Z. fairchildiana* from native- and degraded-forest habitats in two reproductive seasons

first distance classes corresponding to neighboring plants (separated by less than 30 m of distance). Distance classes larger than 40 m in the native-habitat subpopulation and 30 m in the degraded-habitat subpopulation had negative average relatedness values (Fig. 4). Negative relatedness coefficients indicate that relatedness among pairs of individuals in these distance classes is lower than the coefficient expected for a random pair of individuals. Therefore, the plots used in this study seem to be appropriate to describe the fine-scale SGS within patches, as beyond the largest distance used for comparisons individuals are practically unrelated.

Relatedness between pairs of individuals decreased with the logarithm of geographical distance in both habitats (Fig. 4). Both relatedness coefficients showed that the slope of the linear regression of pairwise relatedness on the logarithm of the distance (b_F) is lower in the degraded-habitat subpopulation (Table 3). Lower values for b_F and F_1 resulted in lower values for the S_p statistic, i.e., weaker SGS in the degraded-habitat subpopulation (Table 3). Estimates of average relatedness and the S_p statistic were considerably smaller when using the coefficient proposed by Ritland (1996). This coefficient is usually biased downwards in the presence of rare alleles in microsatellite loci and may have low accuracy, but it has larger precision (smaller SE) than the Loiselle et al. (1995) coefficient (Hardy and Vekemans 2002). Ritland's coefficient showed a significant difference in the degree of SGS between habitats ($P = 0.029$ using Ritland's coefficient; $P = 0.105$ using Loiselle et al.'s coefficient).

Discussion

This study suggests that *Z. fairchildiana* subpopulations in degraded habitats differ in several life-history traits from

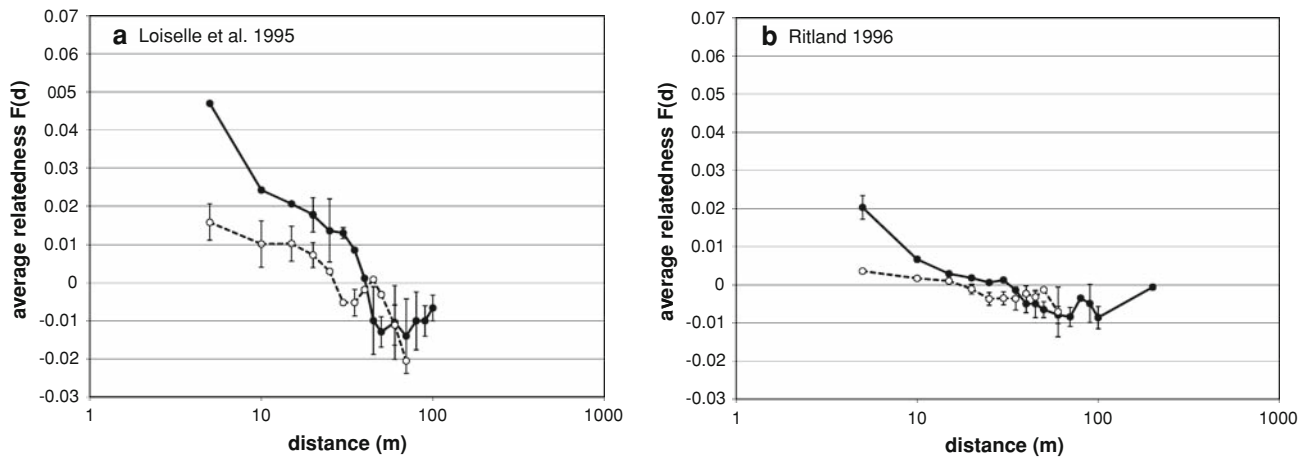


Fig. 4 Average relatedness F (\pm SE) among pairs of individuals in distance classes as a function of the logarithm of the distance between individuals in *Z. fairchildiana* subpopulations from native-forest (closed circles, solid line) and degraded-forest (open circles, dashed

line) habitats. These functions were estimated using the relatedness coefficients as defined in Loiselle et al. (1995) (a) and Ritland (1996) (b)

Table 3 SGS parameters (b_F = slope, F_1 = relatedness for the first distance class, and Sp statistic) for subpopulations of *Z. fairchildiana* from native and degraded habitats

Parameter	Loiselle et al. (1995)		Ritland (1996)	
	Native	Degraded	Native	Degraded
b_F	-0.0144	-0.0079	-0.0060	-0.0029
SE b_F	0.006	0.003	0.001	0.001
F_1	0.0470	0.0159	0.0203	0.0036
SE F_1	0.009	0.005	0.003	0.003
Sp	0.0151	0.0081	0.0061	0.0029
SE Sp	0.007	0.002	0.001	0.001
n	191	244	191	244

Parameters were estimated using the relatedness coefficients defined in Loiselle et al. (1995) and Ritland (1996)

subpopulations in their native habitat, and that life-history differences may be associated with variation in light availability across native- and degraded-forest habitats. Adult plants have higher leaf production in the degraded-forest habitat, and higher leaf production is associated with larger canopy openness. Furthermore, male individuals can start reproducing earlier and can allocate more to fecundity in the degraded-habitat subpopulations. These differences in life history result in a trend for a higher proportion of adults producing cones in a given reproductive season, and a larger availability of males per female in the degraded-habitat subpopulations. The subpopulations are not strongly differentiated at the neutral genetic level, but life-history differences between subpopulations in native and degraded habitats could affect the patterns of distribution of genetic variation within subpopulations. We found weaker SGS in the degraded habitat, suggesting that relatedness among neighboring individuals and the

distribution of genetic variation at a fine-scale within subpopulations could differ between native- and degraded-forest habitat subpopulations, although evaluating the effects of life-history differences among subpopulations from native and degraded habitats on the distribution of genetic variation at a fine-scale requires replicated estimates of the SGS within subpopulations in each habitat.

Differences in life history traits between subpopulations

In the degraded-habitat subpopulations adult individuals produce more leaves, and males start reproducing at a smaller size and have greater allocation to fecundity. Particularly for males, it seems that individuals in the degraded habitat have a faster life-history, in the sense of the “fast–slow continuum” hypothesis (Franco and Silvertown 1996), where fast plants grow rapidly, reproduce early, and invest more in fecundity. High growth and reproductive rates in tropical trees and a faster life-history are usually associated with high resource environments (Baker et al. 2003). Light availability is usually the most limiting factor for growth and reproduction in understory tropical rainforest species (Clark et al. 1992; Brien and Zuidema 2006); and not surprisingly, leaf production was positive associated with canopy openness in *Z. fairchildiana* subpopulations. In addition, canopy cover had no effect on leaf production in juveniles, and juvenile leaf production did not differ between native and degraded habitats (that differ considerably in canopy cover) in the subpopulations. Variation in growth and fecundity rates associated to light levels has been observed in other species of rainforest understories (Cipollini et al. 1994; Cunningham 1997; Svenning 2002). Differences in growth and fecundity may have positive or negative

effects on the demographic dynamics of subpopulations, but regardless of these long-term demographic effects, life-history variation could affect the distribution of genetic variation between and within populations, particularly if they affect mate availability and the patterns of gene movement.

Differences in growth and fecundity rates may have important consequences for the proportion of reproductive adults in a reproductive season and mate availability in the degraded-habitat subpopulations of *Z. fairchildiana*. In cycads, reproduction is highly costly, and only a small percentage of the total adult population produces cones in a given reproductive season (Norstog and Nicholls 1997). Males usually reproduce every 2 or 3 years, but the period between reproducing events is longer for females (Tang 1990; Ornduff 1996). Consequently, favorable conditions and enhanced growth can increase the probability of reproduction, as has been observed for other rainforest *Zamia* species in response to greater light availability (Clark and Clark 1987), and other understory rainforest species in general (Cunningham 1997; Marquis et al. 1997). With higher light availability, and higher growth and investment in reproduction, *Z. fairchildiana* plants in the degraded habitat may be able to reproduce more often, which seems to be the case for males. A faster life-history and higher frequency of reproduction in males will result in a higher proportion of reproducing males in a given reproductive season, as observed in the degraded-habitat subpopulations of *Z. fairchildiana*.

An important consequence of higher investment in reproduction and frequency of reproduction of males in the degraded-habitat subpopulations is that the ratio of male/female cones in a given reproductive season is larger. Male-biased sex ratios within reproductive seasons are common in dioecious tropical species, because of the higher cost of reproduction for females (Wheelwright and Bruneau 1992; Nicotra 1998). Reproduction is highly synchronous in cycads, and females are receptive to pollen only for a few days within a reproductive season (Norstog and Nicholls 1997). Males usually produce more than 1 cone, that mature sequentially to maximize the time during which pollen is released (Clark and Clark 1987). With more males producing cones in a reproductive season, a female may have a larger number of potential pollen donors in a given year in a cycad population. Therefore, mate availability or the number of potential pollen donors may be larger in the degraded-habitat subpopulations of *Z. fairchildiana*, given that females appear not to be pollen limited (all female cones observed during this study were fully pollinated). Greater mate availability could in turn have an important effect on the patterns of relatedness within seeds produced in a female cone, as well as on the spatial distribution of genetic variation within populations.

Effects of variation in life-history on the strength of the SGS within subpopulations

Tropical tree species usually show weak fine-scale SGS, given their great longevity and high pollen and seed dispersal distances (Vekemans and Hardy 2004; Hardesty et al. 2005; Hardy et al. 2006; Cloutier et al. 2007). However, some tree species with large seeds with restricted dispersal show strong SGS within populations (Dutech et al. 2002; Latouche-Halle et al. 2003). The degree of SGS that we observed in the native-habitat subpopulation of *Z. fairchildiana* is comparable to other tropical tree species with animal-dispersed pollen and large seeds that are gravity- or animal-dispersed (reviewed in Hardy et al. 2006). Pollination in *Z. fairchildiana* populations is carried out by weevils, that carry out their life cycle within male cones, but move between male and female cones looking for food and refuge, probably attracted by sugar and amino acid-rich micropyle droplets in female cones (Tang 1987; Norstog and Fawcett 1989). *Zamia fairchildiana* has large seeds dispersed by gravity, and most seeds disperse around the maternal plant. However, some seeds may germinate away from the mother plant because populations are located in steep slopes, and seeds may move down the slope, especially during the heavy rains that are common during the time seeds are dispersed. In addition, rare events of long dispersal may occur by ingestion of seeds by birds (Gomez 1993). Few instances of long seed dispersal and insect-mediated pollen movement might explain the weak SGS within subpopulations of *Z. fairchildiana*. Nevertheless, even with relatively weak SGS in the native-habitat subpopulations, the magnitude of the SGS in the degraded-habitat subpopulation was lower, as estimated by the comparative Sp statistic. Weaker SGS in the degraded-habitat subpopulation could result from increased seed dispersal distances in this habitat (Schnabel et al. 1998; Dutech et al. 2002; Jacquemyn et al. 2005; Jones et al. 2006), but given that most seeds are dispersed by gravity and the similar topography between the sites of the subpopulations, seed dispersal may have little impact on the differences in the strength of SGS observed between native- and degraded-habitat subpopulations.

Potential differences in pollen or seed dispersal distances between native- and degraded-forest habitats for *Z. fairchildiana* populations are difficult to evaluate, given the limited knowledge of its pollination and dispersal biology. Nevertheless, observed higher mate availability and greater adult density in the degraded-habitat populations could at least in part explain the differences we found on the strength of the SGS between subpopulations. Higher mate availability can potentially represent more pollen donors for females in a given reproductive season (Murawski and Hamrick 1991; Stacy et al. 1996), and increase the

proportion of half-sibs in the seeds of female cones. Since most seeds from a female cone are dispersed around the mother plant, seed shadows in the degraded-habitat subpopulation might contain individuals that have smaller average relatedness, which in turn could explain the reduced strength of the SGS in this habitat. Other studies have suggested that differences in the magnitude of SGS between populations with restricted seed dispersal could be associated with different levels of correlated mating resulting from varying mate availability that alter the average relatedness (or the proportions of full- and half-sibs) of seeds from a parental individual (Wells and Young 2002; Torimaru et al. 2007). In addition, greater overlap between seed shadows in populations with larger adult density (Vekemans and Hardy 2004) and more reproducing individuals in favorable environments (Ueno et al. 2006) can also result in weaker SGS, as observed in this study. A more detailed comparison of seed dispersal distances and seed shadow distribution, and the average relatedness in sibships affected by variation in mate availability (together with a wider sampling of subpopulations and sites), should contribute to establish the relative importance of these factors in explaining the differences in the strength of SGS among subpopulations of *Z. fairchildiana*.

Implications for conservation

This study suggests that environmental differences as a result of anthropogenic disturbance in forest habitats of *Z. fairchildiana* can significantly affect the life history of subpopulations, particularly their growth rate and allocation to fecundity, and the availability of mates for a female in a given reproductive season. The long-term ecological effects of a faster life history (especially for males) remain to be evaluated, as they could be positive if they increase overall plant fitness, but also negative if higher growth and allocation to fecundity decrease adult survival and/or longevity (adult survival is the demographic rate with the highest impact on population fitness in cycads; Negron-Ortiz et al. 1996; Negron-Ortiz and Gorchoy 2000; Perez-Farrera et al. 2006). In either case, short-term variation in life history and mate availability among subpopulations of *Z. fairchildiana* could have some effects on the distribution of genetic variation within populations, as suggested by the weaker strength of the SGS in the degraded-habitat subpopulation. Many of the environmental modifications in degraded forests are drastic, and many aspects of forest structure and composition in degraded habitats are likely to become permanently altered when compared to native habitats, even if humans disappear from the landscape (Chazdon 2003; Tabarelli et al. 2004). Therefore, observed differences in the ecological and genetic dynamics of subpopulations may become permanent in the human-

dominated landscape where populations of *Z. fairchildiana* are found. The potential short- and long-term effects of habitat modifications on the life history and the spatial distribution of genetic variation within populations should be explored in more detail in these populations, and may offer useful insight for conservation strategies taking into account the distribution of genetic variation within and between populations.

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