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Evolutionary Ecology

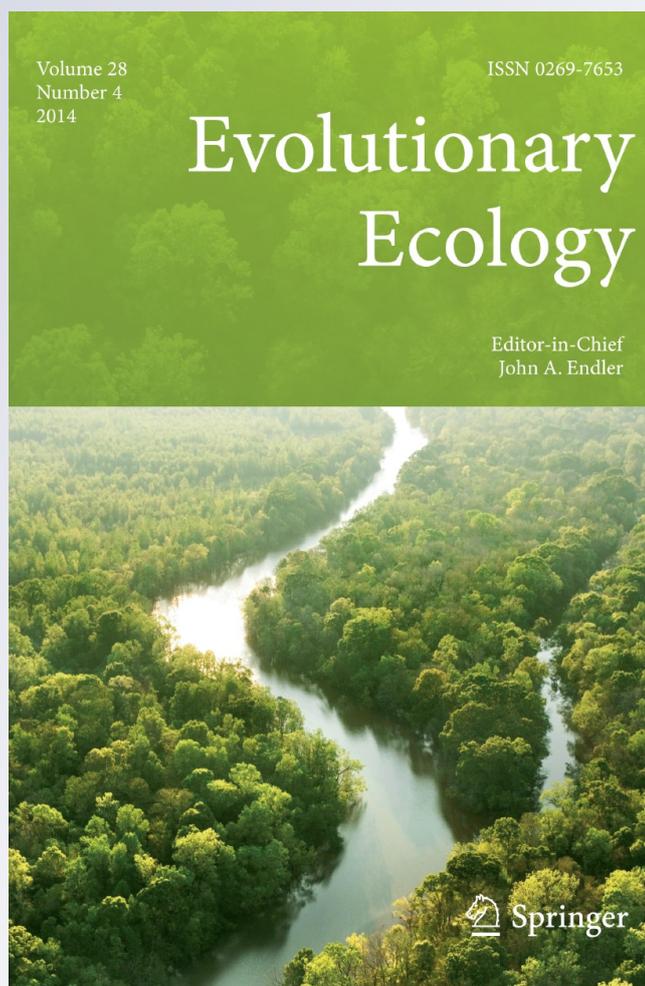
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Genetic variation and the potential response to selection on leaf traits after habitat degradation in a long-lived cycad

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Abstract Rapid evolution may be common in human-dominated landscapes where environmental changes are severe. We used phenotypic selection analyses and a marker-based method to estimate genetic variances and covariances to predict the potential response to selection in populations of a long-lived cycad recently exposed to drastic environmental changes. Patterns of selection in adult fecundity showed that different traits were under directional selection in subpopulations from native-undisturbed habitats and the novel degraded-forest habitat. Plants from a native-habitat subpopulation tend to maximize fitness through larger leaf area or smaller specific leaf area (SLA). In contrast, larger leaf production increased fitness in a degraded-habitat subpopulation, and canopy openness appears to be a major agent of selection for this trait. Leaf production and SLA showed significant additive genetic variance and no genetic trade-offs with examined traits, suggesting that these traits can respond to selection. Directional selection coefficients and heritability values were large, therefore significant phenotypic changes between subpopulations in few generations are possible. These results suggest that recent environmental change can result in strong directional selection in subpopulations of this cycad, and that these subpopulations have the potential to diverge at the genetic level in leaf traits after anthropogenic habitat degradation.

Keywords Adaptation · Natural selection · Quantitative genetics · *Zamia fairchildiana*

Introduction

Many studies suggest that significant phenotypic changes in populations can happen very fast, i.e. within a few generations, especially when environmental changes are drastic

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(reviewed in Hendry and Kinnison 1999; Reznick and Ghalambor 2001; Hairston et al. 2005; Carroll et al. 2007; Kinnison et al. 2007). This could be the result of strong directional selection in traits that may increase fitness under the novel environmental conditions (Kinnison and Hendry 2001; Reznick and Ghalambor 2001; Kinnison and Hairston 2007). Drastic environmental changes are commonplace in human-dominated landscapes, where anthropogenic activities can severely modify habitats. The accelerated rate of change in anthropogenic landscapes results in population extinctions in many cases, but it could also promote rapid evolution if populations can respond to selection and persist in the disturbed habitats (Palumbi 2001; Ashley et al. 2003; Stockwell et al. 2003; Zimmer 2003). Even if habitat modifications are transient, severe environmental changes spanning a few generations and the resulting strong selective pressures might have considerable effects on populations, modifying the genetic structure across populations or eroding genetic variation that could be important to respond to future environmental changes.

To predict potential phenotypic changes as the result of selection, information on the magnitude and direction of selection and on the genetic variance and covariances of traits is required. This information is rare for long-lived organisms, because estimating patterns of selection and heritabilities/genetic correlations of ecologically-relevant traits is challenging (Grant and Grant 2002; Geber and Griffen 2003). Nevertheless, phenotypic selection analyses in combination with estimates on genetic variances and covariances using molecular markers can be used to predict the response to selection to particular episodes of environmental change in wild populations. Directional selection is usually strong immediately after environmental perturbations and it usually remains strong for a few generations (Hendry and Kinnison 2001; Hoekstra et al. 2001). Therefore, detecting selection may be feasible in populations that have been recently affected by drastic habitat changes.

Zamia fairchildiana, similar to many other cycads in the Neotropic, is typical of the understory of lowland rainforests. The understory of lowland rainforests is a highly heterogeneous habitat, where light availability varies considerably in space and over time (Montgomery and Chazdon 2001). In these habitats, light is usually the most limiting resource for understory plants (Clark et al. 1992; Chazdon 1996). Consequently, changes in light availability can greatly affect growth rates, survival and fecundity of these species (e.g. see Clark and Clark 1987; Brienens and Zuidema 2006). *Zamia fairchildiana* can also be found in highly modified or degraded-forest habitats. Forest fragmentation and exploitation practices by humans (e.g. logging, hunting) result in degraded forests that differ significantly in physical structure and species composition when compared to native-undisturbed habitats (Noble and Dirzo 1997; Tabarelli et al. 2004). In particular, degraded-forest habitats for *Z. fairchildiana* have lower canopy cover, which could have important lasting effects on the levels and spatial distribution of light and other environmental factors in the forest understory (Nicotra et al. 1999; Montgomery and Chazdon 2001). Therefore, native and degraded forests may represent distinct habitats for populations of *Z. fairchildiana*, at least in terms of the magnitude and heterogeneity of canopy openness.

Environmental variation in light, humidity and nutrient availability, among other variables, can have strong effects on phenotypic traits related to growth rate and overall plant fitness. The “whole-plant economics spectrum” proposes that leaf functional traits and other traits related to stem and root physiology can greatly impact whole plant performance (Poorter et al. 2014). Alternative “ecological strategies” exist among species along a continuum from fast- to slow-growing species with long leaf life-span, structurally expensive leaves with low nitrogen content, low specific leaf area (SLA) and low photosynthetic rate (Reich et al. 2003; Wright et al. 2004; Shipley et al. 2006). These strategies are highly

consistent for most angiosperms within and across biomes, and are hypothesized to have evolved as adaptations to environmental heterogeneity at different spatial scales (Westoby and Wright 2006; Donovan et al. 2011). Nevertheless, little is known about the role of natural selection or genetic and other constraints on the evolution of variation for functional traits within plant populations, or the relative contribution of phenotypic plasticity versus genetic variation to phenotypic divergence among populations (Albert et al. 2011; Donovan et al. 2011; Wright and Sutton-Grier 2012).

In southwestern Costa Rica, subpopulations of *Z. fairchildiana* currently show low genetic differentiation at the neutral molecular level (Lopez-Gallego and O'Neil 2010). However, genetic divergence in ecologically-relevant traits between subpopulations from native and degraded habitats could arise if environmental changes result in differing patterns of directional selection (so that different genotypes have the highest fitness in each habitat) and the genetic variance/covariance structure of the subpopulations allows a response to selection. Particularly, differences in canopy openness and related environmental changes might have an effect on the phenotypic divergence for leaf functional traits (and other traits on the “whole plant economic spectrum”). Differing investment in reproduction and early survival associated with habitat differences has been observed for other Zamiaceae species in tropical rainforests (Clark and Clark 1988; Perez-Farrera et al. 2006).

This study addresses how phenotypic traits related to growth rate vary across environments and could impact fitness in novel forest habitats created by anthropogenic disturbances. We test the hypothesis that differences in canopy openness between the native- and degraded-forest habitats of *Z. fairchildiana* result in differing patterns of directional selection in each habitat, i.e. differences in the strength and/or magnitude of selection on the phenotypic traits. Furthermore, we estimate heritabilities and genetic correlations for the phenotypic traits to determine whether traits can respond to selection in the subpopulations. Few studies combine phenotypic selection analysis with genetic variances and covariances estimations to explore the potential response to selection on leaf traits in natural populations of plants (Donovan et al. 2011), but we were able to show in this study that different traits are under directional selection in contrasting habitats, and that some of these traits could respond to selection in subpopulations of a rainforest cycad exposed to novel environmental conditions created by anthropogenic habitat degradation.

Materials and methods

Study system

Zamia fairchildiana (Gymnospermae: Cycadales: Zamiaceae) inhabits the understory of lowland and mountain wet-forest between 0 and 1500 masl on the Pacific slope of SW Costa Rica and W Panama (Gomez 1982). Throughout the geographical range of the species, populations of *Z. fairchildiana* appear in large tracts of mature, relatively unaltered rainforest (i.e. the native habitat) and also in degraded or disturbed habitats (hereafter referred as the degraded habitat). *Zamia fairchildiana* individuals can reach up to 2 m in height and have a crown of 5–20 compound leaves. The number of leaflets increases progressively with age, from 4–6 leaflets in the seedlings to about 50–60 in the adults. Leaf production occurs in annual flushes (at the beginning of the rainy season by April–May), and every year the stem increases in height during growth episodes. Plants may take around

10 years to reach the minimum size for reproduction, and may live for several decades (therefore generation time might be on the order of several decades).

Cycads are dioecious, i.e. male and female cones are produced on separate individuals. Reproductive events are annual and synchronous, but every year only a small percentage of individuals in the population produce cones. Pollination is carried out by beetles, when the dry season starts, usually by mid-December. Seed development lasts for ca. 12 months, and mature seeds are dispersed locally by gravity when the cone parts rot away, which occurs at the end of the rainy season (November–December). Germination happens during the dry season, and the first leaves of seedlings emerge by the beginning of the following rainy season.

This study was carried out at two sites: a native-habitat site within Corcovado National Park, and a degraded-habitat site in the buffer zone of the same National Park, within the Golfo Dulce Forest Reserve. Patches of *Z. fairchildiana* individuals in the native-habitat site were located near Sirena station (8°28'46"N, 83°35'10"W), and in the degraded habitat site near El Tigre station (8°32'25"N, 83°23'50"W). A linear aerial distance of approximately 20 km separated the two study sites. Three separate patches or colonies of *Z. fairchildiana* individuals were chosen in each of the two study sites (each patch contained several hundred individuals). The patches of individuals within each habitat or site are considered a subpopulation, as a previous study found low neutral genetic differentiation across habitats (i.e. F_{ST} value = 0.11; Lopez-Gallego and O'Neil 2010).

The two study sites have similar topography and macro-climate, with patches of individuals located near streams on steep slopes (around 30 %), which is the common habitat for *Z. fairchildiana*. Nevertheless, the understory of these rainforests can greatly differ in micro-habitat conditions for plants like cycads. The understory in the degraded-forest site has a similar range of variation in canopy openness values but shifted towards greater values than in the native-forest site (Fig. 1). Therefore, environmental conditions related to light availability, moisture, temperature are likely different across these two habitats.

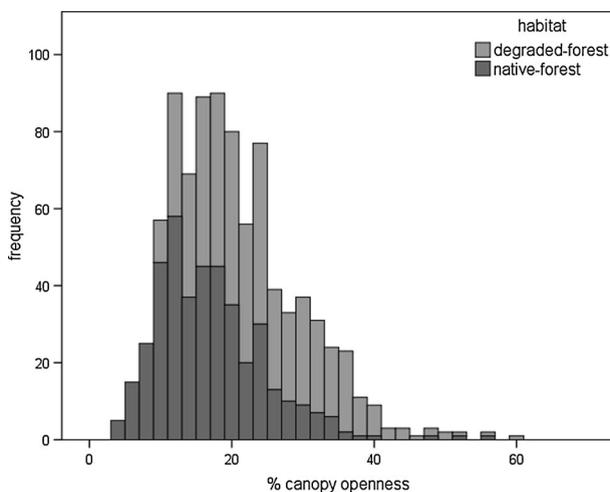


Fig. 1 Distribution of values for canopy openness in native-forest and degraded-forest habitats for subpopulations of *Z. fairchildiana*

Patterns of phenotypic selection

Many plant functional traits have been proposed in the literature to characterize ecological strategies in response to environmental variation (reviewed in Pérez-Harguindeguy et al. 2013). There is ample information for many of these traits in Angiosperm species, but little is known about functional traits variation in Gymnosperms or other plants. Cycads have simple stems compared to angiosperms, but stem size can be a good indicator of stored resources and could be related to overall growth rate (Norstog and Nicholls 1998). Leaf area and SLA (specific leaf area) have been linked to environmental variation at many spatial scales (reviewed in Poorter et al. 2009), and these traits could be closely associated to growth rate in rainforest cycads growing in the highly heterogeneous environment of the forest understory. In addition, growth rate in cycads could be related to leaf production and the number of standing leaves (a product of leaf production and leaf longevity), as most cycads grow in episodic events when they produce a flush of new leaves (Norstog and Nicholls 1998). A few studies have shown that traits like stem size, leaf size and leaf production might have an important effect on fecundity and survival, and therefore overall fitness, in cycads (Clark and Clark 1987, 1988; Ornduff 1991; Tang 1990).

We used phenotypic selection analyses (Lande and Arnold 1983) to estimate the magnitude and direction of selection in chosen phenotypic traits related to plant growth. Selection was estimated for five phenotypic traits: (1) stem length: total height from the base to the lowest leaf base; (2) leaf production: number of new leaves produced in a growing season; (3) number of old leaves: total number of live green leaves; (4) leaflet area: average area calculated with an imaging software using digital photos of four leaflets chosen at random from the middle part of a leaf; (5) specific leaf area (SLA): leaflet area per gram of dry weight, obtained after drying four leaflets until constant weight. We used adult fecundity as a measure of fitness. Fecundity was calculated as the total number of sporophylls (cone parts with seeds or pollen sacs) in all cones in an individual. For these analyses we sampled all reproductive individuals in the subpopulations in each habitat during the reproductive seasons of 2004 and 2005. Selection coefficients were similar in magnitude and sign for females and males and across subpopulations, thus data were pooled together for both sexes. Final sample size for selection analyses was 131 individuals in the degraded-habitat subpopulation and 134 individuals in the native-habitat subpopulation.

Coefficients of linear or directional selection were estimated for each trait in each subpopulation using path analysis (Scheiner et al. 2000). In the path analysis a measure of the overall condition of plants was included to reduce potential biases due to environmental effects that could act both on fitness and the phenotypic traits of interest (Scheiner et al. 2002). We used a measure of overall plant size, as estimated by the maximum number of leaflets (parts of each compound leaf), as a measure of condition. Leaflet number has proven to be a good indicator of size and developmental stage in *Zamia* species (Clark and Clark 1987; Negron-Ortiz et al. 1996). The path model included direct effects of plant size (i.e. condition) on all phenotypic traits, and effects of traits on fecundity. Additionally, there were paths linking leaf traits (i.e. leaflet area and SLA) with leaf production, and leaf production with the total number of leaves (Fig. 2). In a path analysis framework, direct effects of traits on fitness are estimated from direct connections between them, while indirect effects are estimated taking into account connections between traits and fitness that include intermediate traits (e.g. Stinchcombe 2002; Irwin 2006; Ashman and Penet 2007). Therefore, in our model leaflet area, SLA, and leaf production can have both direct and

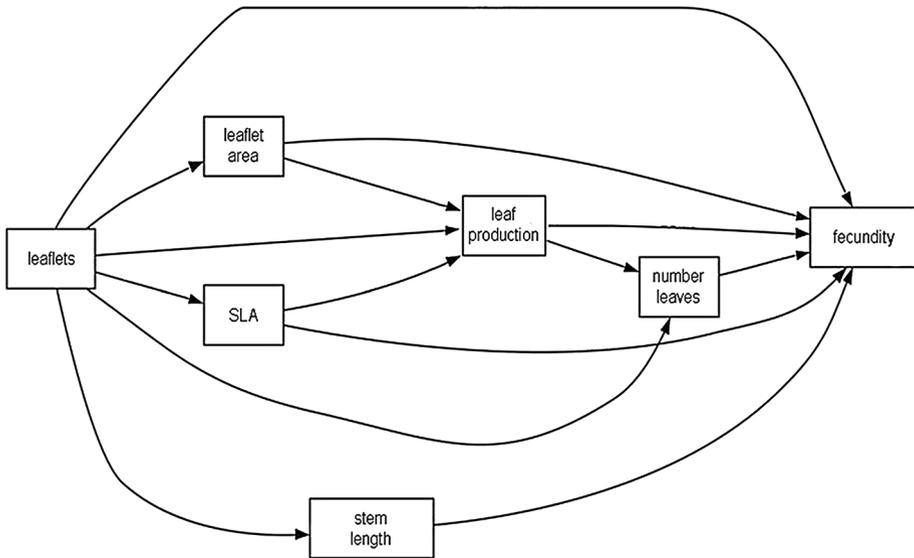


Fig. 2 Path diagram used in the phenotypic selection analyses for subpopulations of *Z. fairchildiana*. The model includes direct and indirect effects of traits on fitness (estimated as plant fecundity) and relationships between overall plant size (leaflets = maximum leaflet number) and phenotypic traits and fitness to account for potential environmental biases

indirect effects on fecundity. The sum of direct and indirect effects is an estimation of the total effect of a trait on fitness, i.e. the selection coefficient for the trait.

Small sample size (lower than 150 individuals) precluded the use of path analysis to estimate selection coefficients for non-linear (i.e. quadratic and correlational) selection in the subpopulations. Therefore, coefficients for non-linear selection were estimated as the partial regression coefficient in a multiple linear regression analysis using squared and cross-product values for the phenotypic traits, respectively (Lande and Arnold 1983; Phillips and Arnold 1989). Linear selection coefficients obtained from the multiple regression analysis are also presented, and should be equivalent to the selection coefficients from the path analysis, except that they do not include any correction for potential environmental biases. Furthermore, in the multiple regression analysis all selection coefficients represent direct effects of traits on fitness, and there are no indirect effects on fecundity through relationships between traits (as in the path analysis).

Finally, we explored the effect of canopy openness on the relationship between phenotypic traits and fitness. For each individual we measured the percentage of canopy openness above the leaf crown of the plant, using a spherical densiometer. To test the hypothesis that canopy openness is an agent of selection in subpopulations of *Z. fairchildiana*, we regressed selection coefficients for traits calculated in each patch of plants on the average canopy openness for all individuals in that patch (three patches in each of two subpopulations, thus $N = 6$). If a significant covariance exists between selection coefficients and an environmental variable across patches of individuals, then it is possible to hypothesize that the environmental variable is a causal agent of selection (Wade and Kalisz 1990). Path analyses and linear regressions were performed using the SEM package and basic packages of the R software (2013).

Estimation of heritabilities and genetic correlations

To predict the potential response to selection in long-lived species, marker-based methods can be used to estimate genetic variance and covariance of traits because they do not require genetic crosses (Ritland 2000; Thomas et al. 2000, Sillanpaa 2011). We used the marker-based method proposed by Ritland (1996) to estimate heritability and genetic correlations for all traits included in the selection analysis, as information on the genealogy and distribution of relatedness values were unknown for the subpopulations studied. With this method heritability is estimated with a linear model that takes into account the effects of additive genetic variance on phenotypic similarity for a trait (dominance and inbreeding depression effects on phenotypic similarity were not included in the model for simplicity). The estimation of the heritability (h^2) requires the calculation of the covariance between phenotypic similarity and relatedness coefficients for pairs of individuals ($C(Z,r)$) using the actual variance of relatedness (V_r) (Ritland 1996). Similar to the heritability estimation, genetic correlations are estimated using the covariance of phenotypic similarity between two traits within a pair of individuals and the relatedness between them ($C(Y_{12},r)$).

Given that heritability estimations in natural populations can have large errors, in many cases these estimates might not be significantly different from zero. Nevertheless, in the method used here the most informative part of the estimate is the term representing the covariance between phenotypic similarity and relatedness between individuals ($C(Z,r)$). If this covariance term is significantly positive, then it is highly probable that heritability is also significantly different from zero (Ritland 1996). Similarly, even if estimations of genetic correlations are not significantly different from zero, the sign of the covariance between two traits in a pair of individuals and their relatedness ($C(Y_{12},r)$) determines the sign of the genetic correlation and can be used to make inferences on constrains for the response to selection in particular traits. In addition to genetic correlations, we estimated phenotypic correlations between traits in the subpopulations for comparisons.

For heritability analyses we sampled all individuals present in two 100×20 m plots in the subpopulation from the native habitat, and two 50×10 m plots in the subpopulation from the degraded habitat (where plant density was higher). Previous analyses of the fine-scale spatial genetic structure of the subpopulations suggested that the spatial scale of these plots was adequate for heritability estimations, as most individual pairs within them had positive values for relatedness coefficients and therefore an excess of zero (or negative) values for relatedness coefficients will not result in a low variance for relatedness. We estimated pairwise relatedness coefficients using six microsatellite loci for all individuals within the plots (see Lopez-Gallego and O'Neil 2010). Polymorphism in the molecular markers used to estimate relatedness was large, i.e. $n(m - 1) \sim 90$ (where n is the number of loci, $n = 6$, and m is the average number of alleles, $m = 16$), compared to a range of 25–100 as recommended for heritability estimation (Ritland 1996). Average relatedness, the actual variance for relatedness, and heritabilities and genetic correlations were estimated using the software Mark (Ritland 2006). The statistical significance of the estimates was obtained based on 10,000 bootstraps by resampling individuals (comparisons between identical individuals were omitted). If more than 95 % of the bootstrap values were positive, then heritability parameters were considered significantly greater than zero (or lower than zero, in the case of negative genetic correlations with more than 95 % negative bootstrap values).

Heritability estimations from natural populations can be biased by an environmental correlation effect (where phenotypic similarity is inflated by the sharing of environments by individuals), particularly when both environment similarity and relatedness among

individuals decrease with increasing spatial distance (Ritland 1996). To explore the extent of the potential environmental correlation, we estimated the degree of spatial autocorrelation for plant size (estimated by the number of leaflets) that may reflect the effects of the environment on plant growth, and for canopy openness above individuals. We estimated the degree of spatial autocorrelation of these two variables using Moran's I index, which measures the degree of correlation between the values of a variable as a function of spatial distances (we used 20 distance classes ranging from 0 to 150 m), as implemented by the software PASSAGE (Rosenberg 2001). Because we found a significant environmental correlation effect, we conducted additional heritability estimations using the residuals from linear regressions of traits on plant size and traits on canopy openness. The analyses with residuals were intended to explore potential biases in heritability estimation generated by the phenotypic similarity being higher among individuals experiencing similar canopy environments; thus addressing the potential effect of the environmental correlation in the subpopulations directly and not assuming a function for the environmental correlation (as suggested by Ritland 1996 when data on the environmental correlation are lacking).

Results

Phenotypic selection analysis

The path model used for the phenotypic selection analysis included direct effects of overall plant size and all phenotypic traits on fecundity and the indirect effects of leaflet area, SLA, and leaf production on fecundity (Fig. 2). Using this path model, plant size had significant direct effects on stem length and number of leaves in both subpopulations, but no effect on leaf production, leaflet area or SLA (Table 1). Plant size affected fecundity in the degraded-habitat subpopulation (path coefficient $\beta = 0.178$, $P = 0.023$), but not in the native-habitat subpopulation (path coefficient $\beta = 0.041$, $P = 0.633$). Therefore, there is evidence of an environmental covariance in the relationship between stem length or number of leaves and fecundity, but only in one subpopulation. Nevertheless, the path analysis was designed to account for this environmental correlation, and selection coefficients for stem length and the number of leaves should not be inflated by this source of variation.

Table 1 Standardized selection coefficients for linear selection obtained from a path analysis for degraded- and native-habitats subpopulations of *Z. fairchildiana*

Trait	Degraded-habitat subpopulation			Native-habitat subpopulation		
	Direct effect	Total effect	Effect of plant size	Direct effect	Total effect	Effect of plant size
Stem length	0.102	0.102	0.242**	0.045	0.045	0.514**
Leaf production	0.346**	0.402**	0.029	0.111	0.125	0.001
No. of leaves	0.451**	0.451**	0.381**	0.094	0.094	0.405**
Leaflet area	0.205	0.304	0.062	0.303*	0.320*	-0.036
SLA	-0.207	-0.293	0.003	-0.473**	-0.474**	-0.072

Selection coefficients are shown for direct effects of traits on fitness and the total effect of a trait on fitness (i.e. combining direct and indirect effects). The direct effect of plant size (number of leaflets) on each phenotypic trait is included

Path coefficients: ** $P < 0.01$; * $P < 0.05$

Table 2 Standardized selection coefficients for linear and quadratic selection obtained from a multiple regression analysis for *Z. fairchildiana* subpopulations from degraded- and native-forest habitats

Trait	Degraded-habitat subpopulation		Native-habitat subpopulation	
	Linear selection	Quadratic selection	Linear selection	Quadratic selection
Stem length	0.127	0.085	0.069	−0.186*
Leaf production	0.409***	−0.010	0.067	−0.024
Number of leaves	0.421***	0.092	0.165	−0.105
Leaflet area	0.115	−0.049	0.172*	0.243**
SLA	−0.105	0.085	−0.197*	0.214*

Partial regression coefficients: *** $P < 0.01$; ** $P < 0.05$; * $P < 0.1$

Different traits were under directional selection in each subpopulation, according to the path analysis (Table 1). Leaf production and the number of old leaves were under strong directional selection in the degraded-habitat subpopulation, but these traits were not under selection in the native-habitat subpopulation. The number of leaves had positive direct effects on fitness, while leaf production affected fitness positively both directly and indirectly (through its effect on the number of leaves) in the degraded-habitat subpopulation. Leaflet area and SLA were under directional selection in the native-habitat subpopulation, where individuals with larger leaflets but smaller SLA had higher fecundity. In the native-habitat subpopulation, leaflet area and SLA had direct effects on fitness and also indirect effects on fecundity (of slightly larger magnitude) because they impacted leaf production and the number of leaves.

The selection analysis using multiple regression showed similar results to the path analysis for directional or linear selection (Table 2). In the multiple regression analysis leaf production and the number of leaves were under directional selection in the degraded-habitat subpopulation. Leaflet area and SLA had marginally significant partial regression coefficients in the native-habitat subpopulation (but significant coefficients in the path analysis). None of the coefficients for quadratic selection were significant in the degraded-habitat subpopulation (Table 2), suggesting that no trait is under non-linear selection in this subpopulation. In the native-habitat subpopulation leaflet area and SLA (with marginal significance) were under non-linear selection, and the selection coefficient for quadratic selection was greater than the coefficient for directional selection (Table 2). The model for correlational selection had no significant effects in the native-habitat subpopulation (data not shown), but the combination of higher leaf production and number of leaves was significantly related to fecundity in the degraded-habitat subpopulation (partial regression coefficient $\beta = 0.440$, $P < 0.001$). When plant size was included as a covariate in the multiple regression analysis selection coefficients did not change substantially, and it did not have a significant effect on fecundity in any subpopulation.

In the regression analysis between mean canopy openness and selection coefficients across patches (three patches per subpopulation), canopy openness explained a large portion of the variation ($R^2 > 0.5$) in the selection coefficients for leaf production (Table 3). The strength of selection on leaf production increased with increasing average canopy openness. Average canopy openness did not explain the variation in the strength of selection on any other phenotypic trait.

Table 3 Effects of average canopy openness on the directional selection coefficients for each trait across patches of *Z. fairchildiana* (within subpopulations)

Trait	R ²	Slope	P
Stem length	0.018	−0.134	0.755
Leaf production	0.724	0.851	0.015
Number of leaves	0.031	0.176	0.705
Leaflet area	0.048	−0.219	0.637
SLA	0.485	−0.697	0.082

R², the slope, and the *P* values are reported for the linear regression analysis done for each trait

Heritability estimates

For heritability estimations the total number of pairwise comparisons within samples was of 14,479 for the native-habitat subpopulation and 16,816 for the degraded-habitat subpopulation. Estimated average relatedness was 0.045 in the native-habitat subpopulation and 0.052 in the degraded-habitat subpopulation (close to the relatedness of first-cousins). The actual variance for relatedness was low ($V_r = 0.001$) but significantly different from zero in both subpopulations ($P = 0.0005$ in the native-habitat subpopulation, $P = 0.0001$ in the degraded-habitat subpopulation). A large number of pairwise comparisons, high marker polymorphism, and the detection of significant actual variance of relatedness allowed the estimation of heritability values in both subpopulations.

Most estimates of heritability were not significantly different from zero in the subpopulations analyzed, except for the trait SLA in the native-habitat subpopulation (Table 4). Nevertheless, the term representing the covariance between phenotypic similarity and relatedness between individuals ($C(Z,r)$) was significantly positive for some traits, which indicated that heritability could also be significantly different from zero. The trait SLA in the native-habitat subpopulation had a heritability significantly different from zero, and the $C(Z,r)$ term was significant (Table 4). The number of leaves in the native-habitat and leaf production in the degraded-habitat subpopulations had significant covariance terms and marginally significant values for heritability (Table 4), suggesting that these traits could have heritabilities greater from zero. Moreover, most traits in both subpopulations had covariance terms that were marginally significant, except for leaflet area, suggesting that most traits may have some additive genetic variance.

Phenotypic and genetic correlations

Since most heritability estimates were not significantly different from zero, genetic correlations were not different from zero either (Table 5). However, some of the estimates of $C(Y_{12},r)$ were significantly different from zero, and involved traits that may have positive additive genetic variances (Table 5). Significant values for the ($C(Y_{12},r)$) term suggested that there was a negative genetic correlation between SLA and the number of leaves, and a positive genetic correlation between the number of leaves and stem length in both subpopulations. Leaf production was not genetically correlated with any other trait in any subpopulation (Table 5).

Phenotypic correlations between SLA-number of leaves and leaf number-stem length were in the same direction as the genetic correlations for these traits (Table 5). Additionally, there were significant phenotypic correlations for traits that showed no evidence

Table 4 Values for heritability (h^2) and the covariance between phenotypic similarity (Z) and relatedness coefficients (r) for pairs of individuals $C(Z,r)$ in two subpopulations of *Z. fairchildiana* from native and degraded habitats

Trait	h^2	$P h^2$	$C(Z,r)$	$P C(Z,r)$
<i>Degraded-habitat subpopulation</i>				
Stem length	0.208	0.251	0.001	0.071
Leaf production	0.605	0.071	0.001	0.001
Number of leaves	0.149	0.323	0.001	0.097
Leaflet area	0.252	0.255	<0.001	0.101
SLA	0.283	0.247	0.001	0.069
<i>Native-habitat subpopulation</i>				
Stem length	0.315	0.205	<0.001	0.068
Leaf production	0.313	0.233	0.001	0.094
Number of leaves	0.498	0.085	<0.001	0.016
Leaflet area	0.023	0.524	0.001	0.278
SLA	0.765	0.027	0.001	0.005

Estimates are reported along with P values for testing the hypothesis that parameters are significantly different from zero

Table 5 Values for phenotypic and genetic correlations between traits in two subpopulations of *Z. fairchildiana* from native and degraded habitats

Trait	Stem length	Leaf production	No. of leaves	Leaflet area	SLA
<i>Phenotypic correlations</i>					
Stem length	–	0.246**	0.563**	0.199**	–0.061
Leaf production	0.225**	–	0.345**	0.054	–0.204**
No. of leaves	0.361**	0.433*	–	0.199**	–0.163*
Leaflet area	0.217**	0.078	0.300**	–	0.729**
SLA	0.134*	–0.066	–0.125*	0.859**	–
<i>Genetic correlations</i>					
Stem length	–	–0.397	0.306*	0.806	0.077
Leaf production	0.673	–	–0.396	–0.012	–0.102
No. of leaves	0.335*	–0.359	–	0.045	–0.847*
Leaflet area	0.494	0.162	0.095	–	0.445
SLA	0.018	0.880	–0.785**	0.349	–

Above diagonal: native-habitat subpopulation. Below diagonal: degraded-habitat subpopulation. For genetic correlations P values represent the significance of the covariance term ($C(Y_{12}r)$) of the genetic correlation, i.e. the covariance between values for the two traits within individuals and their relatedness coefficient

Correlation coefficients: ** $P < 0.01$; * $P < 0.05$

of a genetic correlation, and phenotypic correlations were mostly consistent across the two subpopulations (Table 5). SLA and leaflet area had a strong positive correlation in both subpopulations. Leaf production was positively correlated to the number of leaves in both subpopulations, and had a weak negative correlation with SLA in the native-habitat subpopulation. Finally, plants with a larger stem also had higher leaf production, leaf number, and leaflet area, but these correlations were weak in the subpopulations (Table 5).

Discussion

Differing patterns of selection across subpopulations

There is abundant evidence that functional traits related to growth rate can affect overall plant fitness (Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003), and that these traits can vary in response to environmental variation related to factors like light, water or nutrient availability (Albert et al. 2011; Wright and Sutton-Grier 2012). Patterns of selection in *Z. fairchildiana* subpopulations suggest that individuals have different ways of maximizing fitness in contrasting environments, as different traits were under directional selection across habitats. Individuals in the degraded-forest habitat have been exposed to a drastic environmental change and novel conditions regarding canopy openness, which could result in a strong selective event spanning several decades. Many studies have also found significant effects of natural selection on leaf functional traits in Angiosperms (reviewed in Donovan et al. 2011), but functional traits variation in response to selection on other plants is less known.

For the rainforest cycad *Z. fairchildiana* in its native habitat, the traits leaflet area and SLA showed evidence of stabilizing selection and also directional selection. A combination of directional and stabilizing selection for leaflet area and SLA results in changes in the mean and the variance for those traits, which could be important for plants adjusting to variable environmental conditions in the rainforest understory. In this habitat, where individuals are usually light-limited, greater leaf area could represent a portion of an ecological strategy related to enhancing light capture, as has been observed for other shade-tolerant forest species (Valladares and Niinemets 2008). On the other hand, directional selection for smaller SLA in the native forest habitat could be related to a strategy of keeping long-lived robust leaves (potentially associated to accumulating greater leaf area), enhancing carbon gain in resource-limited environments (Reich et al. 2003; Valladares and Niinemets 2008; Poorter et al. 2009). *Zamia neurophyllidia*, another rainforest cycad like *Z. fairchildiana*, produces very long-lived, well defended leaves, with intermediate physical features between sun- and shade-adapted species (Lee et al. 1990; Clark et al. 1992).

In contrast, within the degraded habitat differences in fitness were not recorded across the observed range of variation in SLA and leaflet area. Some studies suggest that changes in SLA may have a greater impact on growth rates under low- than high-light conditions (Sims et al. 1994; Evans and Poorter 2001). In addition, variation in SLA could be the result of complex interactions with different environmental factors, including light availability and others (Montgomery 2004; Poorter et al. 2009) that are changing in the novel degraded-forest habitat. In the degraded-forest habitat larger leaf production and leaf number had a direct impact on plant fitness (these traits showed directional and also correlational selection). Increases in leaf production and net leaf gain (resulting in a larger number of standing leaf number) are common in rain forest species when individuals are exposed to higher light availability in forest gaps (Blundell and Peart 2001; Osada et al. 2003). In a rainforest cycad like *Z. fairchildiana* producing more leaves to boost the reserves that can be invested in reproduction may be a viable strategy in the degraded habitat, but not in the native habitat where leaf production and reproduction is highly costly because of limited resources (Clark and Clark 1988).

The analysis of a potential cause of selection in subpopulations of *Z. fairchildiana* suggested that canopy openness might be a major agent of selection. Even with a restricted sample of patches of individuals, the magnitude of the selection coefficients increased with higher canopy openness for leaf production. Plant functional traits can vary in response to

many environmental variables related to light, water, and nutrient availability (Reich et al. 2003; Santiago et al. 2004; Poorter et al. 2014), all of which could differ substantially across habitats with different canopy openness. However, variation in canopy openness did not explain all the variation in selection patterns across patches, and other agents of selection may be affecting these subpopulations. For example, insect herbivory has been shown to have an important effect on leaf demography in rainforest *Zamia* species (Clark and Clark 1991; Negron-Ortiz and Gorchoy 2000). Future manipulative experiments could help establishing the relative role of different environmental factors on the relationship between phenotypic traits and fitness (Wade and Kalisz 1990).

The potential response to selection

In the native-forest subpopulation, SLA showed evidence of additive genetic variance and could respond to selection, while in the degraded-habitat subpopulation leaf production and the number of leaves under strong directional selection could also respond to selection. These leaf traits (SLA, leaf production, leaf number) showed evidence for high heritabilities in the subpopulations of *Z. fairchildiana*. Tree species usually show high levels of genetic diversity within populations, given their high outcrossing rates, extensive gene flow between populations, large effective population size, and the fact that they experience large environmental heterogeneity in space and time given their longevity (Petit and Hampe 2006; Albert et al. 2011). In addition, there is evidence from many species that functional traits can exhibit large levels of intraspecific phenotypic variation (Albert et al. 2011). Nevertheless, phenotypic plasticity, and therefore low heritability levels, are common for leaf functional traits in response to many environmental gradients (e.g. Wright and Sutton-Grier 2012). The relative importance of plasticity versus genetic variation in explaining phenotypic variability in these cycad populations remains to be explored, but this study shows that at least some phenotypic variance in leaf traits is the result of additive genetic variance, as shown from the heritability estimates.

On the other hand, genetic correlations show no evidence of genetic trade-offs that could constrain the response to selection of leaf production in the degraded-habitat subpopulation. Genetic correlations among leaf functional traits are highly variable, and it has been suggested that they usually might not limit the evolution of ecological strategies in plant populations (Ackerly et al. 2000; Donovan et al. 2011). However, in our study the number of leaves is strongly correlated to the trait SLA, which could result in correlated changes in SLA as the number of leaves increases in the degraded-habitat subpopulation in response to directional selection. Actual response to selection in all these traits could promote genetic differentiation in leaf traits between subpopulations in native-forest and degraded-forests habitats for *Z. fairchildiana*.

Predictions about the potential response to selection rely on the accuracy of the estimation of heritabilities and genetic correlations. Estimating additive genetic variances/covariances is difficult in natural populations of long-lived plants and few studies have successfully obtained heritability values for populations of long-lived organisms in field conditions (e.g. Andrew et al. 2005; Castellanos et al. 2011). Furthermore, there are few studies that have estimated the amount of genetic variation for leaf functional traits like SLA in natural populations of plants (Ackerly et al. 2000), but most studies have found moderate levels of heritability for leaf traits, i.e. $h^2 < 0.5$ (reviewed in Donovan et al. 2011). *Zamia fairchildiana* shows some fine-scale genetic structure within subpopulations (Lopez-Gallego and O'Neil 2010), therefore showing variation in relatedness between

individuals, which coupled with high marker polymorphisms allowed the use of a marker-based method for estimating heritabilities and genetic correlations in this study.

If heritability estimations in this study are accurate, then high levels of heritability ($h^2 \sim 0.6$) and large selection coefficients ($\beta \sim 0.3\text{--}0.4$, when compared to average values reported by phenotypic selection studies -Kingsolver et al. 2001) could produce changes in the phenotypic mean of leaf production of the order of 20 % per generation (0.3 SD/generation) in subpopulations of *Z. fairchildiana*. Rapid genetic changes like these and fine-scale local adaptation are not rare in plant species (Bone and Farres 2001; Leimu and Fischer 2008; DeKort et al. 2013). For example, local genetic differentiation is common in trees, even with extensive gene flow (Petit and Hampe 2006). In particular, habitat fragmentation and degradation can promote genetic changes in populations through increased population isolation and inbreeding depression effects (Lowe et al. 2005). More precise estimates of heritability values are necessary to make predictions about the possible rate of evolutionary change in subpopulations of *Z. fairchildiana*, as well as potential environmental effects on the genetic variation within subpopulations.

This study suggests that environmental changes resulting from anthropogenic activities could result in important selective events in subpopulations of a rainforest cycad exposed to a novel environment. Such a selective event and continuing altered conditions in the degraded-forest habitat could eventually lead to genetic differentiation between degraded-forest and native-habitat subpopulations in traits than can actually respond to selection, and could alter the genetic structure in the population as genetic divergence arises for some traits. Long-term monitoring of populations is necessary to evaluate the constancy of the selective pressures in native- and degraded-habitat subpopulations and to improve analyses for the potential response to selection in the population. If subpopulations diverge at the genetic level and lose genetic variation because of strong directional selection, subsequent effects on population structure will have implications not only for the definition of evolutionary significant units for conservation, but for the extent to which populations can respond to further environmental changes (e.g. global warming). Therefore, this type of information on the evolutionary dynamics of populations in human-dominated landscapes is relevant for conservation, and it can help understand population responses to rapid and drastic environmental changes in general.

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