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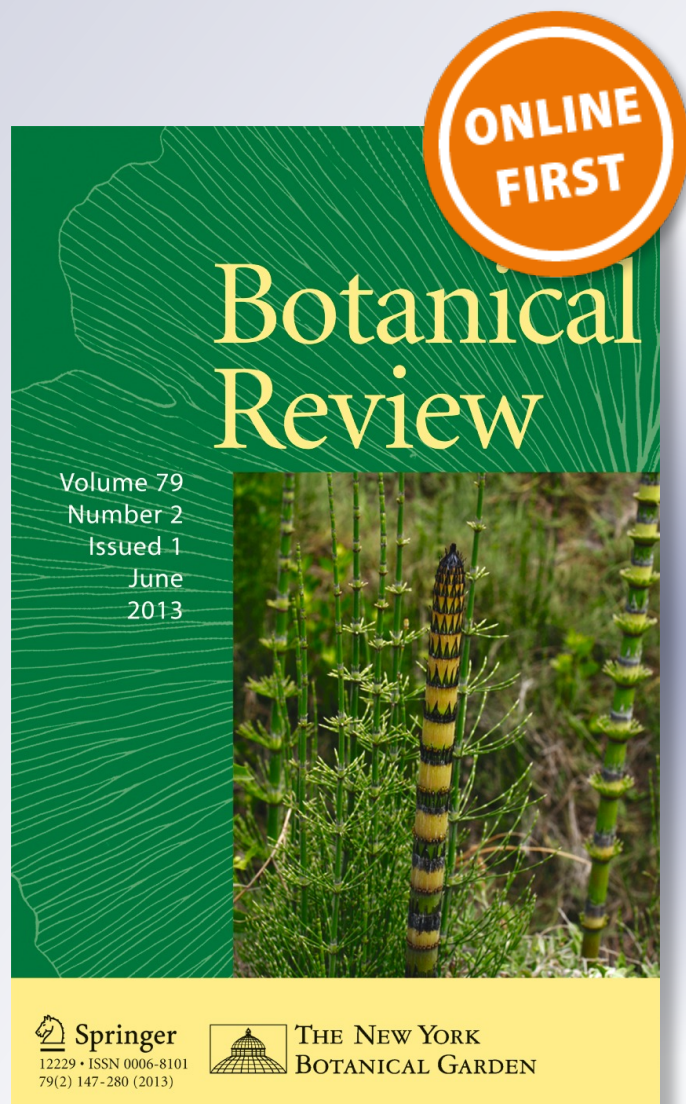
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Genotype-by-Environment Interactions for Seedling Establishment Across Native and Degraded-Forest Habitats in a Long-Lived Cycad

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Abstract Habitat differences might promote adaptive differentiation among populations that can be evidenced by genotype-by-environment interactions (GxE). I examined GxE in seed germination and seedling survival in demes of a rainforest cycad across their native and degraded-forest habitats, and explored the role of maternal effects and resource availability on the observed GxE. A reciprocal-transplant experiment showed a home-site advantage in terms of establishment of the demes. Germination in a manipulative greenhouse experiment mirrored the patterns in natural environments, with GxE in response to light and water availability. Overall germination was lower in the degraded-forest habitat and under high-light and low-water conditions in the greenhouse. Several analysis suggested that maternal effects related to size on germination are weak, but maternal effects are suggested by better survival of larger seedlings in the degraded-forest habitat. With weak maternal effects, GxE in establishment of individuals suggest some adaptive differentiation across demes in this cycad, which could have implications for population persistence in its habitats.

Resumen Diferencias entre habitats pueden resultar en divergencia adaptativa entre poblaciones que se evidencia por interacciones genotipo-ambiente (GxA). Examiné GxA en germinación y supervivencia de plántulas en demes de una cycada de bosques tropicales en sus habitats de bosques nativos y degradados, y exploré el papel de efectos maternos y disponibilidad de recursos en las GxA observadas. Un experimento de trasplante recíproco mostró una ventaja de sitio nativo en términos de establecimiento para los demes. La germinación en un experimento de invernadero simuló los patrones en ambientes naturales, con GxA en respuesta a la disponibilidad de luz y humedad. En general la germinación fue mas baja en el habitat de bosque degradado y en las condiciones de alta luz y baja humedad en el invernadero. Varios análisis sugirieron que los efectos maternos relacionados con el tamaño sobre la germinación son débiles, pero efectos maternos podrían existir dada una mejor supervivencia de plántulas de mayor tamaño en el habitat de bosque degradado. Con efectos maternos débiles, GxA en el establecimiento de individuos sugieren algún grado de divergencia adaptativa entre demes de esta cycada, lo cual podría tener implicaciones para la persistencia de las poblaciones en sus habitats.

Keywords Common-garden experiment · Maternal effects · Population divergence · Reciprocal-transplant experiment

Introduction

In tropical rain forests around the world anthropogenic activities result in forest environments that can differ substantially from the original habitats (Noble & Dirzo, 1997; Tabarelli et al., 2004). Fragmented and degraded forest habitats may represent extreme and even novel environmental conditions for plant populations in the understory. Genetic differentiation associated to such environmental variation at small- and broad-scale is commonly detected in plant populations (reviewed in Bone & Farres, 2001; Linhart & Grant, 1996). For example, tropical trees usually exhibit high levels of genetic variation and seem capable of rapid population differentiation (Petit & Hampe, 2006). Habitat degradation and consequent environmental changes could have strong impacts on fitness and therefore could promote population differentiation (Lowe et al., 2005). Nevertheless, the potential for phenotypic changes in response to habitat degradation and phenotypic/genetic differentiation between undisturbed- and degraded-habitat populations has rarely been considered. Exploring the role of different evolutionary forces in population responses to environmental changes and the potential for population differentiation after habitat degradation is relevant for defining evolutionary significant units (Crandall et al., 2000; Moritz, 1994) and for conservation strategies that take into account the genetic structure of populations (Hufford & Mazer, 2003).

Genetic differentiation between habitats can be tested in common-garden experiments, where genotypes from local demes or subpopulations are compared under similar environmental conditions. To further explore the role of natural selection in population differentiation a reciprocal-transplant experiment under field conditions can be used to test for local adaptation to different habitats (Kawecki & Ebert, 2004). In such reciprocal-transplant experiments crossing reaction-norms for fitness (i.e. functions of fitness values expressed by a genotype in several environments) are considered evidence for adaptive divergence. Crossing reaction norms or genotype-by-environment interactions GxE for fitness traits result from genotypes exhibiting higher fitness in their local environment when compared to genotypes from a different locality (the “home-site advantage effect”). Many studies have found this type of GxE in fitness resulting from home-site advantage effects in plant populations (e.g. Becker et al., 2006; Bischoff et al., 2006; Byars et al., 2007; Ellis & Weis, 2006; Macel et al., 2007), but few studies have compared populations in degraded versus native habitats (but see e.g. Antonovics et al., 1971; Shaw, 1991). These studies are important, as GxE in fitness traits can play a significant role on the ability of populations to persist or colonize degraded habitats, and on the relative role of phenotypic plasticity and genetic differentiation in response to novel environmental conditions (Sultan, 2004). For example, GxE and natural selection on seed germination can represent important factors that determine which genotypes can colonize novel environments (Donohue et al., 2005).

Genotype-by-environment interactions can result from differential fitness of genotypes across environments, but also from environmental-related differences in fitness of individuals. Consequently, environmental effects can not be disregarded

in experiments exploring population differentiation (Kawecki & Ebert, 2004). In particular, maternal effects can greatly affect seed and seedling fitness (Kirkpatrick & Lande, 1989), and contribute to GxE interactions. Maternal environmental effects related to seed reserves or other characteristic affecting germination and seedling performance can result in offspring with higher fitness under the maternal environmental conditions. Maternal effects are usually removed in experiments by rearing mothers in uniform conditions (e.g. Mazer & Gorchov, 1996). Nevertheless, maternal effects can enhance offspring fitness, and if genetic variation is present in populations these effects can evolve as adaptive responses to variable environments (reviewed in Galloway, 2005). In addition, maternal environmental effects can have a major impact on the rate of genetic differentiation between populations (Galloway, 1995; Schmitt et al., 1992). Therefore, instead of ignoring maternal effects they need to be evaluated when considering the magnitude and rate of genetic differentiation in natural environments. Exploring the nature of genotype-by-environment interactions and the presence of environmental effects for traits of interest will provide a more complete picture on the potential for genetic differentiation between demes in differing environments.

Cycads are long-lived tropical and subtropical gymnosperms. Most cycad species are threatened by habitat loss and degradation, and many populations persist in highly modified habitats (Donaldson, 2003). Populations of the cycad *Zamia fairchildiana* are typical of old-growth rainforests in Central America. *Zamia fairchildiana* patches of individuals or demes can also persist in degraded forests affected by selective logging and other human activities, where environmental conditions in the understory differ substantially from the ones in their native habitat. Analysis of the spatial genetic structure of this species in part of its distribution range in Costa Rica have revealed that genetic differentiation is extremely low between demes or subpopulations inhabiting native and degraded-forest habitats (Lopez-Gallego & O'Neil, 2010). Nevertheless, demes in degraded-forest habitats have experienced contrasting environmental conditions for a few generations, which appear to be associated to phenotypic differences in life-history traits and differing patterns of directional selection for growth traits (Lopez-Gallego, 2007). Here, I test for genotype by environment interactions in seed germination and seedling survival between *Z. fairchildiana* demes from native-forest and degraded-forest habitats. Furthermore, I examine the role of maternal effects and resource availability on GxE for seed germination observed in natural environments. To this end, I performed a reciprocal-transplant experiment in the field and a greenhouse experiment using seed families (genotypes) from four demes to estimate genotype-by-environment interactions in establishment of individuals.

Materials and Methods

Study System

Zamia fairchildiana (Cycadales: Zamiaceae) inhabits the understory of lowland and mountain wet-forest between 0 masl and 1,500 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 (hereafter referred as the “native habitat”) and also in degraded or disturbed habitats (hereafter referred as the “degraded habitat”). *Zamia fairchildiana* is a small tree that can reach up to 2 m of height, and has a crown of 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. Leaf production occurs in annual flushes, 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. Cycads are dioecious, i.e. male and female cones are produced in separate individuals. Reproductive events are annual and synchronous, but every year only a small percentage of individuals of the population produce cones. Pollination is carried out by weevils when the dry season starts by 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 by November–December. Seeds begin to form a radicle sometime during the dry season, and the first leaf emerges after the start of the rainy season in March–April. Newly emerged seedlings have one leaf with 4–6 leaflets, and accumulate reserves in the subterranean stem until the next dry season, when most seedling mortality occurs. Seedlings that are older than 1 year also have a high risk of mortality, but survival probability increases with age-stage in individuals.

The field study was carried out in two sites: a native-habitat site within Corcovado National Park, and a degraded-habitat site in the buffer zone of the National Park, within the Golfo Dulce Forest Reserve. Separate demes (patches of individuals) of *Z. fairchildiana* 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 separates the two study sites. Study sites are located around 0–100 m of elevation. Rainfall reaches 4,000–6,000 mm every year and is distributed across the year between a rainy season and a dry season that lasts from December to April.

Two demes of *Z. fairchildiana* were chosen in each of the two study sites. The demes are considered subpopulations because there is low neutral genetic differentiation between them, i.e. $F_{ST}=0.11$ (Lopez-Gallego & O'Neil, 2010, F_{ST} estimated using microsatellite markers). Demes within sites consist of discrete and isolated patches with a few hundred individuals each, that are separated by at least 1 km from each other. All demes were located in places with similar topography, in streams or riverbanks with steep slopes (around 30 %), which is the common habitat for *Z. fairchildiana* in the study sites. The degraded-forest habitat is very similar in terms of its climatic conditions to the native-forest site, but human influence for the last seven decades has resulted in a forest that could differ from the native habitat in terms of light and moisture availability in the understory (for more details see Lopez-Gallego & O'Neil, 2010, and Lopez-Gallego, 2007).

GxE in Germination and Seedling Survival in Natural Environments

To test for genotype-by-environment interactions in seed germination and seedling survival I performed a reciprocal-transplant experiment between sites in native and degraded-forest habitats. I collected ten seed families (hereafter genotypes) from each

deme, corresponding to the largest cones in each deme. A seed family is the set of seeds (usually 25–100) coming from one female cone, and consists of a mixture of half- and full-sibs. Ten cones represented between 80 % and 100 % of the number of female cones in each deme for the reproductive season of 2004. I established a seedling plot in each of the environment types, i.e. one site in native and one site in degraded-forest habitat. The plots were located in a place with a canopy cover similar to the average value for each habitat. In each plot, I randomly distributed twenty seeds per genotype. Seeds were not treated (i.e. the sarcotesta was not removed) before planting. Untreated seeds simulated natural conditions for germination, as most seeds in the wild remain in the soil surface after dispersal and are not consumed by animals before germination. Seeds were arranged in 1 cm deep holes in the soil, where surface litter was minimally disturbed. Within the plot seeds were placed in rows separated by 10 cm from each other and at least 1 m away from adult *Z. fairchildiana* individuals, to minimize potential interactions between individuals.

Six months after seed planting germination (or establishment) rates were calculated for each genotype in each environment as the proportion of seeds that produced a seedling. *Zamia* seeds have no dormancy, and seeds that did not germinate after 6 months were considered dead or non-viable. Seed predation was minimal, as it is common in this species (personal observation). One year after germination and at the end of the first dry season I recorded the proportion of seedlings that survived. This survival period included one full rainy and one full dry season, the last one representing the period where most seedling mortality usually occurs in the populations. To include the potential effects of seed mass on seed germination and seedling survival I also included average seed mass per genotype in the GxE analysis.

Effects of Light and Water Availability on Germination

I performed a manipulative greenhouse experiment to test for GxE interactions on germination in response to light and water availability. For this experiment I collected six female cones in the reproductive season of 2005 from each of four demes: two demes from native and two demes from degraded-forest habitats. I planted 25 to 40 seeds per genotype (depending on total number of seeds in the cone) in pots filled with a special soil mix developed for cycad propagation. The experiment was carried out in the greenhouse of the Montgomery Botanical Center in Miami (FL, USA), where environmental conditions intend to mimic tropical rainforest conditions. Seeds were planted approximately 1 month after they would have been dispersed in the natural population. Seeds were not treated and were placed in the soil with half of the volume above the surface, to simulate natural germination conditions in the field.

Seeds from all genotypes were divided between two light treatments, each one applied to a bench in the greenhouse. The high-light treatment corresponded to 30 % neutral shade and the low-light treatment to 90 % neutral shade. In natural environments the degraded- and native- habitat demes had an average canopy cover of 75 % and 84 % respectively, thus the high light treatment received a substantially larger amount of irradiance compared to natural conditions. Within each light treatment, half of the seeds received a low-water and the other half a high-water treatment. Seeds in the high-water treatment were watered to saturate the soil every week, while seeds in the low-water treatment were watered every 3 weeks. Germination was monitored

for 6 months and I estimated germination rate for each genotype across environmental treatments. Effects of seed mass on the probability of germination were included in the analysis for this experiment.

Maternal Effects Related to Size on Germination

I explored maternal-environmental effects in detail by evaluating the effect of the size of the mother, its light environment, and its average seed mass on seed germination, seedling size, and seedling survival under natural-field and controlled-greenhouse conditions. The light environment in the field for each mother was estimated as canopy openness values above the plant, using a spherical crown densiometer (Model C, Forestry Suppliers). Mother and seedling size were measured as total leaf area. Leaf area was estimated using four leaflets randomly chosen per plant, and then multiplying average leaflet area by the total number of leaflets in the individual. Leaflet area was calculated for each leaflet using a digital picture of it and the ImageJ imaging software (Rasband, 2012). Average seed mass for each mother was obtained by weighing to the nearest 0.01 g all seeds in the female cone produced by the mother.

Germination data for the seeds produced by a mother were obtained from the reciprocal transplant experiment in the field and the greenhouse experiment. Seedling survival data were recorded in natural environments using the seedlings from the reciprocal-transplant experiment that were 1 year old at the time of the census. In addition, I estimated survival in seedlings older than 1 year in two demes per habitat. I marked all the seedlings (individuals with less than 10 leaflets, excluding plants that germinated that year) present in a 100×20 m plot in the native habitat, or a 50×10 m plot in the degraded habitat (where individual density was higher). For seedlings within the plots, leaflet area was estimated from a measurement of leaflet width of the largest leaflet, using a regression equation of leaflet width on leaflet area developed with a preliminary sample of seedlings from both habitats ($r^2=0.91$, $P<0.001$, $N=64$).

Statistical Analysis

To estimate genotype (i.e. family) by environment (i.e. habitat or site) interactions in seed germination and seedling survival in natural environments I used a linear mixed ANOVA model. This model had habitat as a fixed factor, and deme and genotype as random factors. Significance for the fixed factor was evaluated with F-tests and for the random factors with Wald tests, using REML estimation. G×E in the greenhouse experiment were estimated with a similar mixed model, except that instead of habitat, light and water treatments were fixed factors in the analysis. Both models for the estimation of G×E included seed mass as a covariate, but seed mass had no effect on germination or seedling survival and models without the seed mass effect are presented.

Maternal effects on seed germination and seedling size in field environments were estimated using an ANCOVA model, with habitat where seeds were planted as the main factor, and mother size, mother canopy openness and average seed mass as covariates. In such a model, variation in seedling size due to the seedling environment is removed, and the direct effect of mother traits can be evaluated (Galloway, 1995).

Maternal effects on germination and seedling size in the greenhouse experiment were analyzed with a similar ANCOVA analysis, with light and water treatments as fixed factors. Direct effects of individual seed mass on the probability of germination for that seed were evaluated with a logistic regression. Similarly, the effect of seedling size on seedling survival was obtained from a logistic regression analysis, using the maximum number of leaflets/plant as a covariate (to account for effects of developmental stage). Logistic regression is a more appropriate measure of the effect of a trait like size on fitness components that have dichotomous values, like germination or survival (Janzen & Stern, 1998). All statistical analysis were carried out using the SPSS software (SPSS, 2009).

Results

GxE in Germination and Seedling Survival in Natural Environments

In the reciprocal-transplant experiment there was a significant GxE or family-by-habitat interaction for seed germination (Table 1). There was also a genotype effect and a habitat effect on seed germination (Table 1), where germination rate was lower in general in the degraded-forest habitat. The GxE resulted from genotypes originated in the native habitat having higher germination rate in this habitat than in the degraded habitat and vice-versa (Fig. 1a). Almost all genotypes from the native habitat had a germination rate higher than 50 % in the native habitat and lower than 50 % in the degraded habitat. A few genotypes from the degraded habitat had the same germination rate in both habitats or higher germination in the native habitat, and in general the difference in germination rate between habitats was smaller for these genotypes (the slope of the lines is smaller in Fig. 1a).

Genotype and habitat had no effect on seedling survival, but the sample size in this test was small (the number of seeds that germinated within a family was between two and twelve), and therefore the power of these analysis was low (Table 1). Nevertheless, a GxE graph shows that almost half of families had seedlings that survived better in the habitat where their seeds originated (Fig. 1b). The rest of the

Table 1 Genotype, environment, and GxE effects on germination rate and seedling survival for *Z. fairchildiana* demes in a reciprocal-transplant experiment between native and degraded-forest habitats

| Source | df | <i>F</i> | <i>P</i> |
|--------------------|----|----------|----------|
| Seed germination | | | |
| Deme | 01 | 00.69 | 0.516 |
| Genotype | 16 | 02.23 | 0.004 |
| Habitat | 01 | 10.95 | 0.051 |
| Genotype x Habitat | 18 | 02.44 | 0.001 |
| Seedling survival | | | |
| Deme | 01 | 00.51 | 0.606 |
| Genotype | 16 | 00.81 | 0.669 |
| Habitat | 01 | 02.32 | 0.267 |
| Genotype x Habitat | 18 | 01.51 | 0.083 |

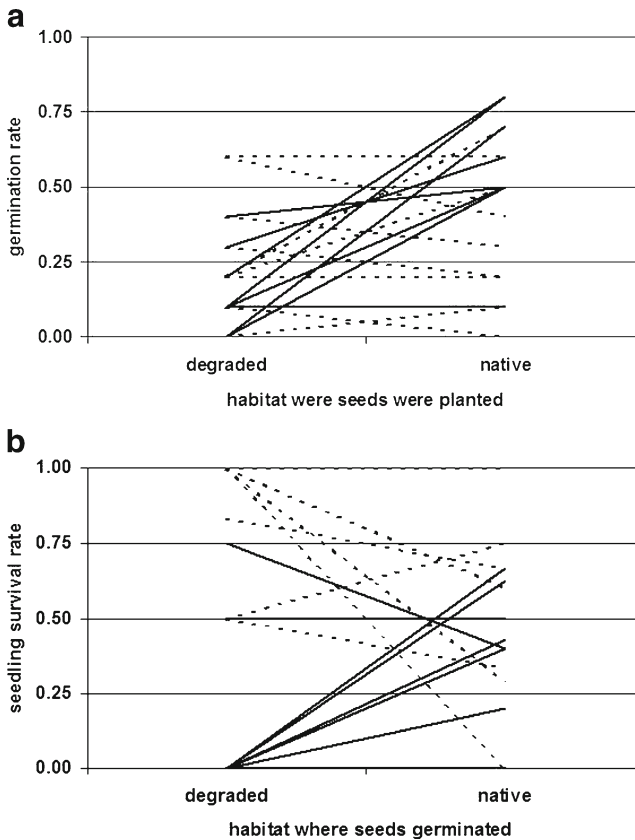


Fig. 1 Germination rate (a) and seedling survival rate (b) for genotypes (families) used in the reciprocal-transplant experiment across native and degraded-forest habitats. *Solid lines*: genotypes originated in the native-forest habitat. *Dashed lines*: genotypes originated in the degraded-forest habitat. See Table 1 for statistical analysis

families had few seedlings or a higher survival in the opposite habitat where their seeds came from originally. Most of the seedlings from genotypes that originated in the native habitat had zero survival in the degraded habitat, but genotypes that originated in the degraded habitat were able to survive in both habitats. When seed size was included in the GxE analysis it had no effect on seed germination or seedling survival across habitats, and it did not alter the significance of the main effects.

Effects of Light and Water Availability on Germination

In the greenhouse experiment, the light and water treatments and the genotype had an effect on germination rate (Table 2). More importantly, there were genotype-by-light and genotype-by-water treatment effects (Table 2). The GxE interactions resulted from smaller differences in germination rate between treatments for the degraded-habitat genotypes (their slope was smaller in Fig. 2). Seeds from families that originated in the native habitat germinated better in low light, and very poorly under high light conditions (Fig. 2a). Seeds from families in the degraded habitat had higher

Table 2 Genotype, environment, and GxE effects on germination rate for *Z. fairchildiana* demes in a greenhouse experiment with light and water treatments using seed families from degraded and native habitats

| Source | df | <i>F</i> | <i>P</i> |
|------------------|----|----------|----------|
| Seed germination | | | |
| Deme | 01 | 00.53 | 0.666 |
| Genotype | 20 | 02.64 | <0.001 |
| Light treatment | 01 | 00.56 | <0.001 |
| Water treatment | 01 | 01.29 | <0.001 |
| Genotype x Light | 23 | 03.09 | <0.001 |
| Genotype x Water | 23 | 03.32 | <0.001 |
| Light x Water | 01 | 00.43 | 0.512 |

germination in the low light as well, but the difference in germination rate between the two habitats is smaller for these families (Fig. 2a). Germination under low water availability was low for all genotypes, regardless of the habitat in which they originated, but germination rate differences between habitats is smaller for degraded-forest genotypes (Fig. 2b).

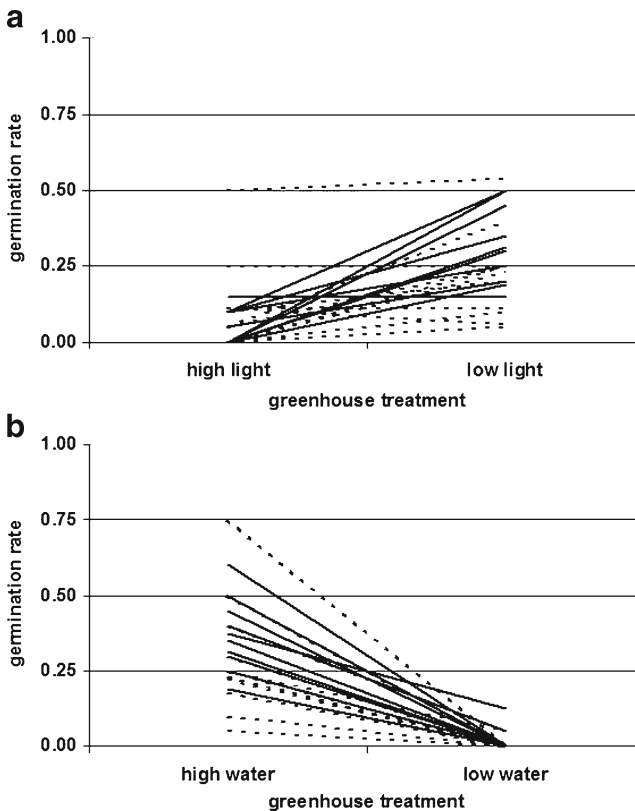


Fig. 2 Germination rate in the light treatments (a) and water treatments (b) for genotypes (families) used in the greenhouse experiment. *Solid lines*: genotypes originated in the native-forest habitat. *Dashed lines*: genotypes originated in the degraded-forest habitat. See Table 2 for statistical analysis

Maternal Effects on Germination

The conditions experienced by mothers in their natural environment did not have an effect on seed mass, and seed mass was similar across mothers growing in the two habitats (GLM $F=0.74$, $P=0.399$). When including all genotypes used in the reciprocal-transplant and the greenhouse experiments, seed mass was not associated with mother size or seed number (whole model $r^2=0.09$, $P=0.42$ for degraded-habitat mothers and $r^2=0.14$, $P=0.31$ for native-habitat mothers). Mother size, light environment, or average seed mass did not affect germination rate in the reciprocal transplant experiment under natural conditions (Table 3). Genotypes with larger seeds had larger seedlings in general, but this trend was not associated with mother size or its light environment (Table 3). The results from maternal-effects analysis in the manipulative greenhouse environment were similar to the results from the experiment in the field. Seed germination was not affected by mother size, light environment, or seed mass (Table 3). The light and water treatment had an effect on germination, where seeds in low light and high water had greater germination than in the other treatments (Fig. 2), but the treatments had no effect on seedling size (Table 3). Like in the reciprocal transplant experiment, seed mass had a positive effect on seedling size (Table 3). Finally, in the field seedling size affected seedling survival in 1 year old seedlings, but only in seedlings growing in the degraded-forest habitat (Table 4).

Discussion

GxE in Seedling Establishment Between *Z. fairchildiana* Demes from Native and Degraded-Forest Habitats

Genotypes of *Z. fairchildiana* from native and degraded-forest habitats had differing germination and survival responses to contrasting environments. Genotype-by-

Table 3 Maternal effects in germination and seedling size related to mother size, mother light availability, and seed size for *Z. fairchildiana* demes in a reciprocal-transplant (RTE) and a manipulative greenhouse experiment (MGE)

| Source | RTE | | MGE | |
|----------------------|----------|----------|----------|----------|
| | <i>F</i> | <i>P</i> | <i>F</i> | <i>P</i> |
| Seed germination | | | | |
| Mother leaf area | 00.60 | 0.444 | 01.43 | 0.239 |
| Mother canopy | 01.35 | 0.254 | 00.39 | 0.535 |
| Seed mass | 00.04 | 0.851 | 00.18 | 0.677 |
| Habitat or treatment | 07.38 | 0.010 | 23.66 | <0.001 |
| Seedling size | | | | |
| Mother leaf area | 00.12 | 0.734 | 00.04 | 0.845 |
| Mother canopy | 00.52 | 0.476 | 00.55 | 0.466 |
| Seed mass | 05.41 | 0.027 | 04.07 | 0.054 |
| Habitat or treatment | 02.22 | 0.147 | 00.07 | 0.794 |

Table 4 Effect of seedling leaf area on seedling survival for *Z. fairchildiana* demes in field environments. Survival rate was estimated for seedlings that were 1 year old (1 year) and for seedlings that were older than 1 year (>1 year) in demes from native and degraded-forest habitats. Logistic-regression values for the slope (β) and Wald tests of significance are reported

| Habitat | Age | β | d.f. | Z | P |
|----------|----------|---------|------|------|-------|
| Native | 1 year | 0.001 | 01 | 0.01 | 0.959 |
| | >1 year | 0.016 | 01 | 3.01 | 0.083 |
| Degraded | 1 year | 0.034 | 01 | 4.98 | 0.026 |
| | > 1 year | 0.003 | 01 | 1.78 | 0.182 |

environment interactions in the reciprocal-transplant experiment in field environments and in response to light and water treatments in the greenhouse were the result of different slopes of reaction norms of genotypes. The results evidenced a typical home-site advantage effect, where local genotypes have the highest fitness in each habitat. Forest degradation resulting in contrasting environmental conditions in the understory of native and degraded-forest habitats could be associated to the observed GxE (although other site effects could be involved as well). In particular, differential responses in germination rate of genotypes from native and degraded-forest habitats may be associated with the ability of seeds and seedlings of this species to tolerate desiccation.

Seeds from all cycads are recalcitrant, i.e. they have no dormancy and very low tolerance to desiccation (Norstog & Nicholls, 1997). Germination cues in tropical rainforests are complex, and may involve light, moisture, and temperature; however for most non-pioneer species water availability has the predominant role in regulating germination timing across the wet versus the dry season (Everham et al., 1996; Garwood, 1983; Vazquez-Yanes & Orozco-Segovia, 1993). Recalcitrant seeds of rainforest species are usually large, get dispersed during the rainy season, and germinate quickly (Daws et al., 2005; Farnsworth, 2000), as shown by *Z. fairchildiana* seeds in their native habitats. Lower overall germination of *Z. fairchildiana* seeds in the degraded-forest habitat, as well as under the high light and low water treatments in the greenhouse, support the idea that sensitivity to desiccation is important. Decreased germination under the high-light and low-humidity conditions are common for rainforest species under the lower canopy of gaps and degraded forests in tropical forests (Bruna, 2002; Kyereh et al., 1999). Similarly, seedling survival of rainforest species is usually lower under the higher desiccation conditions of gaps or forest fragments (Engelbrecht & Kursar, 2003; Fisher et al., 1991; Turner, 1990). Drought has been shown to negatively affect seedling survival in other *Zamia* species (Tang, 1990), and is a strong selective agent in early life-cycle stages in tropical rainforests (Engelbrecht & Kursar, 2003; Tobin et al., 1999). Consequently, genetic and environmental effects on desiccation tolerance may be important in explaining the GxE in germination and seedling survival observed in *Z. fairchildiana* populations.

GxE in response to light and water treatments in the greenhouse suggested that these two environmental factors may play a role in explaining the home-site advantage observed in natural populations of *Z. fairchildiana*. Light is an important factor

affecting germination in many plants, but its effects seem to be less important for non-pioneer tropical tress (Everham et al., 1996; Kyereh et al., 1999; Raich & Khoon, 1990). Nevertheless, it is possible that besides the effects of increased irradiance on desiccation risk for seeds and seedlings, light levels have an impact on germination in *Z. fairchildiana*, as many cycads are adapted to open habitats where light is generally an important regulator of germination (Mathews, 2006). Light effects on GxE on germination will explain the lower germination rate of degraded-habitat families in the native habitat or low light conditions in the greenhouse, where desiccation risk should not be high. Alternatively, different sets of genes may regulate germination in response to different factors (Donohue et al., 2005), like desiccation risk and light conditions. The relevance of desiccation tolerance and other mechanisms affecting the rate and timing of germination needs to be explored in this species. The light treatments in the greenhouse could not be decoupled completely from the moisture levels experienced by the seeds in the experiment. Water treatments manipulated soil moisture availability, but lower air humidity under high light conditions can also affect seed desiccation. Therefore, it is difficult to evaluate the precise role of light availability on seed germination in *Z. fairchildiana* genotypes and this issue needs further attention.

Different responses in germination by native- versus degraded-habitat families resulted from a clear trend in which degraded-habitat families showed a less contrasting response across habitats or greenhouse treatments. This could result from genotypes in the degraded-habitat that are still capable of performing well in their original habitat. In addition, genotypes in the degraded habitat could be regarded as more generalist. There is a similar trend by which generalist species have the ability to maintain relatively high fitness in poor environments and maximize fitness under favorable conditions (Sultan, 2001). At the population level, a more generalist genotype, regarding desiccation tolerance for example, may be able to exploit better the novel environmental conditions in the degraded habitat, while maintaining a good germination rate in the original conditions of the native habitat. Genetic variation for desiccation tolerance has been observed in species with recalcitrant seeds (Peroni, 1995). Nevertheless, little is known about the mechanisms determining variation in desiccation tolerance in recalcitrant species (Farnsworth, 2000). It is known that increased levels of abscisic acid (ABA) inhibit germination in dormant seeds and increase their tolerance to desiccation, a behavior that can be artificially induced in recalcitrant seeds (Finch-Savage & Clay, 1994). Increased levels of ABA in degraded-habitat genotypes may enhance their tolerance to desiccation, but reduce their germination rates in both habitats, which will explain the lower slopes in their germination reaction norms. Finally, costs of desiccation tolerance (and intolerance), e.g. generated by a longer time to germinate that will increase the probability of seed mortality (Tweddle et al., 2003) need to be explored, as they could help explain crossing reaction norms, and particularly the lower germination rate of degraded-habitat genotypes in the native habitat.

Maternal Effects on Seed Germination for *Z. fairchildiana*

Maternal environmental effects can affect germination and seedling survival, thus they could explain in part the presence of GxE, and also mask genetic variation or

reduce the rate of genetic differentiation between populations (Galloway, 1995; Schmitt et al., 1992). In this study size-related maternal environmental effects on germination appeared to be weak and to have little influence on the GxE across habitats. In contrast to germination, size-related maternal effects were important for seedling survival, but notably, only in the degraded-forest habitat. Positive effects of seed size on germination and seedling survival are common in long-lived trees (e.g. Bonfil, 1998; Campbell, 1997; Castro, 1999; Kang et al., 1992; Seiwa, 2000). However, size-related and other maternal effects on early performance are not universal and can not only depend on the species, but also be affected by external environmental conditions (Mazer & Schick, 1991; Munir et al., 2001; Paz et al., 1999). Other studies have found that seed size effects were more important on seedling performance than on germination in perennial plants (Eriksson, 1999; Herrera, 2000), although the reasons for this are not clear. Maternal effects in *Z. fairchildiana* populations may become important for young seedlings under the harsher environmental conditions of the degraded habitat, e.g. if they allow seedlings to develop larger root systems and decrease water stress (Fisher et al., 1991). These maternal effects may be important for population persistence in the degraded-forest habitat, as viability selection through young seedlings is very strong in *Zamia* populations.

Other maternal environmental effects, not related to seed or seedling size, could also affect the patterns of GxE in offspring traits (e.g. see Andalo et al., 1999; Galloway, 2001; Sultan, 1996; Wulff et al., 1994). For example, maternal effects related to water availability and desiccation tolerance could result in GxE. Mother plants producing high levels of ABA in response to desiccation stress in the leaf tissues could produce seeds that have high ABA content and are more tolerant to desiccation (Farnsworth, 2000). This type of maternal effect is prevented in some species with recalcitrant or viviparous seeds, like mangroves, by compartmentalizing the production of phytohormones and substances regulating germination and desiccation tolerance (Farnsworth & Farrant, 1998). However, these mechanisms may be absent in more ancestral plants like cycads. Few studies have focused on maternal environmental effects related to water-availability environments (but see Latta et al., 2004; Luzuriaga et al., 2006; Rice et al., 1993). Furthermore, seeds that are desiccation intolerant are relatively rare compared to seeds that can tolerate some drying during their development and have dormancy (Pammenter & Berjak, 2000; Tweddle et al., 2003), and thus there is virtually no information on potential genetic or maternal environmental effects of desiccation tolerance on germination or seedling performance. In addition, light-related maternal effects could result in seeds that germinate better under the same light conditions that mothers experience. Long-term observational and manipulative experiments will be required to fully address the impact of genetic and maternal environmental effects on GxE in offspring traits in *Z. fairchildiana* populations.

In the presence of weak maternal effects, GxE in seedling establishment for the studied *Z. fairchildiana* demes could result in adaptive population differentiation across demes from native and degraded-forest habitats (given the home-site advantage effect). Further examination of environmental effects besides size-related maternal effects on GxE and replicated experiments in additional sites will provide more solid evidence about the role of habitat changes due to forest degradation on the

population divergence observed. However, evidence that canopy cover changes after habitat degradation could represent a selective event (Lopez-Gallego, 2007) supports the hypothesis that population divergence could have occurred after habitat degradation. Seed germination and seedling survival have an important impact on population fitness, as most selection via mortality occurs at these life-cycle stages in *Zamia* populations. Strong selection in early life stages is common in trees, and it can result in genetic differentiation among populations (Petit & Hampe, 2006). Genetic differentiation between native- and degraded-habitat populations has been detected at the seedling stage in other rainforest species in fragmented habitats (Aldrich et al., 1998). Nevertheless, GxE observed in this study could be the result of other site differences between the two locations besides the degradation of the forest. Long-term data from replicated reciprocal-transplant and other experiments and detailed evaluations of environmental effects on GxE will provide further evidence of the potential for long-term genetic differentiation in *Z. fairchildiana* demes in contrasting habitats. Exploring the potential for local adaptation to degraded habitats and its consequences for the genetic structure and evolutionary dynamics of populations should be an important concern in conservation, as rapid evolution might be widespread among plants and evolutionary processes should be a central component of conservation strategies (Smith et al., 1993; Stockwell et al., 2003).

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