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Effects of habitat degradation
on the evolutionary dynamics of populations
in a rainforest cycad (Gymnospermae)

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Conservation Biology

by

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ABSTRACT

In addition to habitat loss and fragmentation, habitat degradation can have important consequences for biodiversity and population persistence, including effects on ecological and genetic processes beyond decreased demographic viability and the loss of genetic variation. Particularly interesting is the potential for evolutionary changes and adaptation to degraded habitats, that can affect population viability even in the short-term. Here, I explore how environmental changes after habitat degradation affect the evolutionary dynamics of populations of the rainforest cycad *Zamia fairchildiana,* specifically how habitat degradation affects gene dispersal, inbreeding, directional selection, and genotype-by-environment interactions, and the potential for genetic differentiation between populations. Colonies of *Z. fairchildiana* showed little genetic differentiation in neutral molecular markers across study sites, thus can be considered as subpopulations. Subpopulations in the disturbed habitat are experiencing different environmental conditions when compared to subpopulation in their native habitat. Disturbed-habitat subpopulations showed a faster life-history. This faster life history is associated with a weaker spatial genetic structure and higher levels of inbreeding in the disturbed-habitat subpopulations. In addition, higher light availability in the disturbed habitat seems to be a major agent of selection on traits like leaf production that have the potential to respond to selection in these subpopulations. Different traits were under selection in the native-habitat subpopulations, suggesting the potential for genetic differentiation between native and disturbed-habitat subpopulations. Genotype by environment interactions in seed germination and seedling survival, in response to light and water availability, further suggested that subpopulations can adaptively diverge between habitats, but the relative role of genetic and environmental factors, particularly maternal effects, on the magnitude and rate of genetic differentiation between subpopulations remains to be evaluated. These results suggest that habitat degradation can have important consequences for the evolutionary dynamics of populations of this cycad, not necessarily typical of habitat loss and fragmentation. This study identified factors and processes important for population persistence in degraded habitats, but population responses to habitat degradation are complex. Thus further studies and long-term experiments are required for better understanding the effects of habitat degradation on population viability.

KEYWORDS

Biparental inbreeding, Cycads, Fine-scale spatial genetic structure, Genotype-by-environment interactions, Habitat degradation, Life-history strategy, Marker-based heritability, Maternal effects, Response to selection, *Zamia*

GENERAL INTRODUCTION

HABITAT DEGRADATION AND POPULATION VIABILITY

Habitat loss and degradation are the major threats to species persistence worldwide (Myers 1997). The consequences of habitat loss and declining population sizes have received considerable attention in conservation biology. It is well documented that habitat loss and fragmentation result in species extinctions, and that the amount and distribution of remaining habitat area and the scale of fragmentation can influence the patterns of species loss (reviewed in Fahrig 2003; Saunders et al. 1991; Turner 1996). The mechanisms underlying species extinctions are diverse, but usually involve declining population abundance and migration rates, invasion by exotic species, and changes in forest dynamics or the trophic structure of communities (Laurance et al. 2002). In addition to habitat loss, the degradation of the environmental conditions in the remaining habitat (i.e. habitat degradation) can affect species extinction rates and the extinction-colonization dynamics of metapopulations (Fleishman et al. 2002). Nevertheless, evaluating the effects of habitat degradation on species diversity is complex, because it is difficult to separate the effects of habitat loss, fragmentation, and degradation; and many factors, including the disturbance history of the ecosystem, may affect species and community responses to habitat degradation (Caley et al. 2001; Danielsen 1997; Ewers and Didham 2006). For example, studies of lepidoptera and birds suggest that habitat disturbance can increase or decrease species richness and that trends depend on the scale of the analysis (Hill and Hamer 2004).

Although habitat loss represents a more critical issue, habitat degradation may have relevant consequences for population viability, beyond the typical effects of habitat loss and fragmentation of decreased demographic viability and loss of fitness and genetic variation. Furthermore, most ecosystems around the world are directly or indirectly impacted by human activities (Sanderson et al. 2002), and in many cases habitats may be highly degraded, even if large areas of habitat remain in the landscape. For example, in a global assessment of the forest ecosystems of the world it was

estimated that only 36% of the total forest cover can be considered primary forest, i.e. forest of native species with no visible influences of human activity; and that primary forests are declining rapidly because of deforestation and forest modification by selective logging and other human interventions (FAO 2005). In the case of tropical forests, deforestation and forest fragmentation, as well as extractive activities, result in remaining habitat patches that can be highly degraded, or that differ drastically from the original habitat in forest structure and species composition (Laurance 2004; Noble and Dirzo 1997; Tabarelli et al. 2004; Wagner 2000). Therefore, habitat degradation of the environmental conditions in the remaining habitat, in addition to habitat loss, needs to be considered when evaluating population viability in human-impacted landscapes.

Evaluating the effects of habitat degradation on population persistence requires a wide-ranging approach. Decreased demographic viability resulting from habitat fragmentation and degradation is widespread in plant populations, e.g. many population-level studies have shown decreased fecundity and recruitment in fragmented habitats (e.g. Aguilar and Galetto 2004; Bruna and Oli 2005; Kery et al. 2000; Kolb 2005; Wolf and Harrison 2001). However, the direct impact of increased mortality or decreased reproductive output on the long-term persistence of populations in fragmented habitats needs further exploration (Hobbs and Yates 2003). Furthermore, few studies have evaluated directly the effects of modified habitat quality on the demographic viability of plant populations (but see Bawa and Seidler 1998; Brys et al. 2005; Colling and Matthies 2006). Despite general trends of negative effects of habitat degradation on plant survival and fecundity, population responses are likely to be complex, and other aspects of population viability, besides demographic rates, may be affected by habitat fragmentation and degradation. A viable population not only has a positive growth rate and low probability of extinction by stochastic processes, but should also exhibit high levels of individual fitness and genetic variation that confers the ability to respond adaptively to environmental changes (Soule 1987), which may be compromised by habitat degradation.

To fully understand the effects of habitat degradation on population viability, genetic factors, as well as ecological and demographic factors, need to be considered. Genetic processes can affect population viability in many ways (reviewed in Amos and Balmford 2001; Frankham 1995; Hedrick 2001; O'Brien 1994). Theoretical and empirical research suggests that population viability may be affected by reduced fitness as a consequence of inbreeding depression (Hedrick and Kalinowski 2000), the accumulation of non-beneficial mutations (Lande 1995; Lynch and Gabriel 1990), or decreased evolutionary potential after loss of genetic variation mostly by genetic drift (Ellstrand and Elam 1993). Both ecological and genetic factors were considered relevant for population viability in the early conservation biology literature (Frankel and Soule 1981; Franklin 1980; Schonewald-Cox et al. 1983; Shaffer 1981), but demographic issues have become prevalent in population viability analyses in the last decades (Beissinger 2002; Menges 2002; Reed et al. 2002). Demographic viability has been suggested to be more relevant for conservation (Caro and Laurenson 1994; Lande 1988; Schemske et al. 1994), and there has been debate on the relevance of genetic and ecological criteria to define significant units for conservation (Crandall et al. 2000; Fraser and Bernatchez 2001; Moritz 1994). However, combining ecological and genetic information in evaluating population viability is promising (Allendorf and Ryman 2002; Oostermeijer et al. 2003), and will provide valuable information not only for conservation biology, but also to understand population dynamics in general.

HABITAT DEGRADATION AND THE EVOLUTIONARY DYNAMICS OF POPULATIONS

Genetic-related concerns in conservation have focused on the loss of fitness by inbreeding depression, and the risks of extinction in populations with extremely depauperated genetic diversity (e.g. by pest susceptibility), that can affect population viability within a few generations after environmental change. Many studies in plants have detected significant inbreeding depression soon after habitat degradation, but the loss of genetic diversity usually happens at a slower rate, depending on the life-span of organisms, the severity of population size reductions, and the role of gene flow in the rate of loss of genetic variation (reviewed in Lowe et al. 2005; Young et al. 1996).

Genetic variation in populations of interest is usually evaluated with neutral molecular markers (like isozymes, AFLPs, microsatellites, SNPs), and many studies have estimated the levels of diversity and genetic differentiation between plant populations in degraded habitats using these genetic markers (e.g. England et al. 2002; Galeuchet et al. 2005; Honnay et al. 2005; Hooftman et al. 2004; Jacquemyn et al. 2003; Murren 2003). These studies can provide information about patterns and mechanisms behind the loss of genetic variation, e.g. by identifying genetic bottlenecks, reduced effective population sizes, and changes in gene flow. In addition, neutral molecular markers can be used to assign individuals to particular populations, infer paternity relationships, and reconstruct phylogenetic and phylogeographic trends, all of which have important applications in conservation biology (reviewed in Avise 1994; Haig 1998; Hedrick 2002; Morin et al. 2004).

Although the levels of genetic diversity and genetic differentiation exhibited by neutral molecular markers may be informative, the potential effects of the amount and distribution of genetic variation on population viability might be better evaluated using quantitative genetics (Frankham 1999; Lynch 1996; Storfer 1996), given that genetic variation in quantitative traits is not well estimated by neutral molecular markers (McKay and Latta 2002; Merila and Crnokrak 2001; Reed and Frankham 2001). Quantitative genetics can be used to evaluate genetic issues in ecologically-relevant traits, and can help linking ecological and genetic factors affecting population viability to contribute a more comprehensive understanding of the ecological and evolutionary mechanisms governing population persistence, which should be the major target of conservation (Moritz 2002; Myers 1997; Smith et al. 1993). Loss of genetic variation and genetic differentiation in traits linked with the phenotype and fitness of individuals can be directly associated with population parameters like demographic rates and the evolutionary potential to respond to future environmental change (like global warming for example (Bawa and Dayanandan 1998)). Furthermore, quantitative genetic tools can be used to explore the role of natural selection and phenotypic plasticity or genotype-by-environment interactions on the response of populations to environmental change and the process of genetic differentiation between populations.

Of particular interest is the potential for evolutionary changes in populations exposed to novel or extreme environmental conditions in degraded habitats. Populations that are demographically viable and have enough genetic variation could respond adaptively to environmental change in degraded habitats. The potential for evolutionary changes and adaptive genetic differentiation has received little attention in conservation. Nevertheless, there is increasing evidence that evolution by natural selection can happen at a time scale comparable to ecological processes, thus it may have important implications for conservation in the short-term (Palumbi 2001; Stockwell et al. 2003; Zimmer 2003). The accelerated rate of environmental change in degraded habitats can promote rapid evolution in populations, if it results in strong directional selection on traits that have genetic variation to respond to selection (Kinnison and Hendry 2001). Evolutionary changes in degraded-habitat populations will influence not only their persistence on those habitats, but also the spatial genetic structure of populations, as a result of genetic differentiation between populations from modified habitats and the populations that remain in the native, undisturbed habitats (Palumbi 2001; Reznick and Ghalambor 2001; Stockwell et al. 2003). Plants can exhibit strong genetic differentiation at small spatial and temporal scales (reviewed in Linhart and Grant 1996; Petit and Hampe 2006), and it is possible that rapid evolutionary changes in degraded habitats are widespread for plant species. Identifying the conditions that favor adaptive evolutionary changes and genetic differentiation between populations is therefore important in conservation, especially because adaptation in response to habitat degradation may become essential for population persistence when habitats are severely degraded in the long-term (Burger and Lynch 1995; Lynch and Lande 1993). Furthermore, adaptation to degraded habitats could also have negative effects on population viability, because populations will lose genetic variation in the process of adaptation, and because genetic differentiation between populations in contrasting habitats might result in outbreeding depression with continuing gene flow.

In addition to the effects on population persistence and the genetic structure of populations, evolutionary changes by natural selection and other forces like genetic drift

can have implications for many aspects of conservation biology (McKay and Latta 2002; Stockwell et al. 2003). Evolutionary changes can play a major role in the success of invasive species and the susceptibility of communities to invasion (reviewed in Ellstrand and Schierenbeck 2000; Lambrinos 2004; Lee 2002; Mooney and Cleland 2001), the decline of populations subject to overexploitation (Conover and Munch 2002; Haugen and Vollestad 2001), or the metapopulation dynamics of species where dispersal patterns evolve in response to habitat degradation (Sih et al. 2000). But the outcome of evolutionary changes on populations and communities may be affected by the rate of habitat degradation, or the characteristics of the landscape, for example evolutionary changes may happen preferentially in some populations (e.g. sink vs. source, or core vs. peripheral populations) (Holt and Gomulkiewicz 2004; Lowe et al. 2005). Only by integrating ecological, molecular, and evolutionary approaches will we be able to understand how habitat loss, fragmentation, and degradation can affect ecological, genetic, and evolutionary process that interact to determine population viability and species persistence in human-dominated landscapes.

RESEARCH GOALS AND JUSTIFICATION

Cycads are one of the most threatened groups of plants in the world (Donaldson 2003). The persistence of cycad species is threatened mainly by habitat destruction and in some cases by overexploitation of populations (for ornamental uses). Many species persist as small isolated populations, often in highly degraded habitats. Consequently, information on how habitat degradation may affect the ecological and evolutionary dynamics of populations is crucial for cycad conservation. Demographic studies have shown that the population growth rate of cycad populations mostly depends on high adult survivorship and episodic recruitment (Negron-Ortiz et al. 1996; Perez-Farrera et al. 2006; Raimondo and Donaldson 2003), similar to the demographic patterns showed by woody perennials in general (Franco and Silvertown 2004; Silvertown et al. 1993). Therefore, habitat loss and the reduction of population size, and especially adult mortality, should have strong negative effects on the population growth rate of cycads. Population isolation may also have negative consequences for cycad populations.

Pollination and seed dispersal in natural populations seems to be limited to short distances (Donaldson 1997; Mound and Terry 2001; Tang 1987; Tang 1989), and population genetic studies have suggested that population isolation after habitat fragmentation may result in loss of genetic variation (Gonzalez-Astorga et al. 2006; Keppel et al. 2002).

The negative effects of population decline and isolation can result in high population extinction, as observed in many cycad species. Nevertheless, many species are able to persist in fragmented and degraded habitats, and habitat degradation could have important effects on their population biology, beyond negative demographic effects or the loss of genetic variation. There is virtually no information on how habitat degradation can affect the life-history, the distribution of genetic variation and genetic structure, or the evolutionary potential of cycad populations. Of particular interest is the potential for adaptive evolution in degraded habitats, as for many species, adaptation to disturbed environmental conditions may be the only way to guarantee species persistence in the long-term. I chose *Zamia fairchildiana* as a model to investigate the consequences of habitat degradation on the ecological and evolutionary dynamics of cycad populations, and the potential of cycad species to adapt to disturbed habitats. *Zamia fairchildiana* is a cycad typical of the rainforests of Central America (Norstog and Nicholls 1997). In contrast to most other *Zamia* and cycad species, *Z. fairchildiana* still has large populations in their native habitat, that is relatively undisturbed by anthropogenic influences in parts of the distribution range of the species. *Z. fairchildiana* also has large populations in fragmented and degraded habitats, and therefore it constitutes an ideal species for exploring population responses to habitat degradation.

Here, I combine information from observational studies, molecular and quantitative genetic analyses, and manipulative experiments in natural and controlled environments to explore how environmental changes after habitat degradation affect the life-history of Z. fairchildiana *and the evolutionary dynamics of populations, the effects of habitat degradation on evolutionary forces like gene dispersal, inbreeding, directional selection, and genotype-by-environment interactions, and the potential for adaptive*

genetic differentiation between populations. This research is presented in three parts. First, I explore how environmental changes in degraded habitats affect the growth and fecundity rates of populations, and the subsequent effects on the distribution of genetic variation and inbreeding levels within populations. Second, I compare the patterns of directional selection between native and degraded habitats, and estimate levels of heritability for ecologically-relevant traits (mostly related to growth), to evaluate the potential response to selection in populations of *Z. fairchildiana.* Third, I describe genotype-by-environment interactions in seed germination and seedling survival, that depending on the relative contribution of genetic and environmental effects, particularly maternal effects, determine the potential for adaptive genetic differentiation between populations from native and degraded habitats in *Z. fairchildiana.* Finally, I integrate all the results to discuss population responses to habitat degradation, and highlight the need for further information that will provide a more detailed understanding on the overall effect of habitat degradation on the ecological and evolutionary dynamics of this species.

STUDY SYSTEM

Study species

Cycads are the most ancient seed plants that still alive, the most basal lineage in the phylogeny of gymnosperms and seed plants (Hajibabaei et al. 2006), therefore they represent an important group of plants from an evolutionary point of view. Fossil evidence suggests that Cycads appeared towards the end of the Paleozoic era and dominated the world flora during the Mesozoic. The extant species closely resemble many of the fossils in the Mesozoic, thus Cycads are considered 'living fossils' (Norstog and Nicholls 1997). Nevertheless, Cycads possess complex interactions with bacterial root symbionts, and insect herbivores, and pollinators (Schneider et al. 2002), and insect pollination probably first appear in this lineage of plants (Klavins et al. 2005). Nevertheless, we know very little about the biology of Cycads in their natural habitats. In

particular, there is scanty information about the diversity of genera like *Cycas* in Asia, and *Zamia* and *Ceratozamia* in America (Donaldson 2003).

Currently, there are aproximately ca. 300 taxa of Cycads (species and subspecies) distributed in the tropical and subtropical regions around the world (Donaldson 2003). These cycad taxa are grouped in 11 genera, five of which appear in the Neotropics. In the Neotropics, the genus *Zamia* is the richest in species. Furthermore, *Zamia* species are very diverse in habitats (from desertic to rainforest ecosystems) and habits, i.e. species with subterraneous and aerial stems, and a rich variety of leaf morphologies. Most species of *Zamia,* as is common for Cycads, are endemic, and have restricted distribution within one country (Stevenson et al. 2003).

Zamia fairchildiana (Cycadales: Zamiaceae) inhabits the understory of lowland and mountain wet-forest between 0-1500 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 (hereafter referred as the 'native habitat') and also in degraded or disturbed habitats (hereafter referred as the 'disturbed habitat'). *Zamia fairchildiana* is classified as a vulnerable species in the Red list of the IUCN because of habitat destruction (Donaldson 2003), but the species still has large populations in undisturbed and disturbed habitats in Costa Rica and Panama.

In the Osa Peninsula in SW Costa Rica, *Z. fairchildiana* is a small tree in the understory, that can get up to 2 m of height, and has a crown of 5-20 compound leaves (Figure iA, iB). The number of leaflets increases progressively with age, from 4-6 leaflets in the seedlings to about 50-60 in the adults. Leaflet number has been used to describe the developmental stage (or age-stage) of plants in demographic studies in other *Zamia* species (Clark and Clark 1987; Negron-Ortiz and Breckon 1989). Plants may take around 10 years to reach the minimum size for reproduction, and may live for several decades (L. D. Gomez, personal communication). Leaf production occurs in annual flushes, and every year the stem increases in height during growth episodes.

Leaf production peaks around May-June, coinciding with the first peak in rainfall during the year. At this point, most activity by the specialist herbivore *Eumaeus minyas* (Lepidoptera: Lycaenidae) can be observed on young leaves (Figure iiA, iiB). Leaves are heavily prickled, presumably as a defense for herbivory, and the number of prickles in leaves is variable among individuals (although it tended to be higher in the native-habitat individuals). Cycads also produce potent toxins in leaves and other plant parts that act as a chemical herbivory defense (Castillo-Guevara and Rico-Gray 2003). The larvae of *E. minyas* and other cycad herbivores are thought to gain chemical protection from the ingestion of the cycad toxins (DeVries 1976; Nash et al. 1992). Leaves of rainforest *Zamia* species have a very long life-span compared to other plants, and have structural features intermediate between sun- and shade-adapted species (Lee et al. 1990), but their photosynthetic ability decays after a few years, in part due to the heavy cover of epiphylls on the leaflets (Clark et al. 1992).

Cycads are dioecious, i.e. male and female cones are produced in separate individuals (Figure iC, iD). The mechanism of sex determination is unknown, and sex changes are extremely rare (Norstog and Nicholls 1997). *Zamia fairchildiana* females produce one 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, as is common in *Zamia* species (Clark and Clark 1987; Negron-Ortiz et al. 1996). Cone production starts around August, when the second annual peak in rainfall begins. Pollination is carried out by small beetles (Figure iiC, iiD), when the dry season starts by December. During this study pollinators of *Z. fairchildiana* were collected for the first time, and taxonomic specialists have determined that they represent two new species (currently in the process of description, W. Tang, personal communication) of the genera *Pharaxonotha* (Coleoptera: Erotylidae) and *Rhopalotria* (Coleoptera: Belidae).

Seed development lasts for ca. 12 months, and mature seeds have a sarcotesta (outer seed layer) with a bright red to orange color. Most seeds are dispersed locally by

gravity when the cone parts rot away, which occurs at the end of the rainy season (November-December) the following year after pollination. 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. Seedlings quickly develop root nodules containing nitrogen-fixing bacteria that eventually form specialized roots or 'coralloid roots' in the adults. Newly-emerged seedlings have one leaf with 4-6 leaflets, and accumulate reserves in the subterraneous stem until the next dry season, when most seedling mortality occurs. Seedlings that are older than one year also have a high risk of mortality, but survival probability increases with age-stage in individuals.

Figure i. Juvenile (A) and adult (B) individuals of *Z. fairchildiana* in habitat; and female (C) and male (D) cones in reproductive adults.

For this study, three patches or colonies of individuals of *Z. fairchildiana* were chosen in each of two study sites, each site representing one type of habitat: the native-undisturbed habitat, and the degraded habitat. The patches are considered subpopulations because the neutral genetic differentiation between them is very low, i.e. the F_{ST} value across habitats is small (see Chapter 1). Subpopulations within habitats consist of discrete and isolated patches with a few hundred individuals, and are separated at least 1 km from each other. All subpopulations were located in sites with similar topography, in stream or river banks with steep slopes (around 30%), which is the common habitat for *Z. fairchildiana* in the study sites. The only conspicuous difference between subpopulations from the two habitats is that plant density was higher in the patches of individuals in the degraded habitat.

Figure ii. Herbivores and pollinators of *Z. fairchildiana.* A. Larvae and B. Adults of *Eumaeus minyas*, an specialized herbivore. Pollinators of the genera *Pharaxonotha* (C) and *Rhopalotria* (D). Pictures of pollinators provided by Dr. W. Tang, scale bar=1 mm.
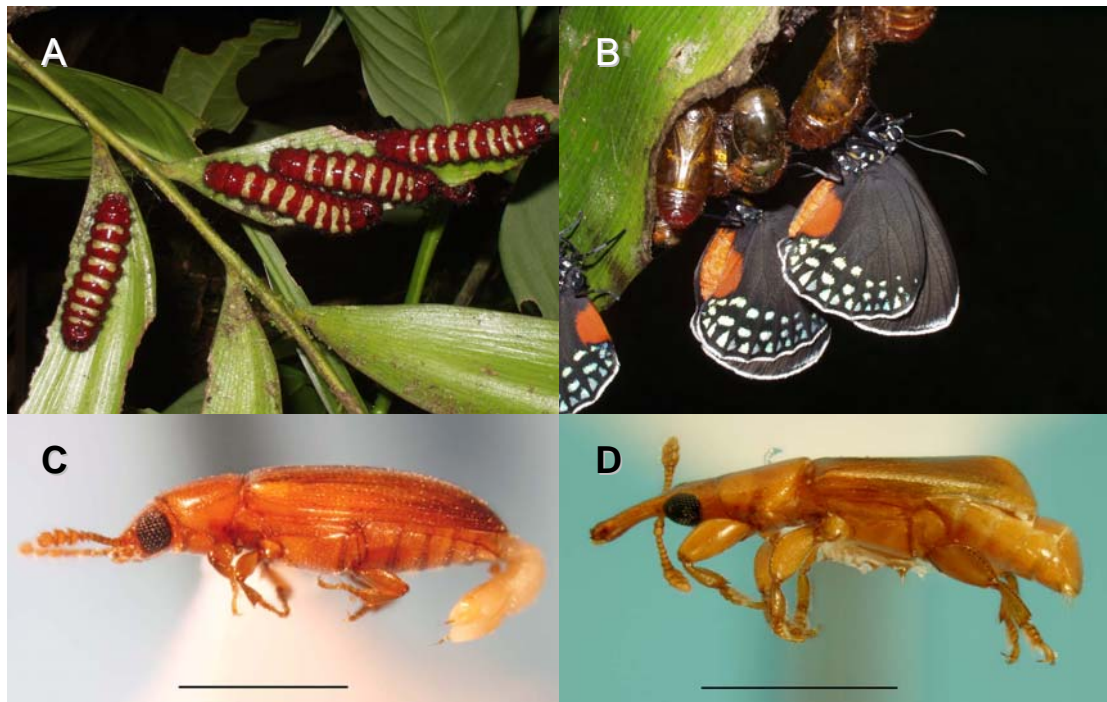
Study sites

The study was carried out in two sites: a native-habitat site within 'Corcovado National Park', and a disturbed-habitat site in the buffer zone of the National Park, within the 'Golfo Dulce' Forest Reserve. Separate patches of individuals of *Z. fairchildiana* in the native-habitat site were located near 'Sirena' station' (8°32'25''N, 83°23'50''W), and in the disturbed habitat site near 'El Tigre' station (8°28'46''N, 83°35'10''W), both ranger stations of the 'Area de Conservación Osa' (ACOSA, SINAC, Costa Rica). The two study sites were separated by a linear aerial distance of approximately 20 kilometers (Figure iii).

Study sites had 0-150 m of elevation. Soils in the Osa Peninsula are predominately of tectonic and erosive origin, dominated by Ultisols in the dissected terrain (Cleveland et al. 2004), where *Z. fairchildiana* occurs. The mean annual temperature is 26°C. Rainfall reaches 4000-6000 mm every year. Rainfall is distributed across the year between a rainy season and relatively mild dry season that last for about four months (Janzen 1983). There is a peak in rainfall in August-November and the dry season lasts from December-April (Figure iv).

Figure iii. Average (±SE) monthly rainfall in the native- and disturbed-habitat sites for the last seven years. Closed circles: native-habitat site, Open-circles: disturbed habitat site. Daily rainfall data were provided by the ACOSA administration of Costa Rica.
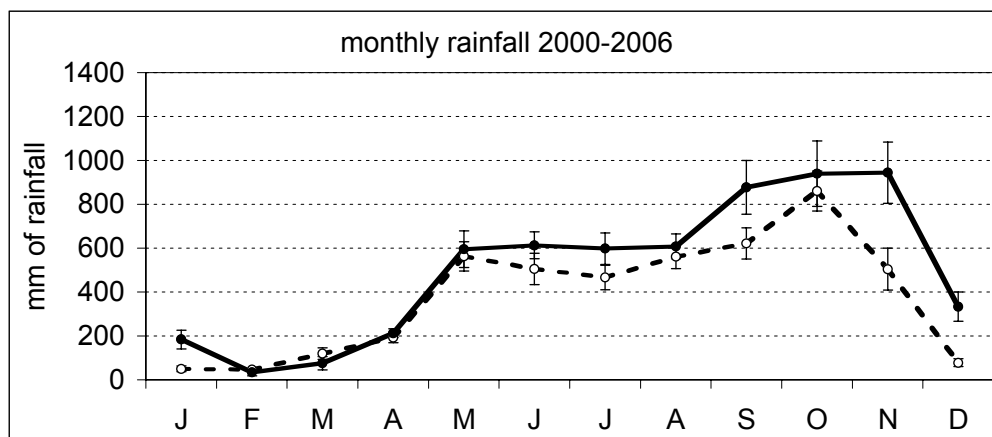
The native habitat is a wet forest with high species diversity and high endemism, e.g. ca. 50 tree species than can reach more than 10 cm dbh. The forest type in the native- and disturbed-habitat sites was classified as 'dense broad-leaved evergreen well-drained lowland forest' by an ecosystem assessment study carried out by the Costa Rican National Biodiversity Institute, INBio (Kappelle et al. 2003). The forest has a dense canopy that is approximately 25 m tall and the understory is relatively open. There is a high density of large trees and lianas (greater then 10 cm dbh), and very few species are deciduous. Tree and liana species are predominantly bird- and mammal-dispersed, which is a feature, together with tree species composition, that makes the rainforest at the Osa Peninsula similar to the rainforest in the Choco biogeographical region of South America (Panama-Colombia-Ecuador Pacific drainage) (Gentry 1988).

The disturbed-habitat site is dominated by fragmented forest that has been altered by anthropogenic disturbances like selective logging, hunting, and mining. The vegetation type in the disturbed habitat is similar to the native habitat. Human pressure on the forest, by deforestation and other activities, has been present in the Osa Peninsula since the 1930s, when gold mining and banana plantations were established, but has been greatest after the 1950s, when colonization was facilitated by the construction of the Panamerican highway (Rosero-Bixby et al. 2002; Sader and Joyce 1988). Corcovado National Park, in the Osa Peninsula, was created in 1975, in forests where human disturbance in the 20th century was never severe. In the native-habitat site, Sirena Station, a few subsistence farmers established between the 1940s and before the declaration of protected area, but these places were restricted to the coastal strip, and the forest cover in the dissected terrain (where *Z. fairchildiana* inhabits) was never cleared or greatly disturbed (Phillips 1985).

Figure iv. Map of the Osa Peninsula in SW Costa Rica showing forest cover (lowland mixed dense forest in dark green, and montane forests in lighter shades of green) and the location of the two study sites. Sirena station (within Corcovado National Park) was the native-habitat site, and El Tigre station (within Golfo Dulce Forest Reserve) was the disturbed-habitat site. The forest cover map was extracted from the Ecomapas project report, by INBio of Costa Rica (Kappelle et al. 2003).
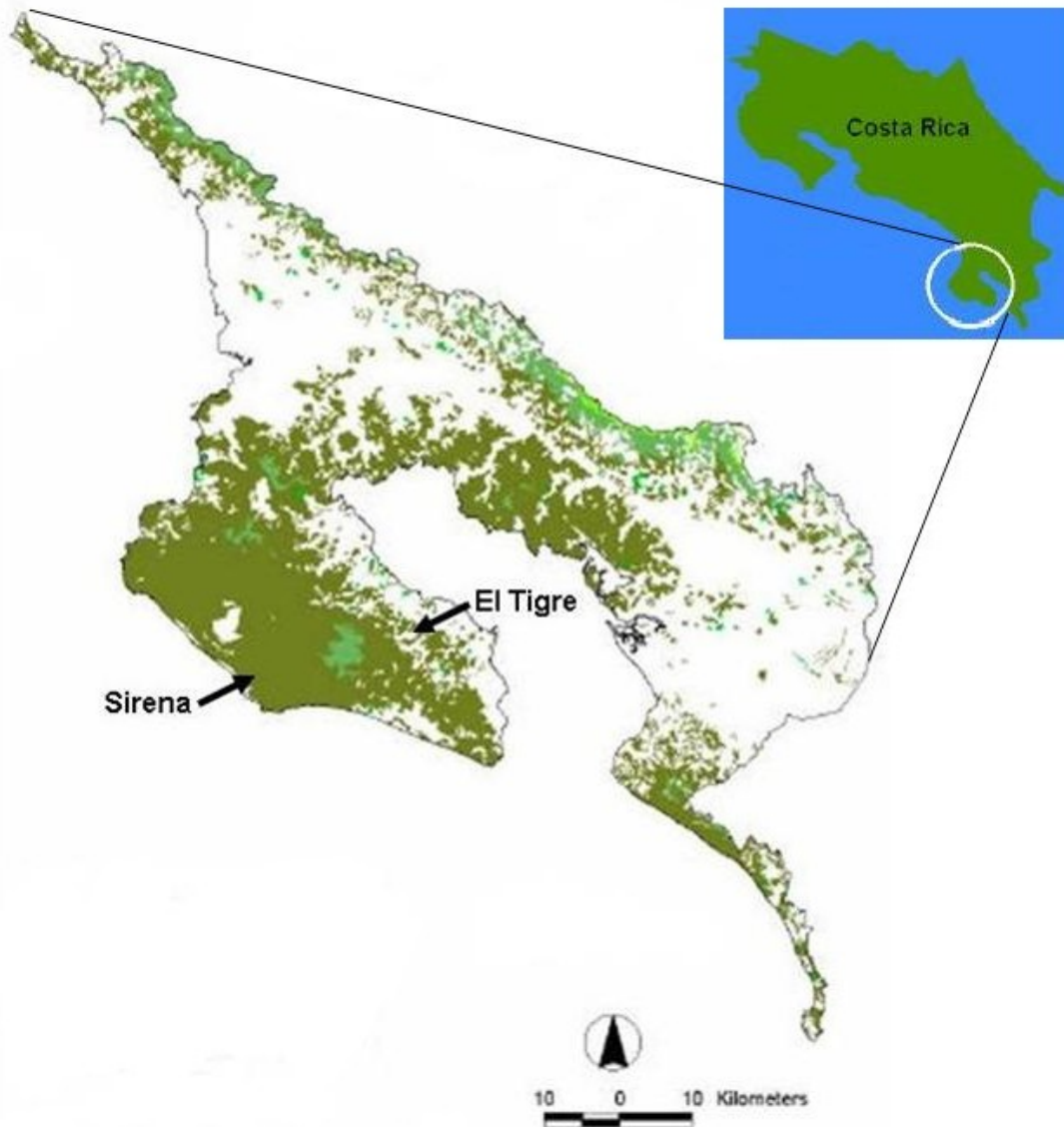
CHAPTER 1

"Life-history changes after habitat degradation and the fine-scale spatial genetic structure in populations of a rainforest cycad"

ABSTRACT

Fine-scale spatial genetic structure (SGS) of populations resulting from gene dispersal limitation is common in plants, and can have important implications for population biology as it affects effective population size, the levels of inbreeding, and the patterns of viability selection. Here, we explore how life-history differences between populations from contrasting habitats may affect the strength of spatial genetic structure and inbreeding in a tropical rainforest cycad (*Zamia fairchildiana*). Patches of individuals across the landscape showed very low genetic differentiation at the neutral molecular level, i.e. low F_{ST}. However, subpopulations recently exposed to higher light availability in degraded habitats showed substantial differences in their life-history. In the degraded-habitat subpopulations individuals grew faster, reproduced earlier, and invested more in reproduction than plants from subpopulations in their native habitats. Disturbed-habitat subpopulations also showed higher frequency of reproduction and greater mate availability. The degraded-habitat subpopulations showed weaker SGS, i.e. a smaller slope in the linear regression of genetic relatedness on linear distance, suggesting that gene dispersal is less restricted in this habitat. In addition, contrary to what is expected for populations with weak SGS, higher levels of biparental inbreeding were found in the disturbed-habitat subpopulations. Changes in the strength of SGS and the levels of inbreeding after habitat degradation will affect the distribution of genetic variation within populations, and may have important consequences for population viability, therefore they should be of concern in conservation biology.

Habitat loss and degradation are the major threats to species persistence worldwide (Myers 1997). 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 populations can be altered as a result of anthropogenic activities. If this is the case, then information on the spatial genetic structure of populations is necessary to delineate appropriate significant units for conservation (Fraser and Bernatchez 2001; Moritz 1994), and for restoration programs that seek to minimize negative effects of movement of individuals between populations (e.g. because of outbreeding depression (Frankham 1995)). Consequently, studies focused on determining the scale of genetic structure between populations are common in conservation biology (e.g. England et al. 2002; Galeuchet et al. 2005; Honnay et al. 2005; Hooftman et al. 2004; Jacquemyn et al. 2003; Murren 2003). One issue that has received far less attention is the potential effects of habitat modifications on the distribution of genetic variation at a fine-scale or at the within-population level.

Fine-scale spatial genetic structure (SGS), i.e. the non-random distribution of genotypes in space, is a wide-spread 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. Spatial genetic structure can result from past demographic events, adult and seed source density, or micro-environmental selection (Enos 2001; Jones and Hubbell 2006), but dispersal limitation is probably the main process affecting fine-scale SGS within populations (Vekemans and Hardy 2004). SGS has important consequences for population biology, as it affects effective population sizes, levels of inbreeding, and patterns of viability selection (Schnabel et al. 1998). Therefore, changes in SGS within populations after habitat degradation may be important to consider in the context of conservation biology, not only because of its potential effects on population viability, but also because it will determine adequate sampling strategies to maximize the representation of the genetic variation in a population (for restoration or captive-breeding purposes for example).

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Higher levels of inbreeding in populations affected by habitat loss and degradation are common (reviewed in Lowe et al. 2005). Changes in the SGS within populations could also be widespread in human-dominated landscapes if life-history or other traits influencing gene dispersal are altered in degraded habitats. In the case of tropical rainforests, deforestation, habitat fragmentation and degradation, and extractive activities result in forest that differ drastically from the original habitat in forest structure and species composition (Noble and Dirzo 1997; Tabarelli et al. 2004; Wagner 2000). Habitat degradation can affect plant survival and reproductive rates, and many studies have shown decreased recruitment and lower levels of genetic variation in degraded-habitat populations of plants (reviewed in Lowe et al. 2005; Young et al. 1996). In addition, habitat degradation can alter the patterns of mating and gene dispersal. For example, habitat fragmentation and degradation can affect patterns of pollen movement and seed production within populations (Ghazoul 2005; Nason and Hamrick 1997). However, there are very few studies that have explored the consequences of altered mating patterns and gene flow on the fine-scale SGS within populations (but see Chung et al. 2004; van Rossum and Triest 2006; Young and Merriam 1994). These studies can help predict the effects of habitat degradation on the levels and distribution of genetic variation within populations and other genetic factors affecting population fitness.

Zamia fairchildiana is a long-lived cycad (Gymnospermae) typical of the understory of tropical rainforests in the Costa Rica and Panama (Gomez 1982). In part of its distribution range, populations of *Z. fairchildiana* persist in forest affected by fragmentation and other anthropogenic activities (i.e. disturbed habitats), where environmental conditions differ considerably from their native habitats. This cycad species, as most understory plants in tropical forests, is particularly affected by changes in light availability (Clark and Clark 1987), as light is by far the most limited resource in these habitats (Brienen and Zuidema 2006; Chazdon et al. 1996; Clark and Clark 1992). In this paper we describe how differences in canopy cover between native and disturbed habitats are associated with significant differences in life-history traits, and how these differences may be affecting the levels of SGS and inbreeding within

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populations of *Z. fairchildiana*. In particular, we evaluate how differences in growth and fecundity rates could affect SGS and the levels of inbreeding through their effects on the frequency of reproduction and mate availability within populations.

METHODS

Sampled populations

Six populations of *Z. fairchildiana* where chosen for monitoring growth and fecundity rates. Three populations were located in old-growth, undisturbed forest within Corcovado National Park (Sirena Station). The other three populations were located in disturbed forests, near El Tigre station that lies outside the National park, in an area affected by deforestation, logging, hunting, and mining for the last five to six decades. Out of the three populations, the largest population in each habitat was selected for genetic analyses (populations P2 and P8 in Table 1.4)

Estimation of growth, fecundity, and mate availability

In three populations/habitat, we sampled all individuals present in a 100 x 20 m transect in the native habitat or a 50 x 10 m transect in the disturbed habitat. Transects were smaller in the disturbed habitat to keep similar samples sizes across populations, since plant density was higher in this habitat. To estimate the growth rate, we counted the number of new leaves in the leaf flush/plant produced in the growing seasons of 2005 and 2006. Growth rates depend on plant size and may be affected by light availability. We estimated total leaf area as the product of the total number of leaflets in all leaves by the average leaflet area. Leaflet size is uniform 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 young but mature leaf. We also estimated the percentage of canopy openness around the plant, using a spherical densitometer. Leaf production for juveniles (smaller than the minimun size for reproduction), reproductive and non reproductive individuals was compared between

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habitats using a repeated-measures GLM, with year and habitat as factors. We used leaf area as a covariate in the analysis, to control for size effects on leaf production. Population did not have an effect on growth or fecundity measures, and data for populations within habitats were pooled in all statistical analyses.

In the reproductive seasons of 2004 and 2005, all adult individuals were checked for cone production, to account for all reproductive plants/population. Allocation to fecundity was estimated as the slope of the regression between fecundity and plant size (Aarssen and Taylor 1992). The minimum size for reproduction is estimated by the intercept in this regression. Plant size was estimated as total leaf area, as described before. Fecundity was measured as the product of the number of cones (only one in females) and cone size, i.e. the number of sporophylls (cone parts bearing seeds or pollen sacs). We performed ANCOVA analyses, combining fecundity year for both years, to test for differences in fecundity allocation between habitats, i.e. to compare the slope of the relationship between plant size and fecundity. We also calculated the proportion of females and males out of the total adult population that produce cones in a reproductive season. A sex ratio of 1:1 was assumed. Genotypic sex ratios are difficult to estimate in cycads because many individual plants do not produce cones in a given season, but the only two studies to date have estimated the sex ratio to be 1:1 (Ornduff 1987; Ornduff 1996). However, if only cone producing plants are counted there is usually a sex bias towards more males in a given reproductive season in cycad populations, as reproduction is more costly for females, and they reproduce less often. We estimated mate availability for females in both reproductive seasons, as the number of males/females in each population. Statistical analyses were performed in SPSS (SPSS 2003).

Development of microsatellites for genetic analyses

We developed microsatellite loci following the protocol by Hamilton (1999) using DNA extracted from two individuals of *Z. fairchildiana* from the Montgomery Botanical Center collections (Miami, US). Genomic DNA was extracted from dry leaf samples

using a DNeasy Plant Mini-kit (QiaGen). DNA was digested with two restriction enzymes (Nhe I and Rsa I) and then ligated to SNX linkers that allowed the recovery of enriched DNA fragments after hybridization with probes containing AG and AT repeats. DNA fragments that successfully hybridize with the probes were cloned using a TOPO-TA cloning kit (Invitrogen). A total of 25 clones were sequenced in an ABI 3100 automated sequencer (Applied Biosystems). Out of the 25 clones sequenced, 12 were suitable for primer development. We developed primers for these loci using the software Primer 3 (Rozen and Skaletsky 2000) (Table 1.1). Six loci showed consistent amplification and were highly polymorphic for the two populations under study.

For genetic analyses, we chose only one population per habitat, in order to perform an detailed sampling of individuals in three transects/population. A total of 200 and 250 individuals in the native and disturbed habitat, respectively, were sampled in three 100 x 20 m transects in the native habitat and three 50 x 10 m transects in the disturbed habitat. The transects were located in different locations, based on topographic features, within each population. Approximately 20 g. of dry leaf tissue/individual was used to extract DNA using a DNeasy Plant Mini-kit (QiaGen). Each loci was amplified in a 10 μl PCR reaction using forward primers with an M13 tail that allowed them to anneal with a universal fluorescent-labeled M13 primer (following Schuelke 2000). PCR reactions had concentrations of ca. 20 ng of template DNA, 0.02 μM of forward- and 0.20 μM of reverse- and labeled M13-primers, 0.4 mM of each dNTP, 0.2 U of *Taq* DNA polymerase, 50mM tricine, and 2mM MgCl$_2$. I employed touchdown PCR profiles, with an initial denaturation step at 94°C for 3 m; followed by 30 cycles at 94°C for 30 s, 54°C for 30 s, decreased by 1°C in cycles 2 through 10, and 72°C for 45 s; and a final extension step at 72°C for 20 min. PCR products were run in an ABI 3730 automated sequencer (Applied Biosystems) and allele scoring was performed using the software Gene Mapper (Applied Biosystems 2004). Loci were tested for Hardy-Weinberg equilibrium (HWE) with the software Arlequin (Schneider et al. 2000), and the presence of null alleles using the software Micro Checker (van Oostechout et al. 2005).

Table 1.1. Primer sequences (R: reverse primer, F: forward primer) for six microsatellite loci for the cycad *Z. fairchildiana*.

Locus	Primer sequence
Zf-01	**R:** AGGACGATCAGAAATGGAAG **F:** GTGGCAAGTGTCCCTGTTG
Zf-02	**R:** GGCCACCCTGGATTTCTAA **F:** AAGTCCTGGCATTGCACCT
Zf-03	**R:** AGCATTCAAAGGTGGCAAGT **F:** GGACGATCAGAAATGGAAGC
Zf-04	**R:** GGTGGAAAACTAATGGGTCAAA **F:** CCCTAAAGGTCCCTTTGCTT
Zf-05	**R:** CCCTAAAGGTCCCTTTGCTT **F:** TGGGTCAAAATATGTTATGCTTT
Zf-06	**R:** TGACCTTGGATGTGGAAAGA **F:** AGAGCACTTAAACCCAGGACA

Estimation of SGS parameters and inbreeding coefficients

To quantify the strength of SGS, we used the Sp statistics (Vekemans and Hardy 2004). This measure allows making direct quantitative comparisons of the magnitude of SGS among populations. The Sp statistics combines information on the slope of the regression of pairwise relatedness on the natural 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 using the six microsatellite loci. We estimated the average relatedness among pairs of individuals 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. We estimated pairwise relatedness using coefficients proposed by Loiselle et al. 1995 and Ritland 1996 (Loiselle et al. 1995; Ritland 1996a), as these coefficients usually perform well in the estimation of SGS with highly polymorphic loci (Vekemans and Hardy 2004). Geographical distance between individuals was calculated as the Euclidian distance from two-dimensional spatial coordinates obtained for plants within the transects used for genetic analyses.

To estimate the inbreeding coefficient (F_{IS}) for each population we used the same relatedness coefficients as for the SGS analysis. Finally, to estimate the degree of genetic differentiation showed by the microsatellite markers, we calculated the R_{ST}

value for the single comparison between the two populations (Michalakis and Excoffier 1996). We performed the estimation of the Sp statistics and F_{IS}-F_{ST} coefficients using the software SpaGeDi (Hardy and Vekemans 2002). Jackknifying over loci was used to obtaine multilocus averages and SE for all parameters. In addition, 1000 permutations of the location of individuals (for SGS parameters) and genes within individuals (for inbreeding coefficients) were used to calculate P-values to test for statistical significance of the estimates.

RESULTS

Growth, fecundity, and mate availability

Average canopy openness was significantly higher in the disturbed habitat (GLM F=55.64, P<0.001, Figure 1.1). The coefficient of variation for canopy values is lower in the disturbed habitat (CV=37.6% versus CV=47.5% in the native habitat). Juveniles, non reproductive adults, and reproductive plants had a higher leaf production in the disturbed habitat in the two growing seasons (Table 1.2, Figures 1.2A and 1.2B). Higher leaf production was positively associated with canopy openness in both habitats and both growing seasons (r>0.45 and P<0.05 in all tests). The minimum size to reproduction was smaller in the disturbed habitat for females and males, as evidenced by the smaller intercept in the fecundity allocation curve (Figure 1.2C and 1.2D). Fecundity allocation was higher in the disturbed habitat for males (ANCOVA F=4.31, P=0.041, Figure 1.2D), but females had similar fecundity allocation in both habitats (ANCOVA F=1.03, P=0.313, Figure 1.2C).

Figure 1.1. Distribution of canopy openness values in subpopulations of native (closed circles) and disturbed (open circles) habitats.
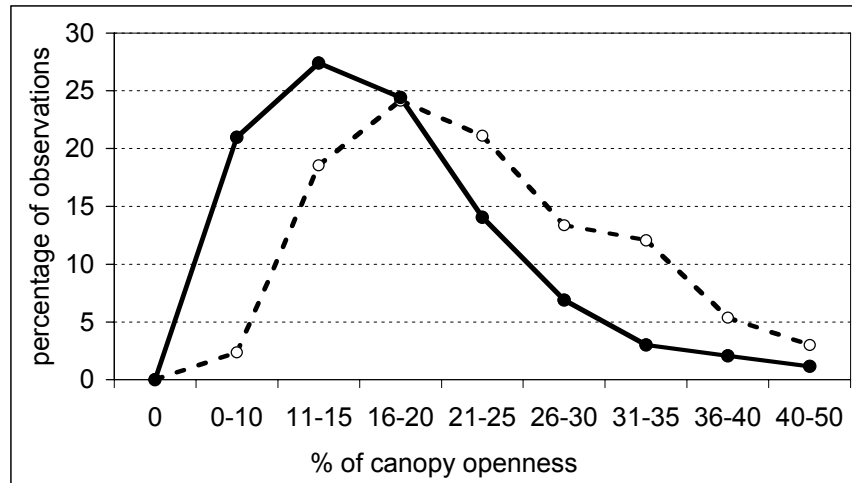
Table 1.2. Effect of habitat on leaf production in different life stages in subpopulations of *Z. fairchildiana.* A rm-ANOVA was used, with habitat as a main factor and leaf area as a covariate.

Life cycle stage	SS	d.f.	F	P
Juveniles	1.23	1	4.49	0.036
Non-reproductives	79.67	1	54.77	<0.001
Females	8.71	1	4.22	0.045
Males	14.78	1	7.56	0.007

Population sizes were smaller in the disturbed habitat (Table 1.3). In both habitats, no more than 6% of the total number of females, or 15% of the total number of males produced cones in a single reproductive season. There was a trend for a higher proportion of plants producing cones (both males and females) in the disturbed habitat, which resulted in a trend for a higher ratio of males/female in this habitat in both years. However, differences were not statistically significant (P values were significant or marginally significant, Table 1.3). The trend suggests that more males reproduced in the disturbed-habitat populations, but males did not produce more cones or larger cones, or had a higher allocation to fecundity.

Figure 1.2. Leaf production (mean ± 2SE) in the growing seasons of 2005 (A) and 2006 (B), and fecundity allocation for females (C) and males (D) in two habitats. Open circles and dashed lines: disturbed-habitat individuals. Closed circles and solid lines: native-habitat individuals. N values on the X axis in A and B are shown for each life-cycle stage.
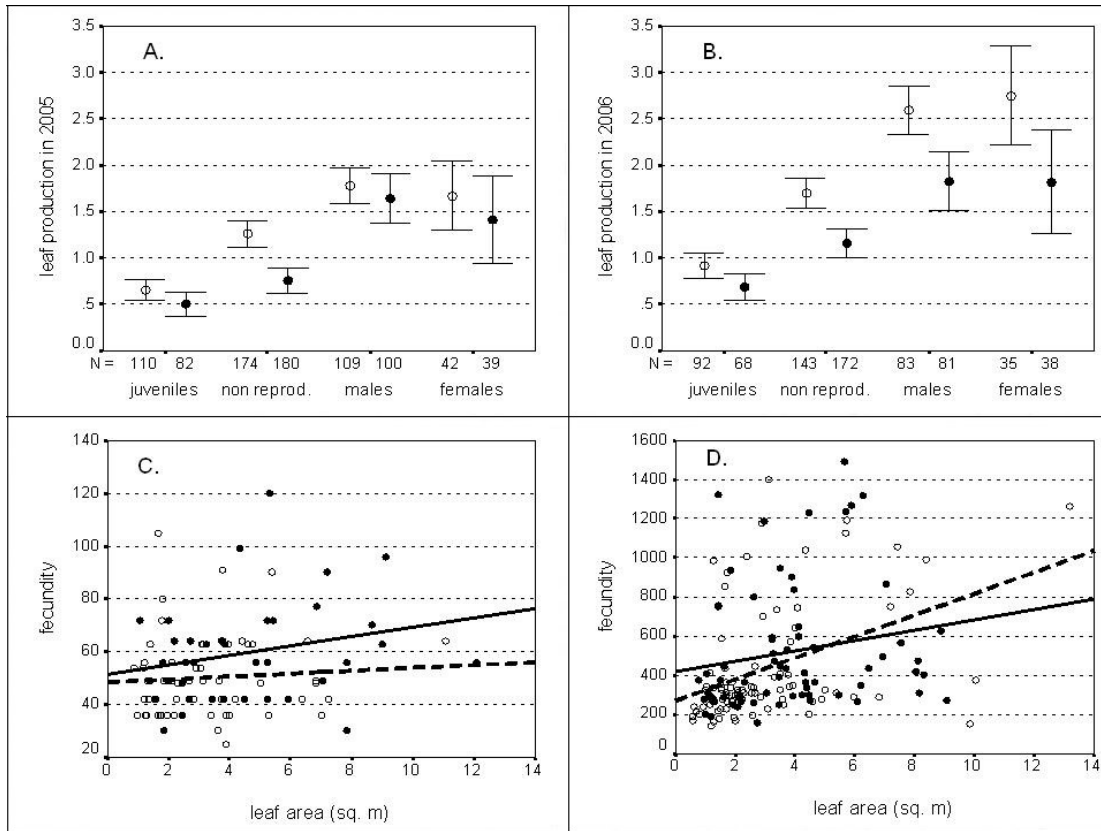
Table 1.3. Proportions of females and males, and male/female ratio in two reproductive seasons for three subpopulations in native and disturbed habitats. The last two rows are mean values for each habitat. P values are reported for t-tests comparing the parameters between habitats.

Population	# adults	females		males		males/females	
		2004	2005	2004	2005	2004	2005
P2	420	0.076	0.062	0.176	0.157	2.31	2.54
P3	316	0.044	0.070	0.114	0.146	2.57	2.09
P4	156	0.038	0.026	0.103	0.128	2.67	5.00
P6	402	0.050	0.030	0.090	0.065	1.80	2.17
P7	510	0.039	0.027	0.090	0.063	2.30	2.29
P8	656	0.037	0.049	0.085	0.082	2.33	1.69
disturbed	297.3	0.053	0.052	0.131	0.144	2.517	3.210
native	522.7	0.042	0.035	0.088	0.070	2.144	2.047
P value	0.05	0.22	0.21	0.10	0.001	0.07	0.09

Spatial genetic structure and inbreeding levels within populations

The levels of heterozygozity were slightly lower, and the number of alleles slightly smaller in the disturbed-habitat population (Table 1.4). Most loci showed deviations from Hardy-Weinberg equilibrium (Table 1.4). Separate HWE tests for transects suggested that some of the deviations from HWE may be the result of a Walhund effect. Within one population, groups of plants in different slopes or separated by a small gorge can be relatively isolated at the genetic level (data not shown). The presence of null alleles could also have contributed to lack of HWE in two loci, but strong genetic drift given the likely small effective population sizes may explain deviations from HWE in different directions in several loci. Nevertheless, inferences on the SGS within populations and inbreeding estimates should not be affected by this, as the relatedness coefficients used to estimate inbreeding do not assume HWE (Vekemans and Hardy 2004).

Average relatedness among pairs of individuals was low in both habitats, i.e. lower than F=0.06, even for neighbor plants (Figure 1.3). Furthermore, both relatedness coefficients showed that average relatedness is lower in the disturbed habitat, particularly for the first distance classes corresponding to neighboring plants (separated by less than 10 m of distance). In both habitats, distance classes larger than 40 m in the native habitat and 30 m in the disturbed habitat had negative average relatedness values (Figure 1.3). Negative relatedness coefficients indicate that relatedness among pairs of individuals in these distance classes is lower than the coefficient expected for a pair of random individuals. Therefore, the transects used in this study were appropriate to describe the fine-scale SGS within populations.

Relatedness between pairs of individuals decreased with the logarithm of geographical distance in both habitats (Figure 1.3). 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 disturbed habitat (Table 1.5). Lower values for b_F and F_1 resulted in lower values for the Sp statistics, i.e. weaker SGS, in the disturbed habitat (Table 1.5). Estimates of average relatedness and the Sp statistics were considerably

smaller when using the coefficient proposed by Ritland 1996 (Figure 1.3), as this coefficient is usually biased downwards in the presence of rare alleles in microsatellite loci. However, this coefficient has larger precision (smaller SE) and 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). Consistency among the results given by two different relatedness coefficients also suggests that the sampling strategy and the statistical analyses of SGS were robust.

Table 1.4. Genetic diversity for six microsatellite loci for the cycad *Z. fairchildiana.* Number of alleles (N_a) and levels of observed heterozygozity (H_{obs}) are presented for the subpopulation in native and disturbed habitats. *P<0.05, **P<0.01 in Hardy-Weinberg equilibrium tests.

Locus	native habitat		disturbed habitat	
	N_a	H_{obs}	N_a	H_{obs}
Zf-01	15	0.83*	14	0.76
Zf-02	7	0.96**	7	0.93**
Zf-03	17	0.83	17	0.73**
Zf-04	28	0.75**	27	0.80**
Zf-05	18	0.54**	16	0.35*
Zf-06	16	0.90**	14	0.81**

Inbreeding coefficients were significantly different from zero in both populations examined, using the Ritland coefficient (Table 1.5). In addition, both relatedness measures showed higher levels of inbreeding in the population from the disturbed habitat (Table 1.5). As cycads are dioecious, this estimates represent the magnitude of biparental inbreeding, i.e. inbreeding resulting from mating between related individuals. Finally, the R_{ST} value for the comparison between the two populations was very low, i.e. R_{ST} =0.011 (SE among loci = 0.005). This indicates that all patches of individuals used in this study belong to a large population, with low differentiation at the neutral molecular level, thus they represent subpopulations.

Figure 1.3. Average relatedness F (±SE) among pairs of individuals in distance classes (d) as a function of the logarithm of the distance between individuals. These functions were estimated using the relatedness coefficients as defined in Loiselle et al. 1995 (A) and Ritland 1996 (B). Dashed line: disturbed-habitat subpopulation. Solid line: native-habitat subpopulation.
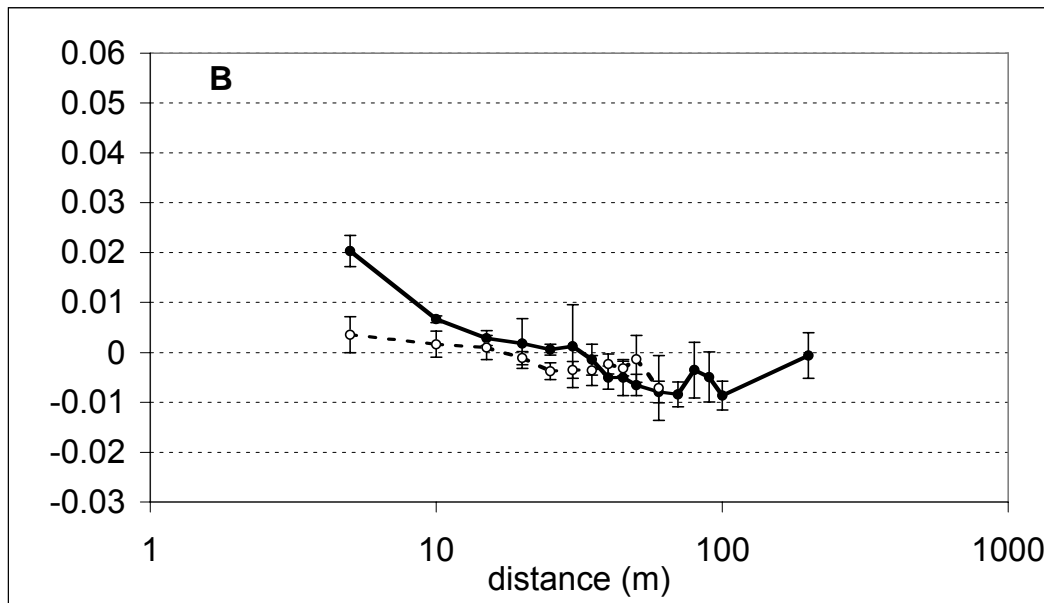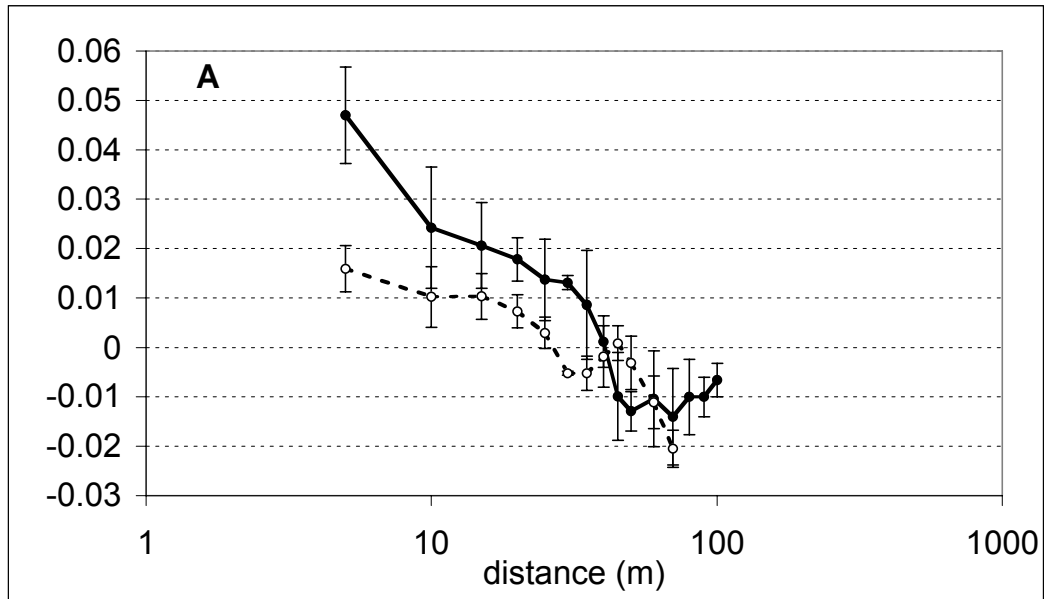
Table 1.5. SGS parameters (b=slope, F1=relatedness for the first distance class, and Sp statistics) and inbreeding coefficients (F_{IS}) for two subpopulations of *Z. fairchildiana* in native and disturbed habitats. Parameters were estimated using the relatedness coefficients defined in Loiselle et al. 1995 and Ritland 1996. N corresponds to the number of individuals successfully genotyped out of the total initial samples of 200 and 250 in the native and disturbed-habitat subpopulations respectively.

Parameter	Loiselle et al. 1995		Ritland 1996	
	native	disturbed	native	disturbed
b	-0.0144	-0.0079	-0.0060	-0.0029
SE b	0.006	0.003	0.001	0.001
F1	0.0470	0.0159	0.0203	0.0036
SE F1	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
F_{IS}	0.007	0.009	0.076	0.121
SE F_{IS}	0.075	0.091	0.022	0.068
N	191	244	191	244

DISCUSSION

Differences in life-history strategy between habitats

In the disturbed habitat, individuals produce more leaves, females and males start reproducing at a smaller age, and the allocation to fecundity is greater in females. It seems that populations in this habitat have a faster life-history strategy, in the sense of the 'fast-slow continuum' hypothesis (Franco and Silvertown 1996), where plants grow fast, reproduce early, and invest more in fecundity. Higher growth and reproductive rates in tropical trees may be associated with high resource environments (Baker et al. 2003). In both habitats, around 50% of the variation in leaf production is explained by canopy openness. Canopy openness and other structural characteristics of these rainforests have strong influences on light availability in the understory (Montgomery and Chazdon 2001; Nicotra et al. 1999). Light resources are very heterogeneous in the understory of tropical rainforests, but light availability is on average higher in the disturbed habitat. Irradiance is the major factor affecting growth rates in tropical rainforest trees (Brienen and Zuidema 2006; Clark and Clark 1992). Consequently, differences in growth and reproductive rates between native- and disturbed-habitat populations of *Z. fairchildiana* may be mostly associated with light availability in these habitats. Variation in light levels is usually associated with

differences in growth and fecundity rates in rainforest understory plants, and these differences can have significant demographic effects at the population level (Cipollini et al. 1994; Svenning 2002).

Differences in life history may have important consequences for the frequency of reproduction and mate availability in the disturbed-habitat populations of *Z. fairchildiana.* In cycads, reproduction is highly costly, and only a small percentage of the total adult population produces cones in a reproductive season. Plants accumulate carbohydrates in the stem for several months before producing new leaves or cones (Norstog and Nicholls 1997). Consequently, favorable conditions and enhanced growth can increase the probability of reproduction, as higher light availability does in rainforest *Zamia* species (Clark and Clark 1987). Furthermore leaf production may be reduced after reproduction, especially in females that deplete their resources during seed formation (Clark and Clark 1988). This pattern of reserves accumulation and depletion before and after reproduction has been observed in other species of the understory of rainforests, and it has been associated with light availability (Cunningham 1997; Marquis et al. 1997). With higher light availability, and higher growth and investment in reproduction, *Z. fairchildiana* plants in the disturbed habitat may be able to reproduce more often. However, plants do not invest in producing more or larger cones in this habitat, but in a higher frequency of reproduction. Higher frequency of reproduction will result in a higher proportion of reproducing males and females in a given reproductive season, as observed in disturbed-habitat populations. Nevertheless, other factors besides irradiance levels, like soil water and nutrient availability, may influence the variation in growth and fecundity rates in populations of tropical trees (Baker et al. 2003), and the relative role of different environmental factors remains to be evaluated.

An important consequence of the higher investment in reproduction and frequency of reproduction in the disturbed habitat 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 (Espirito-Santo et al. 2003; Nicotra 1998; Wheelwright and

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Bruneau 1992). In arborescent cycads, males usually reproduce every two or three years, but the period between reproducing events is larger for females (Ornduff 1991; Ornduff 1996; Tang 1990). Higher light availability may reduce the period between reproducing events, particularly for males of *Z. fairchildiana* in disturbed habitats. This will result in a higher male/female ratio in disturbed-habitat populations, as observed. Reproduction is highly synchronous in cycads, and females are receptive for pollen only for a couple of days (Norstog and Nicholls 1997). Males usually produce more than one cone, that mature sequentially, to maximize the time during which pollen is released (Clark and Clark 1987). With more males releasing pollen within the population, females may have a larger number of potential pollen donors. Below we explore how differences in life history and particularly higher reproduction and mate availability could have implications for the levels of SGS and inbreeding in populations of *Z. fairchildiana.*

Differences in the levels of SGS and inbreeding between habitats

Populations of *Z. fairchildiana* in the study area had low genetic differentiation at the neutral molecular level (i.e. a low R_{ST} or F_{ST}). Low genetic differentiation implies that the populations studied in the area (even if separated by more than 20 kilometers in a region with very roughed topography) behave effectively as one large population, and represent subpopulations. These subpopulations are likely descendant from a single ancestral population and/or have some gene flow between them. However, some subpopulations in the study region are currently being exposed to novel habitat conditions, particularly with respect to canopy openness. Subpopulations in the novel habitat show clear differences in life-history in comparison to subpopulations in the original or native habitat. These differences in life-history and other ecological factors may be affecting the strength of SGS and the levels of inbreeding within subpopulations, which could important consequences for the distribution of genetic variation in this population.

The strength of SGS within a population depends directly on the patterns of pollen and seed dispersal, and indirectly on ecological factors affecting the distribution

of pollen, seeds, and recruits within the population. SGS is present in most plant species where gene dispersal has been studied, and only species with light seeds and pollen that are dispersed by wind show a random distribution of genotypes within populations (Vekemans and Hardy 2004). Trees usually show weak SGS, given their great longevity and high pollen/seed dispersal. However, some tree species with large seeds that are gravity dispersed show stronger SGS within populations (e.g. Quercus species, Berg and Hamrick 1995). *Zamia fairchildiana* has large seeds dispersed by gravity. However, 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), although cycad seeds are highly toxic for most vertebrates. Long seed dispersal distances, and the fact that cycads are dioecious, and consequently obligate outcrossers, will result in weak SGS within populations, as observed in this study. The degree of SGS of the native habitat populations of *Z. fairchildiana* is comparable to other tropical tree species, with animal-dispersed pollen and large seeds that are gravity- or animal-dispersed (Hardy et al. 2006).

The presence of SGS suggests that seed dispersal distances are still restricted when compared to the area occupied by the whole populations, and perhaps more importantly, that pollen movement is also restricted within populations. Pollen movement usually has a major effect on gene dispersal and may have a stronger impact on SGS than seed dispersal (Hardy et al. 2006). Pollination in *Z. fairchildiana* populations is carried out by weevils (personal observation). Pollinators carry out their whole life cycle within male cones, and feed mostly on the sporophyll tissue of the male cones. Pollinators move between male cones, looking for feeding and mating places, and sometimes visit female cones, probably attracted by sugar and amino acid-rich micropyle droplets and to use them as refuges (Norstog and Fawcett 1989; Tang 1987). There is limited information on the flight ability of the weevils that pollinate cycad cones, but differences in female cone rewards and the availability of food/mating places could affect the patterns of pollinators movement within a cycad population. The spatial scale

of genetic structure within subpopulations of *Z. fairchildiana* is similar to gene dispersal distances estimated for other Neotropical rainforest trees with limited pollen and seed dispersal, i.e. pairwise relatedness is extremely low at distances greater than 150 m (e.g. Degen et al. 2004; Hardesty et al. 2005; Latouche-Halle et al. 2003)

Another notable fact is that SGS in *Z. fairchildiana* subpopulations in disturbed habitats is weaker in comparison to the subpopulations in their original native habitat. Few studies have analyzed the SGS of populations of the same species under different environments (but see Dutech et al. 2002; Vekemans and Hardy 2004), but differences in pollen or seed dispersal distances resulting from differing ecological conditions may affect the strength of SGS within populations. Seed dispersal is passive, mostly carried out by gravity, and the topography is similar between the subpopulations compared in this study, therefore seed dispersal distances probably have little effect on differing levels of SGS in the populations. Weaker SGS in the disturbed-habitat subpopulation might be explained by increased pollen movement between plants, and/or by indirect effects of higher reproduction on gene dispersal distances. A higher proportion of reproducing adults in a given year in the disturbed habitat may enhance pollinator movement, as weevils forage for food and nesting resources among reproducing plants. Pollinator behavior and the patterns of pollen movement can be affected by the density of reproductive plants in rainforest trees (e.g. Ghazoul and McLeish 2001; e.g. House 1992). In addition, overall adult density is higher in the disturbed-habitat subpopulation, and density is an important determinant of the SGS across species (Hamrick et al. 1993), through its indirect effects on gene dispersal and the magnitude of genetic drift. SGS is generally stronger in low density populations (Vekemans and Hardy 2004), as observed in this study. Detailed studies of pollinator behavior and direct estimates of pollen flow are required to explore the role of density and male/female reproductive ratios on the SGS on subpopulations of *Z. fairchildiana.*

Patterns of reproductive output may also affect the degree of gene dispersal limitation indirectly. For example, spatial and temporal variation in seed production increases dispersal limitation (Nathan and Muller-Landau 2000). Therefore, a higher

investment in reproduction in the disturbed habitat may result in a more homogenous production of seeds across space, which will in turn increase the overlap between seed shadows, and thus decrease the SGS within the subpopulation. A higher probability of flowering and higher seed production in canopy-gaps sites resulted in weaker SGS within a populations of a subcanopy tree (Ueno et al. 2006). A higher density or more homogeneous distribution of flowering individuals can also result in a larger number of pollen donors for females (Murawski and Hamrick 1991; Stacy et al. 1996). In subpopulations of *Z. fairchildiana* a higher number of males available for mating may increase the proportion of half-sibs within a female cone (if more males pollinate the cone) and that would decrease the degree of relatedness among seed families and neighbor plants. Finally, environmental conditions in the disturbed habitat are less heterogeneous than in the native habitat, as evidenced by a lower variation in values for canopy openness. Lower heterogeneity in light levels has been found in other disturbed habitats of tropical rainforests (e.g. Montgomery and Chazdon 2001). Weaker micro-environmental selection could also result in a more random mortality of seedlings and a decrease in SGS. Higher seed shadow overlap resulting from higher reproduction, or correlated effects like higher pollinators movement and lower relatedness within families might explain the decreasing magnitude of SGS in disturbed-habitat subpopulations of *Z. fairchildiana*, but additional information is necessary to assess the relative role of each of these factors. In particular, comparisons of the degree of SGS between early and late stages in the life cycle could discriminate between hypothesis dealing with limited pollen dispersal versus selective thinning after seed dispersal.

Finally, an important consequence of strong SGS is that related individuals are close in space, and if they are more likely to mate, then biparental inbreeding levels could increase in the population (e.g. Degen et al. 2004; e.g. Gapare and Aitken 2005; van Rossum et al. 2002). However, inbreeding levels are higher in the disturbed habitat, where SGS is weaker. The higher levels of biparental inbreeding observed in the disturbed habitat may result from large variation in the reproductive success of individuals. Large variance in individual fecundity has been shown for other tropical trees (Meagher and Thompson 1987; Schnabel et al. 1998). In addition, some

populations show few successful cohorts (Jones and Hubbell 2006), where a few families (i.e. related individuals) have the highest fitness. A few individuals belonging to successful families that contribute disproportionably to recruitment could explain the higher levels of inbreeding in the disturbed habitat. This will result in lower levels of genetic diversity in the disturbed habitat subpopulations, i.e. lower heterozygozity and allele richness, as observed in this study. The levels of SGS and inbreeding observed in subpopulations of *Z. fairchildiana* depend not only on gene dispersal and ecological features of the population, but also on the strength of genetic drift and stochastic factors affecting the effective population size, that may not be at equilibrium shortly after habitat degradation. Nevertheless, the observed significant differences in life history, and the magnitude of SGS and inbreeding can have important consequences for the distribution of genetic variation in 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 affect the life-history strategies of populations and the distribution of genetic variability within populations. Before the significant alterations of the landscape resulting from recent human settlements, the environmental conditions in the forest habitat where these populations are found were likely very similar. Accordingly, the low R_{ST} value indicates that these two populations of *Z. fairchildiana* are not significantly differentiated, at least at the neutral molecular level. However, environmental modifications in disturbed forests are drastic, and many aspects of forest structure and composition in disturbed habitat are likely to become permanently differentiated when compared to the native habitat (Tabarelli et al. 2004). Subpopulations in the disturbed habitat are smaller, but similar levels of genetic variation (number of alleles and heterozygozity) suggest that genetic drift is not affecting the population significantly in the recent past. Effects of habitat disturbance on the levels of genetic variation may take several generations to be detected, but other genetic effects influencing population viability, like inbreeding depression, can affect populations rapidly (Lowe et al. 2005).

Changes in the distribution of genetic variation within populations that will affect their response to environmental heterogeneity in space and time will have important consequences for population viability in dynamic ecosystems like tropical rainforests. Processes affecting the clustering of related individuals will affect patterns of viability selection through enemy-mediated effects or survival/competition among offspring, and the effectiveness of selection for adaptation to microenvironmental variation within populations (Schnabel et al. 1998). Herbivory can have a strong impact on individual growth and survival in populations of *Zamia* species (Clark and Clark 1991; Negron-Ortiz and Gorchov 2000), and other factors affecting seedling survival may be influenced by the differences in SGS between populations of *Z. fairchildiana.* Higher homozygozity may also affect the fitness of individuals in the disturbed habitat, particularly if there is significant inbreeding depression (Hedrick and Kalinowski 2000). The consequences of habitat fragmentation and local extinction of some species will persist for a long time, and in some places forest will not be able to regenerate even if human disappear from the landscape (Chazdon 2003). Therefore, even if anthropogenic disturbances are transient, long-term environmental changes in degraded forests might affect significantly the life-history and the genetic composition of populations. The potential effects of habitat modifications on the life history and the fine-scale spatial distribution of genetic variation within populations should be of concern in conservation.

CHAPTER 2

"The potential for genetic differentiation in response to selection between populations from native and disturbed habitats in a rainforest cycad"

ABSTRACT

Rapid evolution may be common in human-dominated landscapes, where environmental changes are severe. Predicting evolutionary changes in populations requires information on the patterns of directional selection and genetic variances and covariances of traits that may affect fitness under the novel environmental conditions. Here, we used phenotypic selection analyses and a marker-based method to estimate heritabilities and genetic correlations to predict the potential response to selection in populations of the long-lived cycad *Zamia fairchildiana* exposed to habitat degradation. Patterns of selection in adult fecundity showed that different traits were under strong directional selection in subpopulations from native habitats and degraded forests. In the native-habitat subpopulations, plants maximize fitness by enhancing photosynthetic ability through larger leaf surface area or smaller SLA, and these traits showed a combination of directional and quadratic selection. In contrast, larger leaf production increased fitness in the disturbed-habitat subpopulations, and light availability appears to be a major agent of selection for this trait. Stabilizing selection was unimportant in the disturbed habitat, where light availability is less heterogeneous. Leaf production and SLA showed positive additive genetic variance, and no genetic trade-offs with other traits, suggesting that this traits can respond to selection in each habitat. Nevertheless, genetic correlations between SLA and the number of leaves could result in indirect changes in these traits, and weaken the magnitude of genetic differentiation between environments in these traits. Directional selection coefficients were large, and if combined with moderate levels of heritability could result in significant phenotypic changes between habitats in few generations. Comparisons of phenotypic means between subpopulations showed significant differences in leaf production in the direction predicted by the selection analyses. Other traits showed less phenotypic

differentiation between habitats, as predicted by the genetic analyses. These results suggest that recent environmental change results in strong directional selection in subpopulations of *Z. fairchildiana,* and that the subpopulations have the potential to diverge at the genetic level in traits like leaf production. Anthropogenic habitat changes can result in major selection events, and if persistent for several generations, may promote rapid evolution of populations.

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 (Hendry and Kinnison 1999; Reznick and Ghalambor 2001). Strong directional selection after environmental changes can cause rapid evolution in traits that may increase fitness under the novel environmental conditions and that have genetic variation to respond to selection (Kinnison and Hendry 2001). Drastic environmental changes are commonplace in human-dominated landscapes, where anthropogenic activities can result in rapid and severe modifications in the habitat of plant populations. The accelerated rate of change in ecosystems caused by humans promotes population extinctions in many cases, but it could also promote rapid evolution of populations if they can persist in the disturbed habitats and respond to selection (Palumbi 2001; Stockwell et al. 2003; Zimmer 2003). Studies of the potential for rapid evolution of populations in human-dominated landscapes are therefore relevant for conservation, but they also are important to understand the circumstances that can promote adaptive evolution and the rate of phenotypic change that is possible in natural populations.

To predict whether phenotypic means of populations will change over time as the result of selection, information on the magnitude and direction of selection and genetic variance (and covariances) of the traits is required. This information is rare for long-lived organisms, because patterns of selection may vary over time and estimation of heritabilities and genetic correlations are difficult to obtain (Grant and Grant 1995). However, many species of conservation interest are long-lived, and information on

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potential phenotypic changes as result of habitat modifications may be relevant for population persistence and for conservation. In particular, significant phenotypic changes and potentially rapid evolution will alter the genetic structure of populations (as population become differentiated at the genetic level), and will impact the evolutionary potential of populations to respond to future environmental changes (as genetic variation is eroded by strong selection). Furthermore, the rate of evolutionary change may be important in the success of invasive species, or population viability of managed species (Stockwell et al. 2003).

Although rapid evolution is difficult to document in long-lived species, selection analyses in combination with information on genetic variance and covariances can be used to predict the response to selection to particular episodes of environmental change. Directional selection is usually strong immediately after environmental perturbations and it usually remains strong for a few generations (Hendry and Kinnison 1999; Hoekstra et al. 2001). Therefore, detecting selection may be feasible in populations that are currently being affected by drastic habitat perturbation. Phenotypic selection analyses provide straightforward methods to identify targets of selection in natural populations during such events (Lande and Arnold 1983; Phillips and Arnold 1989; Scheiner et al. 2000). Furthermore, even if habitat modifications are transient, severe environmental changes spanning a few generations and the resulting strong selective pressures might have considerable effects on the genetic composition of populations. To predict the potential response to selection, marker-based methods can be used to estimate genetic variance and covariance of traits in long-lived species, because they do not require genetic crosses (Ritland 2000; Thomas et al. 2000). Marker-based methods have not been widely applied to estimating heritabilities in natural populations of plants, because the method requires substantial variation in the relatedness coefficients within populations (Andrew et al. 2005). Only three studies have demonstrated the presence of heritable variation of quantitative traits in long-lived plants (Andrew et al. 2005; Klaper et al. 2001; Ritland and Ritland 1996), but the method is promising if used in species that fit the requirement of strong spatial genetic structure, which is widespread in plant species (Vekemans and Hardy 2004). Finally,

phenotypic and genetic differentiation between cohorts of individuals within a population that developed in different environmental conditions across time can provide evidence of potential genetic changes in populations in the long term (Linhart and Grant 1996).

Zamia fairchildiana, similar to many other cycads (Gymnospermae) in the Neotropics, is typical of the understory of lowland rainforests (hereafter referred as the native habitat). 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 the most limiting resource for understory plants like *Z. fairchildiana* (Chazdon et al. 1996; Clark et al. 1992). Consequently, changes in light availability can greatly affect growth rates and survival of understory plants (Brienen and Zuidema 2006; Clark and Clark 1992). *Z. fairchildiana* can also be found in highly modified or disturbed forest habitats. Forest fragmentation and exploitation practices by humans (e.g. logging, hunting) result in disturbed forests that differ significantly in the physical structure and species composition when compared to native habitats (Noble and Dirzo 1997; Tabarelli et al. 2004). In particular, disturbed-forest habitats for *Z. fairchildiana* have lower average canopy cover (see Chapter 1). Canopy cover influences the levels and spatial distribution of light in the understory of tropical rainforests (Montgomery and Chazdon 2001; Nicotra et al. 1999). Therefore, native and disturbed forests may represent distinct habitats for populations of *Z. fairchildiana,* at least in terms of the magnitude and heterogeneity of light availability.

In the study site (Osa Peninsula in southwestern Costa Rica), colonies or patches of *Z. fairchildiana* individuals show low genetic differentiation at the neutral molecular level (i.e. low F_{ST} values, see Chapter 1) between them. Consequently, the colonies represent subpopulations of a large regional population at the genetic level. However, subpopulations exposed to the novel environmental conditions in disturbed forests exhibit some phenotypic divergence in life-history traits (see Chapter 1). Genetic divergence in ecologically-relevant traits between subpopulations *Z. fairchildiana* from native and disturbed habitats could arise if environmental differences 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 in the traits. Particularly, differences in light availability (or other environmental factors) between habitats could affect the ability of plants to grow, reproduce and/or the number of offspring they can produce (selection via fecundity), or the ability of seedlings to survive to the juvenile/adult stage (selection via mortality). Selection through other fitness components is possible, but likely to be weak, as juvenile and adult survival are extremely high in *Zamia* populations (Negron-Ortiz and Gorchov 2000; Negron-Ortiz et al. 1996).

In this paper, we test the hypothesis that differences in light levels between the native and disturbed 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 phenotypic traits related to growth and response to light availability. Furthermore, we estimate heritabilities and genetic correlations for the phenotypic traits to determine whether traits can respond to selection, and we compare phenotypic means between habitats to explore the magnitude of phenotypic divergence in traits than can respond to selection. We show that different traits are under directional selection in each habitat, and that some of these traits that can respond to selection and show significant differences in the phenotypic mean across habitats. These results suggest that rapid adaptive divergence is occurring in response to environmental change, particularly light availability, in populations of *Z. fairchildiana.*

METHODS

Estimation of selection coefficients

For selection analyses, we sampled all reproductive individuals in three subpopulations per habitat during the reproductive seasons of 2004 and 2005. Selection via fecundity was estimated for five phenotypic traits: 1) stem length: related to growth rate and the ability of plants to accumulate resources for further growth and reproduction; 2) leaf production: number of new leaves produced in the growing season

of 2004; 3) number of leaves: number of old leaves, which results from a combination of leaf production and leaf longevity; 4) leaflet area: average leaflet 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 (only one in females) in an individual. 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 disturbed habitat, and 134 individuals in the native habitat.

Coefficients of linear (or directional) selection were estimated for each trait using path analysis. In the path analysis a measure of the overall 'condition' of plants was included to reduce potential biases due to environmental correlations with traits and fitness. In this analysis condition is used to account for environmental effects that could act both on fitness and the phenotypic traits of interest (Scheiner et al. 2002). We used the maximum number of leaflets (parts of each compund leaf) as a measure of condition, because this variable increases with plant age and has proven to be a good indicator of the developmental stage of the plant in *Zamia* species (Clark and Clark 1987; Negron-Ortiz and Breckon 1989). The path model included direct effects of condition on all phenotypic traits, and of traits on fecundity. Additionally, there were paths linking physiological leaf traits (i.e. leaflet area and SLA) with leaf production, and leaf production with the total number of leaves (Figure 2.1). In a path analysis framework, direct effects of trait on fitness are estimated from direct connections between them, while indirect effects go through connections between traits and fitness that include intermediate traits (Scheiner et al. 2000). Therefore, leaflet area, SLA, and leaf production can have both direct and indirect effect on fecundity in the model. 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 quadratic and correlational selection. Coefficients for quadratic and correlational selection were estimated as the partial regression coefficient in a multiple linear regression analysis using squared traits and cross-product values for the phenotypic traits, respectively (Lande and Arnold 1983; Phillips and Arnold 1989). Linear selection coefficients 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 causal relationships between traits, as in the path analysis.

Finally, to explore the effect of light availability on the relationship between phenotypic traits and fitness, we estimated light availability for each individual by measuring the percentage of canopy openness above the leaf crown of the plant, using a spherical densitometer. To test the hypothesis that light availability is an agent of selection in subpopulations of *Z. fairchildiana,* we regressed selection coefficients for traits in each subpopulation on the average canopy openness for that subpopulation (thus N=6). If a significant covariance exists between selection coefficients and an environmental variable across subpopulations, then it is possible to hypothesize that the environmental variable is a causal agent of selection (Wade and Kalisz 1990). Path analyses were performed using the SEM package in R. Linear regression and logistic regression analyses were performed in SPSS (SPSS 2003).

Estimation of heritabilities and genetic correlations

We used the marker-based method proposed by Ritland (Ritland 1996b) to estimate heritability and genetic correlations for all traits included in the selection analysis. With this method, heritability is estimated with a linear model that takes into account the effects of additive genetic variance and the environmental correlation (sharing of environments) on phenotypic similarity for a trait (dominance and inbreeding

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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)) and the actual variance of relatedness (Vr) (Ritland 1996b). *Z. fairchildiana* has weak spatial genetic structure within subpopulations (see Chapter 1), but there was enough variation in relatedness as to allow the use of this marker-based method for estimating heritabilities and genetic correlations. 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 ($C(Y_{12}r)$).

For heritability analyses, we sampled all individuals present in two 100 x 20 m transects in one subpopulation from the native habitat, and two 50 x 10 m transects in one subpopulation in the disturbed habitat (where plant density was higher). We estimated pairwise relatedness coefficients using six microsatellite loci for all individuals within the transects. Polymorphism in the molecular markers used to estimate relatedness was large, i.e. n(m-1)~90 (were 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 1996b). Details about the development and genotyping of microsatellite loci can be found in Chapter 1. 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 10000 bootstraps by resampling individuals (comparisons between identical individuals were omitted). If more than 95% of the bootstrap values were positive (or negative), then heritability parameters were considered significantly greater from zero (or significantly lower than zero, in the case of genetic correlations).

Phenotypic divergence between habitats

If the magnitude and/or direction of selection in a trait differ between subpopulations from native and disturbed habitats, and that trait has responded to

selection (at least for a few generations), then the phenotypic mean for the trait should differ between habitats. However, for subpopulations of *Z. fairchildiana* in the disturbed habitat, the phenotypic mean (and variation) will be influenced by the presence of adults that have survived habitat changes, even if they do not contribute offspring to the new cohorts (and by phenotypic plasticity). Consequently, potential divergence in the phenotypic means across habitats may be better observed at the juvenile stage, that is entirely composed of plants that recruited after the environmental change took place.

To test for the phenotypic divergence between habitats, trait means for all traits included in the selection analyses were compared between habitats, for juveniles and adult individuals. For three subpopulations per habitat, we sampled all individuals present in a 100 x 20 m transect in the native habitat, or a 50 x 10 m transect in the disturbed habitat (where individual density was higher). Individuals in transects were classified as juveniles or adults based on leaflet number. Juveniles were plants with more than 10 but less than 26 leaflets (the minimum size of observed reproductive individuals) and adults had 26 or more leaflets/leaf. Phenotypic means for all traits were compared between habitats and life-cycle stages in a multivariate GLM. The number of leaflets was included as a covariate in the analysis, because phenotypic traits like leaf production and leaflet area increase as the plant gets larger in size, i.e. as the number of leaflets gets larger.

RESULTS

Phenotypic selection analysis

The path model used for the phenotypic selection analysis included direct effects of all phenotypic traits on fecundity and indirect effect of leaflet area, SLA, and leaf production on fecundity (Figure 2.1). Using this path model, condition had significant direct effects on stem length and number of leaves in both habitats, but no effect on leaf production, leaflet area or SLA (Table 2.1). Condition affected fecundity in the disturbed habitat (path coefficient $\beta=0.178$, P=0.023), but not in the native habitat (path coefficient

β=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 the disturbed habitat. Nevertheless, the path analysis was designed to account for this environmental bias, and selection coefficients for stem length and the number of leaves should not be inflated by this bias.

According to the path analysis, different traits were under directional selection in each habitat (Table 2.1). Leaf production and the number of old leaves were under strong directional selection in the disturbed habitat, but these traits were not under selection in the native habitat. The number of leaves had only direct effects on fitness, but leaf production affected fitness both directly and indirectly (through its effect of the number of leaves) in the disturbed habitat. Leaflet area and SLA were under directional selection in the native habitat, where individuals with larger leaflets but smaller SLA had higher fecundity. In the native habitat, 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 as well.

Table 2.1. Standardized selection coefficients for direct and indirect selection in the path analysis for subpopulations of disturbed and native habitats in *Z. fairchildiana*. The effect of condition is the direct effect of plant size (number of leaflets) on each phenotypic trait in the path model. For path coefficients: **P<0.01, *P<0.05.

Trait	disturbed habitat			native habitat		
	direct effects	indirect effects	effect of condition	direct effects	indirect effects	effect of condition
stem length	0.102	0.102	0.242**	0.045	0.045	0.514**
leaf prod.	0.346**	0.402**	0.029	0.111	0.125	0.001
no. 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

Figure 2.1. Path diagram used in the phenotypic selection analyses for subpopulations of *Z. fairchildiana*. The model includes relationships between condition (leaflet number) and all phenotypic traits and fitness. The beta1 to beta5 parameters are the selection coefficients for each trait (due to direct selection).
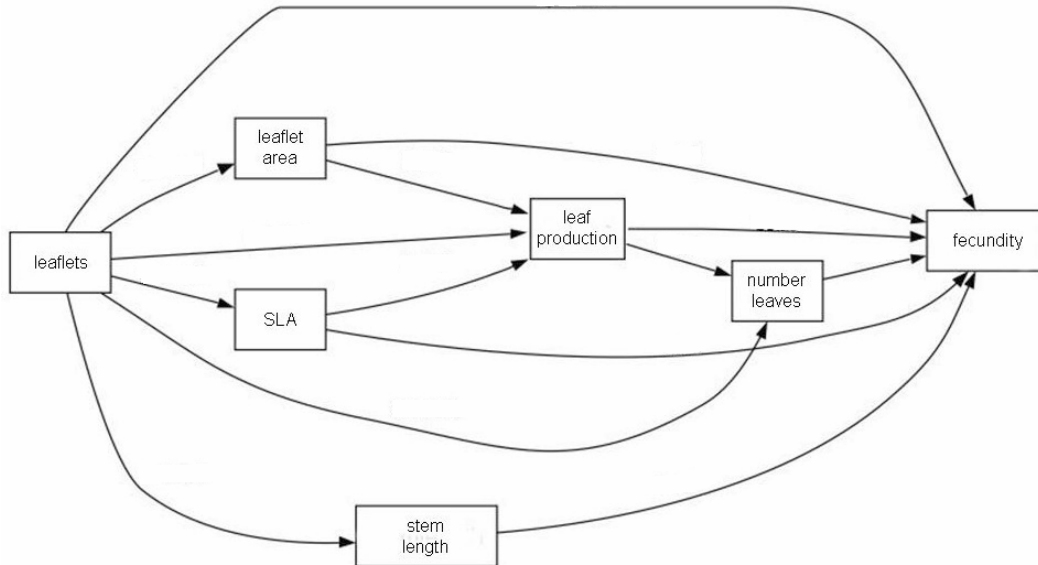
The selection analysis using multiple regression showed similar results to the path analysis for directional (or linear) selection (Table 2.2). In the multiple regression analysis, leaf production and the number of leaves were under directional selection in the native habitat. Leaflet area and SLA had marginally significant partial regression coefficients in the linear selection analysis. Coefficients for quadratic selection were not significant in the disturbed habitat (Table 2.2), therefore no trait is under stabilizing or disruptive selection in this habitat. In the native habitat, leaflet area was under disruptive selection, as evidence by a negative coefficient in the quadratic selection analysis, and the selection coefficient for quadratic selection was greater than the coefficient for directional selection (Table 2.2). Similarly, SLA had a marginally significant coefficient for stabilizing selection. The model for correlational selection had no significant effects in the native habitat, but the combination of higher leaf production and number of leaves was significantly related to fecundity in the disturbed habitat (partial regression coefficient $\beta=0.440$, P<0.001). When condition was included as a covariate in the multiple regression analysis, selection coefficients did not change substantially, and condition did not have a significant effect on fecundity in any habitat.

Table 2.2. Standardized selection coefficients for linear and quadratic selection in the multiple regression analysis for *Z. fairchildiana* subpopulations from disturbed and native habitats. Partial regression coefficients: **P<0.01, *P<0.05, §P<0.1.

Trait	disturbed habitat		native habitat	
	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§

In the regression analysis between mean canopy openness and selection coefficients across subpopulations, canopy openness explained a large portion of the variation (R^2>0.5) in the selection coefficients for leaf production (Table 2.3). 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 of any other phenotypic trait.

Table 2.3. Effects of average canopy openness on the directional selection coefficients for each trait across subpopulations of *Z. fairchildiana*. R^2, the slope, and the P values are reported for the linear regression analysis done for each trait, where N=6, the number of subpopulations analyzed.

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

Heritability estimates

In the heritability estimations, the total number of pairwise comparisons within samples was of 14479 for the native habitat subpopulation and 16816 for the disturbed habitat subpopulation. Estimated average relatedness was 0.045 in the native habitat and 0.052 in the disturbed habitat (close to the relatedness of first-cousins). The actual variance for relatedness was low (Vr=0.001) but significantly different from zero in both habitats (P=0.0005 in the native-habitat subpopulation, P=0.0001 in the disturbed-

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 habitats. However, estimates of heritability were not significantly different from zero for any trait or any of the two subpopulations analyzed. Average values ranged from 0.023 to 0.765, but the 95% confidence intervals around the estimates were very large (Table 2.4). Low reliability of the estimates is likely the result of very low actual variance in relatedness and not of small sample size, inappropriate sampling, or low marker polymorphism.

Table 2.4. Values for heritability (h^2) and its 95% CI estimated using a marker-based method. CI are based on 10000 bootstraps where individuals were re-sampled. P C(Z,r) are P values for the significance of the covariance between phenotypic similarity (Z) and relatedness coefficients (r) for pairs of individuals in two subpopulations of *Z. fairchildiana* from native and disturbed habitats.

Trait	h^2	h^2 [CI]	P *C(Z,r)*
Disturbed habitat subpopulation			
stem length	0.208	[-0.437,1.025]	0.071
leaf production	0.605	[-0.140,1.427]	0.001
number of leaves	0.149	[-0.506,0.925]	0.097
leaflet area	0.252	[-0.544,1.357]	0.101
SLA	0.283	[-0.435,1.050]	0.069
Native habitat subpopulation			
stem length	0.315	[-0.409,1.205]	0.068
leaf production	0.313	[-0.515,1.295]	0.094
number of leaves	0.498	[-0.179,1.332]	0.016
leaflet area	0.023	[-0.823,0.787]	0.278
SLA	0.765	[-0.013,1.800]	0.005

Actual variance of relatedness is necessary for the estimation of heritability, but 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 2000). The number of leaves and SLA in the native habitat, and leaf production in the disturbed habitat, had a significant covariance term at the α=0.05 level (Table 2.4). In addition, all traits in both habitats had covariance terms that were marginally significant (P<0.1 in Table 2), except for leaflet area, suggesting that they may have some additive genetic variance (Klaper et al. 2001). Finally, if heritability estimates are accurate (even with the lack of precision observed),

then levels of additive genetic variance for SLA, leaf number, and leaf production were high in subpopulations of *Z. fairchildiana,* i.e. $h^2 > 0.5$ (Table 2.4).

Phenotypic and genetic correlations

Since heritability estimates were not significantly different from zero, then genetic correlations were not different from zero either. However, 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. 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 2.5). 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 habitats. Leaf production was not genetically correlated with any other trait (Table 2.5).

Phenotypic correlations between SLA-number of leaves and leaf number-stem length had the same direction as the genetic correlations for these traits (Table 2.5). Additionally, there were significant phenotypic correlations for traits that showed no evidence of a genetic correlation, and phenotypic correlations were mostly consistent across the two subpopulations (Table 2.5). SLA and leaflet area had a strong positive correlation in both habitats. Leaf production was positively correlated to the number of leaves in both habitats, and had a weak negative correlation with SLA in the native habitat. Finally, plants with a larger stem, also had higher leaf production, leaf number, and leaflet area, but these correlations were weak.

Phenotypic divergence

Average leaf production was significantly higher in the disturbed habitat for all life stages (Figure 2.2), as predicted by selection analysis in adults. Leaf production had high heritability in the disturbed habitat, and is the trait where the greatest response to selection is expected in this habitat. The number of leaves was similar between native

and disturbed habitat subpopulations for all life stages (Table 2.6). SLA was significantly lower in the native habitat, but only for adult plants. Finally, leaflet area did not differ between habitats for juveniles or adults (Figure 2.2). Statistical power was low (i.e. power<0.30) for all comparisons that showed no significant effects of habitat on the phenotypic traits, especially for juveniles (Table 2.6). Sample size were between 60 and 110 individuals for juveniles, and between 260 and 360 individuals for adults, but the large phenotypic variance in most traits reduced the statistical power of the tests.

Table 2.5. Values for phenotypic and genetic correlations between traits in two subpopulations of *Z. fairchildiana* from native and disturbed habitats. Upper diagonal: native-habitat subpopulation. Lower diagonal: disturbed-habitat subpopulation. For phenotypic correlations: **P<0.01; *P<0.05. 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: * P<0.05.

Trait	stem length	leaf prod.	no. of leaves	leaflet area	SLA
Phenotypic correlations					
stem length	----	0.246**	0.563**	0.199**	-0.061
leaf prod.	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**	----
Genetic correlations					
stem length	----	-0.397	0.306*	0.806	0.077
leaf prod.	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	----

Table 2.6. Effect of habitat on the phenotypic mean for traits in juveniles and adults from six subpopulations of *Z. fairchildiana*. Sum of squares (SS), degrees of freedom (d.f.), and F, P, and power values are shown from a GLM with habitat as a fixed factor, and the number of leaflets as a covariate.

Source	SS	d.f.	F	P	Power
Juveniles					
leaf production	0.972	1	3.76	0.050	0.48
number of leaves	2.224	1	1.43	0.235	0.22
leaflet area	17.054	1	0.56	0.456	0.11
SLA	0.001	1	1.27	0.262	0.20
Adults					
leaf production	90.355	1	61.61	<0.001	0.99
number of leaves	0.007	1	0.01	0.944	0.05
leaflet area	426.544	1	0.96	0.328	0.164
SLA	0.006	1	7.82	0.005	0.80

Figure 2.2. Comparison of four phenotypic traits (mean ± 2SE) in juvenile and adult individuals between subpopulations from native and disturbed habitats. Open circles: means for disturbed habitat subpopulations; Closed circles: means for native habitat subpopulations.
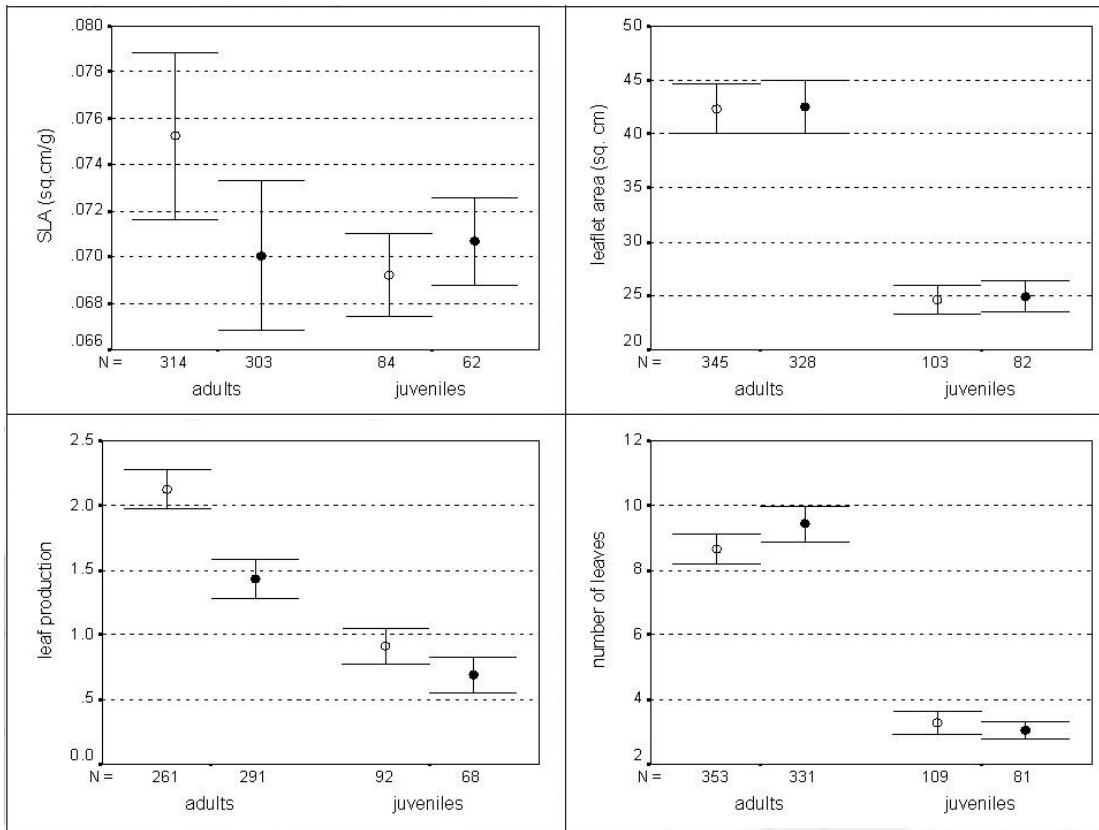
DISCUSSION

Differing patterns of selection across habitats

Patterns of directional selection differed between habitats, as different traits were under selection in each habitat. In the native habitat, plants with larger leaflet area and smaller SLA had higher fecundity. In the disturbed-habitat subpopulations a larger number of leaves and higher leaf production is associated with higher fecundity. Leaf traits affecting the photosynthetic ability of plants (like leaf surface area and SLA) and traits related to leaf demography (like leaf production and leaf longevity, that will determine the number of standing leaves in the plant) are commonly associated with growth rates of individuals and can affect fitness directly or indirectly (Ackerly et al.

2000; Arntz and Delph 2001; Geber and Griffen 2003). However, it is predicted that suits of correlated physiological or leaf traits will change together in response to environmental changes like increasing irradiance. Patterns of selection in *Z. fairchildiana* subpopulations suggest that rather than changes in suits of correlated traits in response to higher irradiance in the disturbed habitat, the subpopulations have different ways of maximizing fitness in each habitat.

This study suggested a direct effect of leaflet area and SLA on fecundity of plants. Greater leaflet area enhances the capture of light, while smaller SLA increases the photosynthetic ability of plants (although it limits light capture at low irradiance). However, leaflet area and SLA have no impact on fitness on the higher irradiance conditions of the disturbed habitat. This may be possible because changes in SLA may have a greater impact on photosynthetic rate and relative growth rate under low- than high-light conditions (Evans and Poorter 2001; Sims et al. 1994). In addition, responses to light availability in rain forest plants are not linear, and with high irradiance, water or nutrient availability may become more limiting than light and constrain growth (Montgomery 2004), so that different traits may become relevant in a higher light habitat. In addition, non-linear selection on photosynthetic traits may be more important in the native habitat, where light conditions are more variable than in disturbed or secondary forests (Montgomery and Chazdon 2001; Nicotra et al. 1999; Numata et al. 2006). A combination of directional and stabilizing/disruptive selection for leaflet area and SLA results in changes in the mean and the variance for those traits that can be important for plants to adjust to variable environmental conditions in space and time in the understory of the native habitats of *Z. fairchildiana.* Quadratic selection is difficult to detect in natural populations, and most available estimates are of small magnitude, most likely because a lack of statistical power in the small samples used for selection analyses (Kingsolver et al. 2001). Long-term monitoring of populations is necessary to evaluate the constancy of the selective pressures in native and disturbed habitat and to improve analyses for other forms of selection in the subpopulations.

Light levels are less heterogeneous in the disturbed habitat, and non-linear selection in this habitat was not important. In contrast, larger leaf production and leaf number had a direct impact on plant fitness. 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 disturbed habitat, but not in the native habitat, where leaf production and reproduction is highly costly because of limited light availability (Clark and Clark 1988). *Zamia neurophyllidia*, another rainforest cycad, produces very long-lived, well defended leaves, with intermediate physical features between sun- and shade-adapted species (Clark et al. 1992; Lee et al. 1990). Long-lived rainforest species, in general, produce leaves with long life spans, that have low photosynthetic ability but resistant physical structure (Reich et al. 1991). It is possible that a release from limited light allows *Z. fairchildiana* plants in the disturbed habitat to produce more leaves at a smaller cost, and that this strategy is more efficient to maximize fitness than adjusting leaf area or SLA to the new environmental conditions. As mentioned before, responses in leaf production and leaf longevity may also be related to other environmental factors besides light availability. For example, relationships between leaf life span and other leaf functional traits have been observed in response to gradients of water availability in tropical rain forests (Santiago et al. 2004).

Analyses of the potential causes of selection in subpopulations of *Z. fairchildiana* suggested that light availability may be a major agent of selection. Even with a restricted sample of subpopulations (N=6 for the regression analysis), average canopy openness was significantly related to variation in the selection coefficients for leaf production. More importantly, this relationships agreed with the trends observed in the phenotypic selection analyses (where no information of the environment was included). The magnitude of the selection coefficients increases with higher light availability for leaf production. That patterns of selection in *Z. fairchildiana* subpopulations agree with functional predictions of the response of plants to light availability, suggest that canopy

cover may be a major determinant of fitness in the species. Nevertheless, only manipulative experiments can establish the relative role of different environmental factors on the relationship between phenotypic traits and fitness (Wade and Kalisz 1990). In addition, light did not explain all the variation in selection patterns across subpopulations, and other agents of selection may be affecting these subpopulations. In particular, insect herbivory has been shown to have an important effect on leaf demography in rainforest *Zamia* species (Clark and Clark 1991; Negron-Ortiz and Gorchov 2000).

Regardless of the agents of selection, directional selection was relatively strong, when compared to average values reported by phenotypic selection studies (Kingsolver et al. 2001). This supports the idea that recent environmental changes result in strong selective pressures on subpopulations of *Z. fairchildiana* exposed to a novel environment. However, strong directional selection observed in populations of *Z. fairchildiana* could be attributable to environmental biases in the analyses. The presence of environmental covariances in the relation between traits and fitness may overestimate selection coefficients (Kruuk et al. 2003; Rausher 1992). Path analysis was employed here in an attempt to capture indirect effects of traits on fitness, but also to control for potential environmental biases (Scheiner et al. 2002). A measure of plant size (the condition variable) had an impact of fitness, and on the traits stem length and the number of leaves in both habitats. Stem length and the number of leaves (which are genetically correlated) increase steadily with plant size, i.e. as the number of leaflets/leaf increases. Similarly, fecundity increases with plant size. Increases in fecundity with light availability and plant size have been observed in other *Zamia* species (Clark and Clark 1987), and understory rainforest plants (e.g. Cunningham 1997). Within subpopulations, plants with higher leaf production will have larger stems and leaf number, as suggested by phenotypic correlations between these traits. This may be a response to resource availability. However, leaf production and leaf number are not under selection in the native habitat, suggesting that the potential environmental covariance for the relationship between these traits and fitness was eliminated by the inclusion of the condition variable in the selection analysis.

Finally, patterns of selection within a generation may be modified by phenotypic correlations between traits. In the native habitat, leaflet area and SLA are not linked at the genetic level, but there is a positive phenotypic correlation between them. Plants with larger leaflets have larger SLA, i.e. thinner leaves with more area per unit of biomass. This same relationship has been found in other *Zamia* species exposed to variable light environments (Newell 1985; Newell 1989). This may be possible if leaf water content is high, allowing plants to have large leaf surface area (to increase light capture) with a small investment in leaf biomass (Shipley 1995). Large leaf area may increase fitness, by increasing efficiency in light capture. However, smaller SLA increases photosynthetic ability, and selection favors leaflets with small SLA as well. At the phenotypic level, plants can not have larger leaf surface area and small SLA at the same time, thus maximizing fitness can only be attained with alternative trait states. There is no genetic constraint on the evolution of these two traits, but simply the combination of trait values that will maximize fitness in the native habitat does not exist in the natural populations. There is no genetic variation for leaflet area in subpopulations of *Z. fairchildiana*, then genotypes with larger SLA will become less common in the subpopulations, even if larger leaflet area increases fitness, because the phenotypic mean of SLA will decrease across generations, since this trait can respond to selection. Phenotypic correlations between all traits and leaf production should not affect the relationship between leaf production and fitness within a generation in the disturbed habitat.

The potential response to selection

Not all the traits that are under directional selection in each habitat can respond to selection. SLA in the native habitat and leaf production in the disturbed habitat showed evidence of additive genetic variance, and therefore these traits could respond to selection. In contrast, leaflet area in the native habitat and the number of leaves in the disturbed habitat may have some additive genetic variance (the covariance term in the heritability estimation is marginally significant), but the response to selection may be

more limited in these traits. Genetic correlations show no evidence of genetic trade-offs that could constrain the response to selection of SLA or leaf production in the subpopulations. There is a strong genetic trade-off between SLA and the number of leaves, but the number of leaves is not under selection in the native habitat, therefore genetic changes in the mean or the variance for SLA should be theoretically possible in this habitat. In the disturbed habitat, leaf production had no genetic trade-offs. Traits that can respond to selection increased fitness through effects on fecundity.

Nevertheless, genetic correlations can cause indirect genetic changes in the phenotypic means of traits that are not directly under selection (Roff 1996). The presence of a genetic correlation between the number of leaves and other traits suggests that this trait has some additive genetic variance, and that it could change at the genetic level in the disturbed habitat and promote correlated changes in both habitats. For example, SLA and the number of leaves have a negative genetic correlation, therefore genetic changes that increase leaf number in the disturbed habitat will result in a decrease in SLA. In the native habitat, a response to selection in SLA will promote a correlated change in leaf number, interestingly in the same direction as in the native habitat. Therefore, correlated responses to selection in traits like SLA and the number of leaves could slow down the process of genetic differentiation between subpopulations, as their correlated response is in the opposite direction as selection for these traits in each habitat. In summary, predictions about the potential response to selection in subpopulations of *Z. fairchildiana* indicate that the phenotypic mean for leaf production should increase in the disturbed habitat, and SLA should decrease in the native habitat, but SLA and the number of leaves could exhibit other correlated responses in each environment.

All these 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 only two methods are available for the estimation of heritabilities for species where information on pedigrees is impractical to obtain (Thomas et al. 2000). Very few studies

have successfully obtained heritability values for populations of long-lived organisms in field conditions (Andrew et al. 2005). Furthermore, there are few studies that have estimated the amount of genetic variation for physiological traits like SLA in natural populations of plants (Ackerly et al. 2000). In this study, SLA, leaf production, and leaf number showed evidence for high heritabilities in the subpopulations of *Z. fairchildiana*. Trees 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). High levels of genetic variability in combination with some spatial genetic structure within subpopulations may have allowed the successful estimation of heritability and genetic correlations in subpopulations of *Z. fairchildiana* (Ritland 2000).

Heritability estimations were not precise, but if they are accurate (i.e. the estimate is close to the actual value in the population), then high levels of heritability ($h^2 \sim 0.6$) and large selection coefficients ($\beta \sim 0.3\text{-}0.4$) 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). 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. Nevertheless, heritability analyses are powerful enough to determine the presence of additive genetic variance in traits under selection like leaf production and SLA, which could therefore diverge at the genetic level between subpopulations from native and disturbed habitats.

Adaptive divergence between habitats

Comparisons of phenotypic means between subpopulations mainly agreed with the predictions in the response to selection, although they likely include variation in phenotypes due to phenotypic plasticity. Phenotypic selection analyses and estimates of the variance/covariance of traits predicted that the phenotypic mean for leaf production should increase in the disturbed habitat, and that SLA should decrease in the native habitat subpopulations. Both predictions were supported by the phenotypic data. Nevertheless, SLA was not different between habitats for juvenile plants, only for adults. This may be the result of maternal effects (see Chapter 3) that could mask genetic variation in this trait in early stages of the life cycle. The phenotypic mean for number of leaves showed no phenotypic divergence between habitats. Lack of differentiation between subpopulations from the two habitats in SLA (in juveniles) and the number of leaves could be the result of a weaker response to selection, but also of correlated responses in these traits, because they have a strong genetic correlation. These comparisons need further exploration, as the statistical power for them was low. Finally, leaflet area showed no differentiation between habitats for juveniles or adults, which was expected from the lack of genetic variation in this trait. These phenotypic analyses can not determine the relative importance of environmental and genetic components on phenotypic variation within and between subpopulations. Nevertheless, the presence of additive genetic variance in leaf production suggest that there is the potential for genetic differentiation between subpopulations for this trait.

Strong genetic differentiation in response to change in the environment is common in plants , even over small spatial and temporal scales (Bone and Farres 2001; Linhart and Grant 1996). This study suggests that there is the possibility of genetic divergence in leaf traits between subpopulations of *Z. fairchildiana* from contrasting habitats. Phenotypic comparisons suggest that differentiation is currently under way in subpopulations, even when populations differentiation at the neutral molecular level is extremely low (low F_{ST}). Local adaptation to differing habitats in life-history traits despite high levels of gene flow has been observed in shrubs and trees (e.g. Aldrich et al. 1998;

e.g. Kittelson and Maron 2001). This study suggests that the environmental changes resulting from anthropogenic activities have the potential to affect the evolutionary potential of populations and alter the genetic structure in this species, as subpopulations locally adapt to differing habitats. This has implications for the definition of evolutionary significant units for conservation, and the extent to which populations can respond to further environmental changes (e.g. global warming). Information from the evolutionary dynamics of populations in human-dominated landscapes is relevant for conservation issues, and it can help understand population responses to rapid and drastic environmental changes in general.

CHAPTER 3

"Genotype-by-environment interactions in seed germination and seedling survival in a rainforest cycad"

ABSTRACT

Genotype-by-environment interactions (GxE) resulting from a 'home site advantage' will promote genetic differentiation between populations, but environmental effects (such as maternal effects) can influence the magnitude and rate of genetic differentiation. In this paper, we explore GxE in seed germination and seedling survival in subpopulations of a rainforest cycad (*Zamia fairchildiana*) from their native and degraded habitats, and the role of maternal effects, light, and water availability on the variation in seed germination between habitats. A reciprocal-transplant experiment in natural environments showed crossing reaction norms for seed germination, and a trend for GxE in seedling survival. In addition, reaction norms for germination had smaller slopes in families originated in the degraded habitat. 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 disturbed habitat, under high light and low water conditions in the greenhouse, that may result in a harsh environment for the desiccation-intolerant seeds of this species. Seed size had little effect on statistical analyses testing for GxE, and separate analyses also suggested that maternal effects of seed size on germination are weak. Seedling size was affected by seed size, and larger seedlings had better survival in the disturbed habitat, suggesting that maternal effects on early seedling performance may be important, but only in one habitat. Seedlings showed the typical shade-avoidance response of angiosperms, as well as and great levels of plasticity for leaflet area in response to light availability. GxE in germination and seedling survival suggest the potential for genetic differentiation between subpopulations of *Z. fairchildiana* from native and disturbed habitats, but the relative role of genetic and environmental effects

on GxE, like maternal effects in seedling survival and other maternal effects not related to seed size, need further exploration.

INTRODUCTION

Genotype by environment interactions (GxE) occur when genotypes have dissimilar responses in phenotype expression across a set of environments. This is evidenced by genotypes having different slopes in reaction norms, i.e. a function of the phenotypes expressed in different environments. GxE can have important implications for the ecological and evolutionary dynamics of populations (reviewed in Agrawal 2001; Miner et al. 2005; Pigliucci 2005). Differential responses of genotypes to environmental variation can result in comparable overall fitness of genotypes and therefore contribute to the maintenance of genetic diversity within populations (Stratton 1994; Sultan and Bazzaz 1993). GxE can affect the rate of phenotypic evolution as well, as they cause the magnitude of phenotypic variation expressed to vary across environments (Bennington and McGraw 1996; Mazer and Schick 1991). Furthermore, if GxE result in norms of reaction that cross, then different genotypes have the highest fitness in each environment, and directional selection could result in genetic differentiation of populations in differing environments. However, if environmental heterogeneity is fine grained compared to the distribution of a population, then GxE can result in selection for greater phenotypic plasticity, instead of genetic differentiation (Schlichting 1986), but the debate about the role of plasticity in promoting or preventing genetic differentiation is ongoing (Price et al. 2003).

In tropical rain forests around the world, anthropogenic activities result in forest environments that can differ substantially from the original habitats (Noble and Dirzo 1997; Tabarelli et al. 2004). Strong directional selection in traits related to fitness in degraded habitats may promote genetic differentiation between populations from these modified habitats and the populations that remain in the native, undisturbed habitats (Palumbi 2001; Reznick and Ghalambor 2001; Stockwell et al. 2003). Many studies have demonstrated that long-lived plants can exhibit strong genetic differentiation at

small spatial and temporal scales (reviewed in Linhart and Grant 1996; Petit and Hampe 2006). GxE can play a significant role on the ability of populations to colonize or persist in 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 germination can represent a strong filter that determines which genotypes can colonize novel environments (Donohue et al. 2005b). Reciprocal-transplant and common-garden experiments designed to test for GxE can provide information about how genotypes respond to habitat degradation, the role of phenotypic plasticity in population responses, and the potential for genetic differentiation between contrasting habitat conditions.

Genotype-by-environment interactions can result from differential fitness of genotypes across environments, but also from environmental-related differences in fitness of individuals. Particularly, maternal effects can greatly affect seed and seedling fitness (Kirkpatrick and Lande 1989). Maternal environmental effects, related to size reserves or other characteristic affecting germination and seedling performance, can result in offspring with higher fitness under the maternal environmental conditions that do not reflect a home site advantage like in crossing reaction norms. Therefore, potential maternal effects need to be considered when estimating GxE in natural populations. These effects are usually removed by rearing mothers in uniform conditions or by including traits like seed size in statistical analyses (e.g. Mazer and Gorchov 1996; Schmid and Dolt 1994). Nevertheless, maternal effects can enhance offspring fitness, and if genetic variation is present in populations, they 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). Consequently, instead of ignoring maternal effects, they need to be evaluated when considering the magnitude and rate of genetic differentiation in natural environments, and even as adaptive responses themselves. Exploring the nature of genotype-by-environment interactions across environments, and the relative role of environmental and genetic effects on GxE, will provide a more complete picture on the potential for genetic

differentiation between populations in differing environments, like undisturbed and degraded habitats.

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 *Z. fairchildiana* are typical of old-growth rainforests in Central America (hereafter referred as the native habitat). *Z. fairchildiana* colonies can also persist in forests affected by selective logging and other human activities (hereafter referred as the disturbed habitat), where environmental conditions in the understory differ substantially from the ones in their native habitat. Analyses on the spatial genetic structure of this species in part of its distribution range in Costa Rica have revealed that genetic differentiation at the neutral molecular level (as estimated by F_{ST} values) is extremely low between colonies of individuals, thus colonies act as subpopulations (see Chapter 1). Nevertheless, subpopulations in disturbed habitats have experienced differing environmental conditions for a few generations, which have promoted changes in life-history traits, the distribution of genetic variation within subpopulations, and the patterns of directional selection for growth traits (see Chapters 1 & 2). Here, we explore the presence of genotype by environment interactions in seed germination and seedling survival between *Z. fairchildiana* subpopulations from native and disturbed habitats. Furthermore, we examine the role of maternal effects, and light and water availability on the variation in seed germination between habitats. To this end, we performed a reciprocal-transplant experiment in natural populations and a manipulative greenhouse experiment with seed families from four subpopulations of *Z. fairchildiana.*

METHODS

Seed families

We chose four subpopulations of *Z. fairchildiana* to collect seed families for the experiments. A seed family consisted of seeds from a single female cone, that usually

bears 50-200 seeds. Most seeds within a cone are viable or contain an embryo, and inviable seeds, that are easily recognizible by their smaller size and smaller weight, were excluded from the experiment. A seed family is a mixture of half- and full-sibs, whose proportions correspond to the number of male individuals that pollinated ovules in the female cone. Two subpopulations were located in native habitats, that consist of old-growth, undisturbed forest within Corcovado National Park (near Sirena Station). The other two subpopulations were located in disturbed forests, near El Tigre station that lies outside the National park, in an area affected by deforestation, logging, hunting, and mining for the last five to six decades.

GxE in germination and seedling survival in natural environments

To test for genotype-by-environment interactions between subpopulations in seed germination and seedling survival, we performed a reciprocal-transplant experiment between subpopulations from native and disturbed habitats. We collected ten female cones in one subpopulation from the disturbed habitat and nine cones in one subpopulation from the native habitat. These cones represented 80 and 100% of the total number of female cones in the subpopulations for the reproductive season of 2004. Twenty seeds per cone were chosen randomly, and ten seeds were planted in two 200x60 cm blocks in each of the habitats. Blocks were placed in sites where canopy cover was similar to the average value for that habitat, and the two blocks were approximately 50 meters apart. Surface litter was removed within the blocks, but the soil environment was not manipulated in any other way.

Seeds were not treated (the outer fleshy layer was not removed), and were planted in 1 cm deep holes in the soil. These conditions simulated natural conditions for germination, as most seeds in the populations remain in the soil surface after dispersal and are not consumed by animals. Within each block, seeds were placed in five rows separated by 10 cm from each other and at least 1 m away from adult *Z. fairchildiana* individuals, to avoid seedling-seedling and seedling-adult competition effects. After six months, germination rates were calculated for each family in each habitat. *Zamia* seeds

have no dormancy, and seeds that did not germinated after six months were considered dead or non-viable. Seed predation was minimal, as is common in this species (personal observation). One year after germination, we 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 occurs.

Light, water, and maternal effects on germination

We explored maternal-environmental effects in natural environments by evaluating the effect of the size of the mother, its light environment (estimated by canopy openness values), and its average seed weight on seed germination, seedling size, and seedling survival. 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 an imaging software (Rasband 2000). Average seed weight for each mother was obtained by weighting to the nearest 0.01 g all seeds in the female cone produced by the mother.

We estimated the effect of seedling size on seedling survival in natural environments in seedlings from the reciprocal-transplant experiment, that were 1 yr old. Additionally, we monitored the survival rate of seedlings >1 yr old for a year in two subpopulations per habitat. We marked all the seedlings (individuals with less than 10 leaflets, excluding germinants from that year) present in a 100 x 20 m transect in the native habitat, or a 50 x 10 m transect in the disturbed habitat (where individual density was higher). For seedlings within the transects, 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).

We performed a manipulative greenhouse experiment to test the hypothesis that light and water availability affect germination rate. We collected six female cones from

each of two subpopulations per habitat in the reproductive season of 2005, for a total of 12 families per habitat. We randomly chose 40 seeds per cone, or the total number seeds in the cone (8 families with 24-36 seeds/cone). Seeds were planted in 40 blocks containing one seed/family in a random order, in pots filled with a special soil mix developed for cycad germination at the Montgomery Botanical Center (MBC). The experiment was carried out in the greenhouse of the MBC in Miami, Florida. Seeds were planted approximately one month after they were dispersed in natural subpopulations. 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.

Seed families were divided between two light treatments, each one applied to a bench in the greenhouse. Within each light treatment, half of the blocks received a low-water and the other half of the blocks a high-water treatment. The high light treatment corresponded to 30% neutral shade and the low light treatment to 90% neutral shade. In natural environments, the disturbed- and native- habitat subpopulations had an average canopy openness of 23% and 16% respectively, thus the high light treatment received a substantially larger amount of irradiance compared to natural conditions. Blocks in the high water treatment were watered to saturate the soil every week, while seeds in the low water treatment were watered every three weeks. Seed germination was monitored for six months, to compare germination rates between families and treatments. Direct effects of individual seed weight on the probability of germination for that seed were considered in this experiment. At the end of six months several measures of seedling size were obtained. *Zamia* species have compound leaves, therefore instead of measuring total leaf length, we obtained the length of the petiole and the rachis (part of the leaf with leaflets). Seedling leaf area was obtained with digital pictures of four leaflets per plant as explained above.

Statistical analyses

To estimate genotype (i.e. family) by environment (i.e. habitat) interactions in seed germination and seedling survival in natural environments we used a linear mixed

ANOVA model. This model had habitat and source population as fixed factors, and family and block (nested within habitat) as random factors. Significance for the fixed factor was evaluated with F-tests, and for the random factors with Wald tests, using REML estimation. GxE 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. A model for the estimation of GxE including seed weight as a covariate was performed to estimate the relative importance of maternal effects (related to seed size) on variation in germination in both experiments.

Maternal effects in natural 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 weight as covariates. In such a model the 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 seed 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 weight 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 and Stern 1998). All statistical analyses were carried out using SPSS (SPSS 2003).

RESULTS

GxE in germination and seedling survival in natural environments

In the reciprocal-transplant experiment there was a genotype or family effect, but not a habitat effect on seed germination (Table 3.1). More importantly, there was a significant GxE or family-by-habitat interaction for seed germination (Table 3.1), i.e.

families from the native habitat germinate better in this habitat than in the disturbed habitat, and vice versa. This can be better visualized in a graph of GxE (Figure 3.1A). Almost all families from the native habitat had a germination rate higher than 50% in the native habitat and lower than 50% in the disturbed habitat. A few families from the disturbed habitat had the same germination rate in both habitats, or even a higher germination in the native habitat, and in general the difference in germination rate between habitats was smaller for these families (the slope of the lines is smaller in Figure 3.1A).

Family 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 2 and 12), and therefore the power of these analyses was low (Table 3.1). The GxE term was marginally significant for seedling survival (Table 3.1). Nevertheless, a GxE graph shows that almost half of families had seedlings that survived better in the habitat where their seeds originated (Figure 3.1B). The rest of the families had few seedlings (and survival rates of 1 or 0 in both habitats) or a higher survival in the opposite habitat where their seeds came from originally. Most of the seedlings from families originated in the native habitat had zero survival in the disturbed habitat, but the opposite was not true for families originated in the disturbed habitat. Therefore, even if statistical tests were not powerful enough to detect significant GxE effects, there is a trend for GxE in seedling survival in these subpopulations of *Z. fairchildiana.* When seed size was included in the GxE analyses, it had no effect on seed germination or seedling survival across habitats, and it did not alter the significance of the main effects.

Figure 3.1. Germination rate (A) and seedling survival rate (B) for families used in the reciprocal-transplant experiment. Solid lines: families originated in the native habitat. Dashed lines: families originated in the disturbed habitat. See Table 3.1 for statistical analyses.
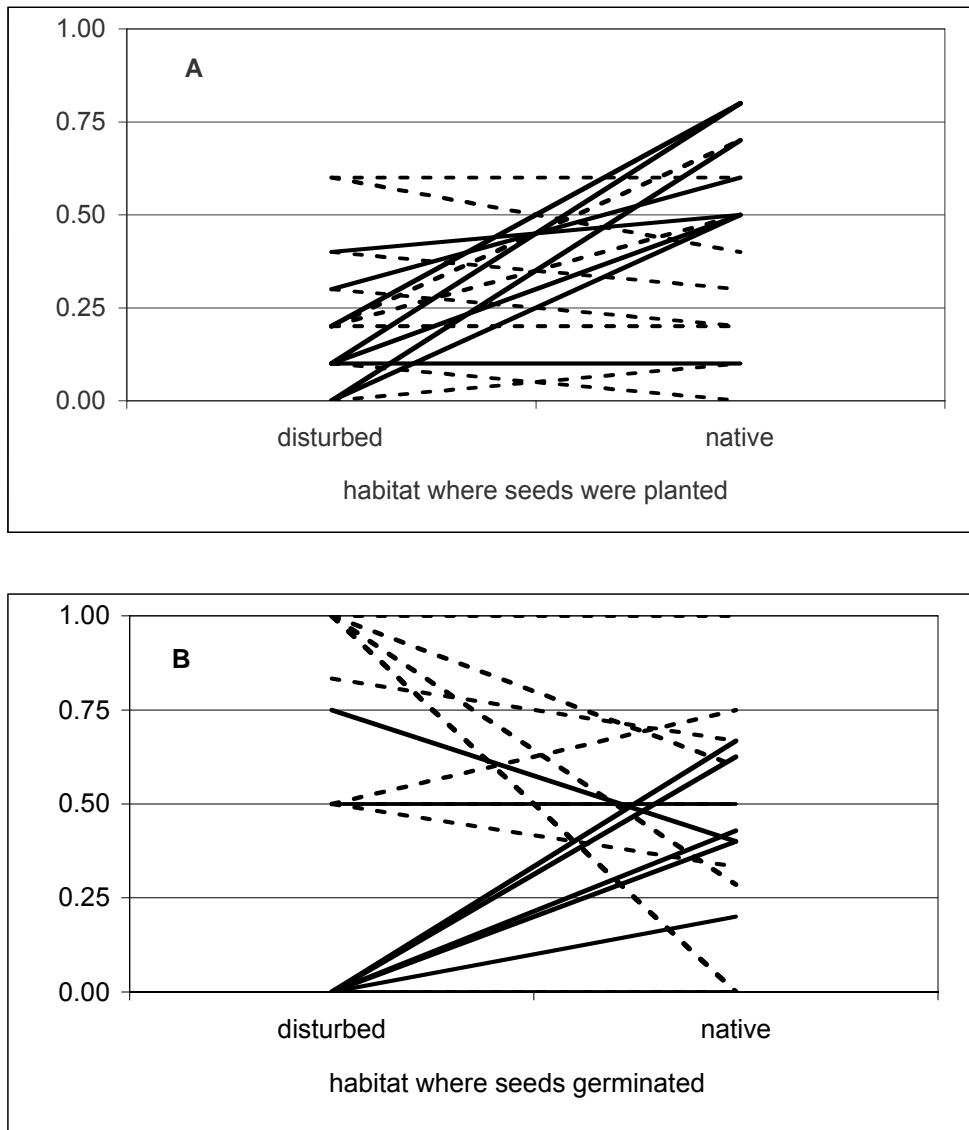
Table 3.1. Genotype, environment, and GxE effects on germination rate and seedling survival in a reciprocal-transplant experiment between native and disturbed habitats. A linear mixed-model was used, with habitat and source population as fixed factors, and family and block nested within habitat as random factors (F and P values from tests are reported).

Source	df	F	P
Seed germination			
Family	16	2.23	0.004
Habitat	1	10.95	0.080
Family x Habitat	18	2.44	0.001
Source population	1	0.69	0.516
Block (Habitat)	2	0.87	0.420
Seedling survival			
Family	16	0.81	0.669
Habitat	1	2.32	0.267
Family x Habitat	18	1.51	0.083
Source population	1	0.51	0.606
Block (Habitat)	2	0.20	0.790

Maternal effects on germination

Maternal effects on germination related to seed reserves were non significant in the reciprocal-transplant experiment in natural environments. When including all families used in the reciprocal-transplant and the greenhouse experiments, seed weight was not associated with mother size or seed number (whole model r^2=0.09, P=0.42 for disturbed-habitat mothers and r^2=0.14, P=0.31 for native-habitat mothers). The coefficient of variation (CV) in seed size of mothers within habitats was 14% and 15% for the disturbed and native habitat, respectively (Figure 3.2A, 3.2B), while the CV in seed number of mothers was 26% and 33%, respectively. Average seed weight of mothers was not significantly different across habitats (GLM F=0.74, P=0.399). Mother size, light environment, or average seed weight did not affect germination rate (Table 3.2). Habitat had no effect on seedling size (Table 3.2). The overall CV in seedling size was 23% for disturbed-habitat families and 29% for native-habitat families (Figure 3.2C). Maternal effects were found in seedling survival, but only in the disturbed habitat. Families with larger seeds had larger seedlings within habitats (Table 3.2), and seedling size affected seedling survival in 1 yr old seedlings growing in the disturbed habitat (Table 3.3).

The results from maternal-effects analyses in the manipulative greenhouse environment were similar to the results from the experiment in natural environments. Seed germination was not affected by mother size, light environment, and seed size (Table 3.2). When the size of individual seeds was considered, seed weight had no effect on the probability of germination for that seed in any light treatment (logistic regression $\beta=0.03$, $P=0.95$ under high light conditions, and $\beta=0.07$, $P=0.76$ under low light conditions), reinforcing the result of lack of size-related maternal effects in germination. The light treatment had a effect on germination, but not on seedling size. Likewise, seed size had an effect on seedling size (Table 3.2). Seedling size was larger in the greenhouse when compared to seedlings in natural environments, but the CV within light treatments were similar to the ones in the reciprocal-transplant experiment, 25% for disturbed-habitat families and 19% for native-habitat families (Figure 3.2D).

Figure 3.2. Seed weight and seedling size (mean ± 2SE) for families used in the reciprocal-transplant experiment (A, C) and the greenhouse experiment (B, D). For seed weight (A, B) open circles represent disturbed-habitat families, and closed circles represent native-habitat families. Seedling size was averaged for individuals within a family growing the native habitat (closed circles in C) or the disturbed habitat (open circles in C); or growing under low light (closed circles in D) or high light (open circles in D) conditions in the greenhouse.

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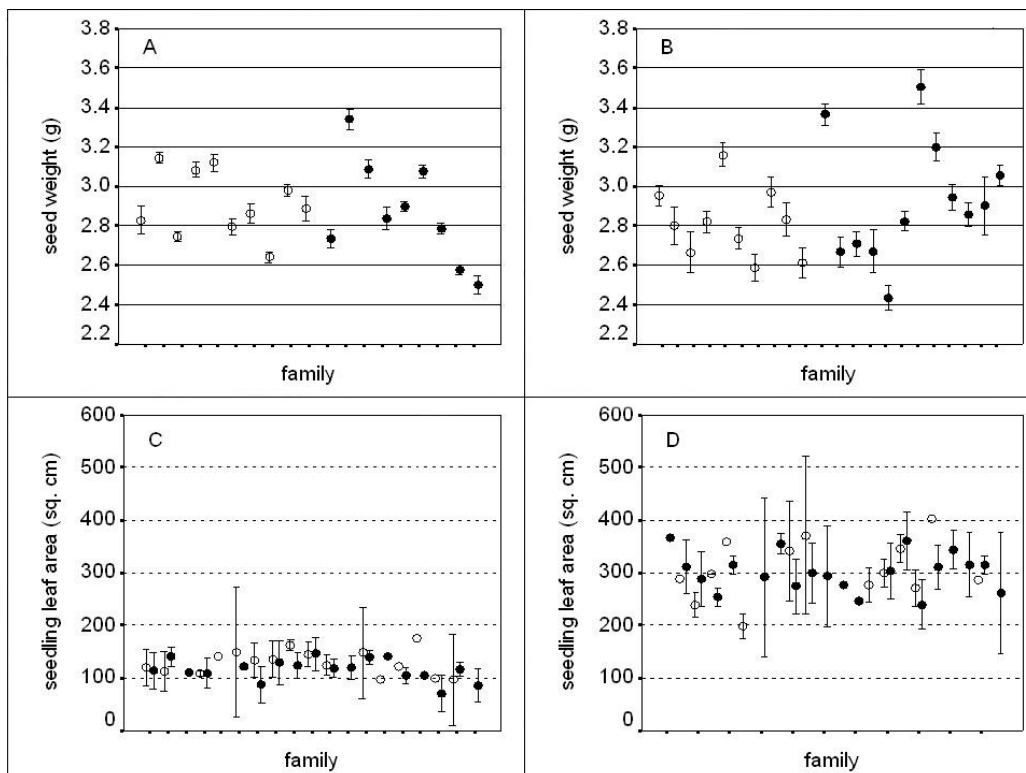
Table 3.2. Maternal effects in germination and seedling size related to mother size, mother light availability, and seed size in a reciprocal-transplant (RTE) and a manipulative greenhouse experiment (MGE). F and P values from F-tests are reported from an ANCOVA with habitat or light treatment as a fixed factor, and mother traits as covariates.

Source	RTE		MGE	
	F	P	F	P
Seed germination				
Mother leaf area	0.60	0.444	1.43	0.239
Mother canopy	1.35	0.254	0.39	0.535
Seed weight	0.04	0.851	0.18	0.677
Habitat or Treatment	7.38	0.010	23.66	<0.001
Seedling size				
Mother leaf area	0.12	0.734	0.04	0.845
Mother canopy	0.52	0.476	0.55	0.466
Seed weight	5.41	0.027	4.07	0.054
Habitat or Treatment	2.22	0.147	0.07	0.794

Table 3.3. Effect of seedling leaf area on seedling survival in natural environments. Survival for seedlings that were 1 yr old was monitored in the reciprocal-transplant experiment, while survival for seedlings that were older than 1 yr was monitored in transects in subpopulations from native and disturbed habitats. Logistic-regression values for the slope (β), and Wald tests of significance are reported.

Habitat	Age	β	d.f.	Z	P
Native	1 yr	0.001	1	0.01	0.959
	>1 yr	0.016	1	3.01	0.083
Disturbed	1 yr	0.034	1	4.98	0.026
	> 1 yr	0.003	1	1.78	0.182

Effects of light and water availability on germination

In the greenhouse experiment, the light and water treatments had no direct effect on germination rate (Table 3.4). However, there was a significant family effect, and more importantly a family-by-light and family-by-water treatment interaction term (Table 3.4). The GxE effects resulted from smaller differences in germination rate between treatments for the disturbed-habitat families (their slope was smaller in Figure 3.3). Seeds from families that originated in the native habitat germinated better in low light, and very poorly under high light conditions (Figure 3.3A). Seeds from families in the disturbed habitat had higher germination in the low light as well, but the difference in germination rate between the two habitats is smaller for these families (Figure 3.3A). Germination under low water availability was low for all families, regardless of the habitat in which they originated (Figure 3.3B). Finally, under high water conditions, the

average number of days to germination for disturbed-habitat families was smaller under high light (85.89 days in high light and 159.61 days in low light), but for native-habitat families the number of days to germination was smaller under low light (132.70 days in low light and 176.88 days in high light).

Table 3.4. Genotype, environment, and GxE effects on germination rate and seedling traits in a greenhouse experiment with light and water treatments using seed families from disturbed and native habitats. A linear mixed-model was used, with treatments and source population as fixed factors, and family and block as random factors (F and P values from tests are reported).

Source	df	F	P
Seed germination			
Family	20	2.64	<0.001
Light treatment	1	0.56	0.455
Water treatment	1	1.29	0.256
Family x Light	23	3.09	<0.001
Family x Water	23	3.32	<0.001
Light x Water	1	0.43	0.512
Source population	3	0.53	0.666
Block	39	0.01	0.998
Seedling petiole length			
Family	18	0.04	0.999
Light treatment	1	14.19	<0.001
Family x Light	21	1.31	0.209
Source population	3	0.94	0.444
Block	39	1.58	0.114
Seedling leaflet area			
Family	18	0.22	0.999
Light treatment	1	0.05	0.826
Family x Light	21	0.96	0.529
Source population	3	0.91	0.457
Block	39	0.02	0.986

As an unexpected result, families compared across light treatments exhibited a plastic response corresponding to the shade-avoidance syndrome of flowering plants. Specifically, seedling leaf petiole was longer in response to low light (Table 3.4). In contrast, seedling leaflet area did not show a plastic response to differing light availability in the greenhouse experiment (Table 3.4). The interaction term between family and light treatment was not significant for these seedling traits (Table 3.4), indicating that there is no genetic variation for these plastic responses in the subpopulations used in this study. Similar to the patterns observed in the greenhouse

experiment, petioles were longer (GLM F=3.88, P=0.0501) and leaflet area was larger (F=6.55, P=0.011) under the low light conditions of the native habitat in seedlings older than 1 yr in a random sample of seedlings from natural environments.

Figure 3.3. Germination rate in the light treatments (A) and water treatments (B) for families used in the greenhouse experiment. Solid lines: families originated in the native habitat. Dashed lines: families originated in the disturbed habitat. See Table 3.4 for statistical analyses.
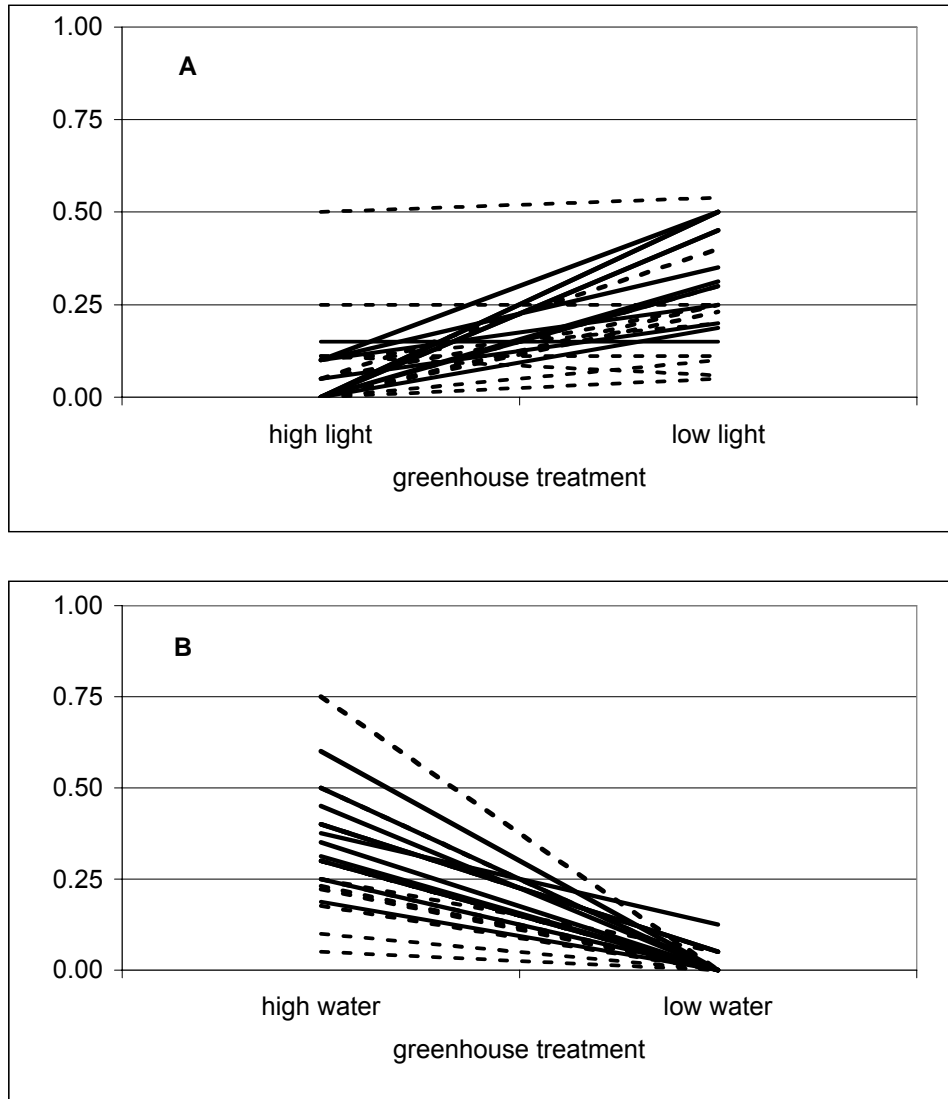
GxE in germination and survival between Z. fairchildiana *subpopulations*

Genotypes (or families) of *Z. fairchildiana* subpopulations from native and disturbed habitats had different germination responses to contrasting environments, explained in part by the different levels of light and water availability. Genotype-by-environment interactions in the reciprocal-transplant experiment in natural environments and in response to light and water treatments in the greenhouse were the result of different slopes of reaction norms of families originated in native versus disturbed habitats. Furthermore, reaction norms of families crossed in the experiment in natural environments, suggesting that genetic differentiation between native and disturbed habitat is possible. Genetic differentiation to local environmental conditions is widespread in plant populations (Linhart and Grant 1996), therefore GxE are expected to be common in reciprocal-transplant experiments. Genotype by environment interactions in seed germination may be explained by a 'home site advantage', where local genotypes have the highest fitness in each habitat because of local adaptation, but maternal effects could also have a role in explaining variation in germination across environments. The potential for genetic differentiation across habitats will depend on the presence of genetic variation in germination responses (e.g. Donohue et al. 2005a), and the role of environmental influences in germination.

Differential responses in germination rate of genotypes from native- and disturbed-habitat families may be associated with the ability of seeds to tolerate desiccation. Seeds from all cycads are recalcitrant, i.e. they have no dormancy and very low tolerance to desiccation (Norstog and 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 and 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 disturbed 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 disturbed 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 and Kursar 2003; Fisher et al. 1991; Turner 1990). Drought has been shown to affect negatively seedling survival in other *Zamia* species (Tang 1990), and is a strong selective agent in early life-cycle stages in tropical rainforests (Engelbrecht and 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.

Light treatments in the greenhouse can not be decoupled completely from the moisture levels experienced by the seeds. 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* families. Nevertheless, there were strong GxE in response to light treatments, and families showed opposing responses in germination date, where disturbed-habitat families germinated earlier under high light and native-habitat families germinated earlier under low light conditions. 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 and 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 disturbed-habitat families in the native habitat or low light conditions in the greenhouse, where desiccation risk should

not be very high. Alternatively, different sets of genes may regulate germination in response to different factors (e.g. Donohue et al. 2005a), 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.

Different responses in germination by native- versus disturbed-habitat families resulted from a clear trend in which disturbed-habitat families showed a less contrasting response across habitats or greenhouse treatments. At the species level, 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 disturbed 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 recalcitrance seeds (Peroni 1995). Nevertheless, little is know 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 and Clay 1994). Increased levels of ABA in disturbed-habitat families 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 disturbed-habitat families in the native habitat.

Maternal effects on seed germination

Genotype by environment interactions in reciprocal-transplant experiments in natural environments suggest the potential for genetic differentiation between

populations. However, maternal environmental effects could affect germination and seedling survival and could mask genetic variation and 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 disturbed habitat. The effects of seed size on germination and seedling survival are well established in long-lived trees (e.g. Bonfil 1998; Campbell 1997; e.g. 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 and Schick 1991; Munir et al. 2001; Paz et al. 1999; Schmitt et al. 1992). 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 disturbed 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 disturbed habitat, as viability selection through young seedlings is very strong in *Zamia* populations. In addition, maternal effects on seedling survival in the disturbed habitat could slow down genetic differentiation between subpopulations, as seedling fitness will be affected by environmentally-induced variation in size.

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 effects 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 and Farrant 1998), but 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 that have dormancy (Pammenter and 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 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.

Phenotypic plasticity in seedling leaf traits in response to light

Interestingly, seedlings in the low light treatment in the greenhouse experiment and in natural environments showed typical signs of etiolation, i.e. an adaptive plastic response under low light in which plants elongate their stem or leaf petioles in an attempt to increase the potential for light capture (Schmitt et al. 2002). This etiolation behavior is common in angiosperms, and is modulated by phytochromes that can sense light quantity and quality levels and induce phenotypic responses. Cycads and other gymnosperms show a more limited ability in shade-avoidance and de-etiolation responses than angiosperms (Mathews 2005; Mathews 2006). Most cycad species inhabit open habitats, however *Z. fairchildiana* and a few other species of *Zamia* are adapted to survive under the deep shade of the understory of tropical rainforests. Tropical light-demanding species usually have large levels of plasticity to the light environment (Chazdon et al. 1996), and it is possible that rainforest *Zamia* species have retained high levels of plasticity and a shade-avoidance behavior. There was no GxE in this shade-avoidance response in families of *Z. fairchildiana*, indicating that there is no genetic variation for the plastic response in the subpopulations. This lack of genetic

variation in the plastic response in families coming from two different habitats may also be explained if the shade-avoidance behavior is an ancestral state for all *Zamia* species that has not been lost in rainforest species like *Z. fairchildiana.*

Leaflet area did not show phenotypic plasticity to light environments in the greenhouse experiment. Leaflet area has no genetic variation in *Z. fairchildiana* subpopulations in either habitat (see Chapter 2). Variation in seedling leaflet area is therefore mostly environmental, and may be related to variation in light levels. Plastic responses in leaflet area may enhance light capture in seedlings, and can affect their survival, at least under some circumstances. Variation in leaf surface area in response to heterogeneous light environments is common in rainforest plants (e.g. Evans and Poorter 2001; Montgomery 2004). Variation in leaflet area also affects the fitness (fecundity) of adults (see Chapter 2), and it would be interesting to explore the levels of plasticity in this trait at the adult stage. Curiously , environmentally-induced (given no genetic variation for this trait) differences in leaflet area were not observed for either seedlings or adults (see Chapter 2). Leaves with long life-spans, like leaves of *Zamia* species, usually show low levels of plasticity (Clark et al. 1992; Kursar and Coley 1999). Shade avoidance responses and the extent of phenotypic plasticity in seedling and adult traits should be further explored in populations of *Z. fairchildiana,* particularly as they may influence the potential for genetic differentiation between native and disturbed habitats.

Adaptive divergence between habitats

The results from the experiments in this study suggest that genetic differentiation between *Z. fairchildiana* subpopulations from native and disturbed habitats is possible, given genetically-based differences and GxE in germination and seedling survival. Nevertheless, the magnitude and rate of genetic differentiation will depend on the strength of maternal effects on fitness of early stages in this population. Maternal-environmental effects related to seed reserves seem to be weak, but other maternal effects could explain GxE in germination or mask the genetic variation in germination

responses. 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 rapid genetic differentiation among populations (Petit and Hampe 2006). If genotypes that are able to perform better under the modified environmental conditions (e.g. because higher tolerance to desiccation) of the disturbed habitats produce more seeds, or seeds that recruit better, then subpopulations in the disturbed habitat may diverge genetically from the subpopulations in the native habitat. Genetic differentiation between native- and disturbed-habitat populations has been detected at the seedling stage in other rainforest species in fragmented habitats (Aldrich et al. 1998). Long-term reciprocal-transplant experiments and detailed evaluations of environmental effects on GxE will provide further evidence of the potential for genetic differentiation in *Z. fairchildiana* subpopulations in life-history and adult traits.

GENERAL CONCLUSIONS

The results from this study suggest that habitat degradation can have important effects on the evolutionary dynamics of *Z. fairchildiana* populations. Habitat loss and fragmentation have not been too severe for this species, but many colonies of individuals (subpopulations) persist in forest habitats affected by human activities that differ considerably in variables like canopy cover from the native habitat for the species. The subpopulations of *Z. fairchildiana* in degraded habitats do not show the typical consequences of habitat fragmentation, like drastic reductions of population size and high degree of isolation, at least in the short-term (few generations after habitat disturbance). The levels of genetic diversity in molecular markers suggest the lack of extreme bottlenecks (that result in loss of rare alleles), or genetic isolation, as evidenced by the lack of genetic structure in neutral molecular markers. In addition, subpopulations in the disturbed habitat do not show signs of decreased reproductive output and recruitment, as usual in tropical trees in fragmented and degraded habitats, although seed germination and seedling survival were lower in the disturbed habitat. Conversely, individuals in disturbed-habitat subpopulations seem to have a 'faster' life-history with rapid growth and high investment in fecundity, and moderate rates of germination and seedling survival. The long-term consequences of these life-history changes remain to be evaluated, and whether this life-history results in larger or smaller population growth rates will depend on the patterns of adult mortality. Faster life-histories are usually associated with high adult mortality and lower life-span, and this could have negative consequences for population growth rate in subpopulations in the degraded habitat. Unless habitat degradation has a major negative impact on adult mortality/longevity, it seems that it has not affected severely the demographic viability of *Z. fairchildiana* subpopulations.

In contrast to the lack of severe negative demographic effects (at least in the short-term), habitat degradation appears to have significant influences on several aspects of the evolutionary dynamics of subpopulations of *Z. fairchildiana.* Life-history differences between native and disturbed-habitat subpopulations seem to result in a

weaker spatial genetic structure and higher levels of inbreeding in the disturbed-habitat subpopulations. Furthermore, environmental changes in the disturbed habitats, particularly higher light availability and increased probability of desiccation, are associated with important differences the patterns of selection and genotype-by-environment interactions (GxE) between subpopulations from native and disturbed habitats. Average fecundity in the disturbed-habitat subpopulations may not be decreased by habitat degradation, but selection and GxE analyses suggest that not all genotypes have the same probability of recruitment. Particularly, differential genotype performance in light environments will have important implications for the genetic composition within subpopulations, the spatial genetic structure, and the evolutionary potential of the whole population.

Furthermore, the results suggest that habitat degradation has the potential to promote adaptive genetic differentiation between native and disturbed habitat subpopulations of *Z. fairchildiana*. Habitat degradation generated strong selective pressures for this species, and subpopulations can respond to these selective pressures. In particular, light seems to be an important agent of selection for the evolution of genetic differences in traits like leaf production, but other environmental factors affecting desiccation tolerance may be important agents of selection as well. The implication of a response to selection in a trait like leaf production may be far reaching, if genetic differentiation between subpopulations in this trait is associated with genetic divergence in the whole life history strategy. GxE on early performance, and in relation to light and water availability, further suggested that different genotypes may have the highest fitness in native versus disturbed habitats, which supports that adaptive genetic differentiation may take place in response to habitat degradation. Nevertheless, environmental effects, and particularly maternal effects, may affect the rate of genetic differentiation between subpopulations in the two habitats. Evaluating the relative importance of genetic and environmental effects in GxE will likely provide interesting information on the interaction between directional selection and maternal effects and other forms of phenotypic plasticity on the process of genetic differentiation between populations. The strength of spatial genetic structure and inbreeding within

subpopulations may also affect the action of selection and the potential for evolutionary changes in this species. All these finding suggest that the response to habitat degradation in *Z. fairchildiana* populations involves a complex interaction of ecological, genetic, and evolutionary factors.

In addition to differences in adult survival or longevity, and the effects of environmental effects and phenotypic plasticity on genetic differentiation, several issues emerge as crucial for a wider understanding of population responses to habitat degradation in *Z. fairchildiana.* The actual consequences of differences in the spatial genetic structure on effective population sizes or the action of viability selection may reveal interesting interactions between patterns of genetic variation and population fitness. The causes of higher inbreeding in the disturbed habitat and its consequences, like inbreeding depression, could also reveal important aspects of the effect of habitat degradation on overall population fitness. Particularly, large variation in fecundity rates among individuals and across time (e.g. a few dominant reproducing individuals) and the consequent reduction in effective population size, may affect the evolutionary potential and potential for genetic differentiation by drift in subpopulations. More accurate estimations of the heritability of ecologically-relevant traits will contribute to evaluating the effects of habitat degradation, via drift or selection for example, on the levels of genetic variation within subpopulations. Genetic differentiation may also be affected by maternal environmental effects, but another interesting possibility is that genetic maternal effects could evolve in the disturbed habitat, wh ere they seem to increase in importance. Long-term experiments will be required to evaluate maternal effects and other forms of phenotypic plasticity and their impact on population differentiation, but estimating the degree of genetic divergence shown by quantitative traits (Q_{ST}) in adults is also possible using molecular markers. Finally, the extent of gene flow in genes underlying ecologically-relevant traits, particularly the ones under selection (which may not be equivalent to the extent of gene flow showed by molecular markers), will have a major impact on the potential for genetic differentiation between subpopulations, and could also have the potential for creating outbreeding depression between diverging subpopulations in contrasting habitats.

Only long-term studies in a long-lived plant like *Z. fairchildiana* will determine that subpopulations of *Z. fairchildiana* are in the process of adaptive genetic differentiation in response to habitat degradation, but this study shows that habitat changes can have major impacts in several aspects of the ecology, genetics, and evolutionary dynamics of populations. The study provides information on particular environmental factors, phenotypic traits, and aspects of the life history of the species that are relevant in population responses to habitat degradation. It demonstrates as well that anthropogenic habitat changes can result in major selection events, that can have important short-term consequences for several aspects of population structure and dynamics, which should be of interest in conservation biology. Adaptive evolution will allow *Z. fairchildiana* populations to persist in degraded habitats, but it will alter the genetic structure of the population, and have other consequences for the evolutionary dynamics of the populations that need further examination. From a conservation biology perspective these are interesting issues to address in a world where most ecosystems are under the pressure of human activities and most species of conservation interest are long-lived like cycads.

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VITA

Maria Cristina López-Gallego was born in Mérida, México, on November 19-1974, daughter of Teresa Gallego-Cadavid and Hernan López-Tello. She lived in México for six years, and then moved to Medellín, Colombia, where she started her education. She received her BSc in 1999 from Universidad de Antioquia in Colombia, with an emphasis in Botany. During her college training, she participated in research on tropical forest diversity, and began to be interested in conservation issues. She then focused on population biology, particularly with cycads as her study system. Her undergraduate thesis explored some aspects of population ecology in populations of a dry forest cycad in a fragmented landscape in Colombia. After obtaining her bachelor's degree, she participated in a research training program in conservation biology, sponsored by the Colombian branch of Wildlife Conservation Society (Fundacion EcoAndina). Within this program she participated in research dealing with montane forest function and restoration in the Colombian Andes. In 2001 she was accepted as a graduate student in the conservation biology program at University of New Orleans. For her PhD she focused again on population biology and explored the effects of habitat degradation on the ecological and evolutionary dynamics of populations of a rainforest cycad in Costa Rica. During her PhD she also obtained experience in teaching, in courses of general biology and evolutionary ecology. Her research with cycad conservation has lead to the establishment of collaborations with US and international conservation institutions like the Montgomery Botanical Center and the IUCN Cycad Specialist Group.