2008-12-18

The Importance of Sexual and Clonal Reproduction for Population Dynamics in the Understory Herb Calathea marantifolia (Marantaceae)

David P. Matlaga
University of Miami, dmatlaga@bio.miami.edu

UNIVERSITY OF MIAMI

THE IMPORTANCE OF SEXUAL AND CLONAL REPRODUCTION FOR POPULATION DYNAMICS IN THE UNDERSTORY HERB *CALATHEA MARANTIFOLIA* (MARANTACEAE)

By

David P. Matlaga

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2008

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

THE IMPORTANCE OF SEXUAL AND CLONAL REPRODUCTION FOR POPULATION DYNAMICS IN THE UNDERSTORY HERB *CALATHEA MARANTIFOLIA* (MARANTACEAE)

David P. Matlaga

Approved:

_____
Carol Horvitz, Ph.D.
Professor of Biology

_____
Terri A. Scandura, Ph.D.
Dean of the Graduate School

_____
Barbara Whitlock, Ph.D.
Assistant Professor of Biology

_____
David Janos, Ph.D.
Associate Professor of Biology

_____
Hans de Kroon, Ph.D.
Professor of Biology
University of Nijmegen

MATLAGA, DAVID (Ph.D., Biology)

The Importance of Sexual and Clonal Reproduction for Population Dynamics in the Understory Herb Calathea marantifolia (Marantaceae) (December 2008)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Carol Horvitz.
No. of pages in text. (130)

Many understory plants regularly produce both sexual and clonal offspring, but it is unclear how variation in light availability influences sexual and clonal offspring production, demographic performance and contribution to population dynamics. I addressed these issues by studying the Neotropical understory herb *Calathea marantifolia* (Marantaceae) across a range of light environments in Costa Rica.

In a field experiment, I investigated how demographic performance was influenced by light availability by planting seedlings and clonal offspring into the centers of tree-fall gaps, the edges of tree-fall gaps or the shaded understory. Both seedlings and clonal offspring grew best in tree-fall gap centers. However, the two kinds of offspring differed in their survival response to light; seedlings survived best in tree-fall gap centers whereas clonal offspring survived best in shaded understory. Overall, seedlings were more sensitive than clonal offspring to light levels.

To study the demographic consequences of physiological integration between parent plants and their clonal offspring, I combined an isotope tracing study with a severing experiment, both performed in natural populations. Little water was transported between offspring and parent plants and the flow of water was predominantly from the parent to clonal offspring that had not yet rooted in the soil. Severing the connections linking parents to offspring reduced the demographic performance of offspring but not of

parents. The effect on offspring was more dramatic when severing occurred prior to rooting rather than post-rooting. Clonal offspring benefited from integration with their parent but this benefit decreased after offspring had rooted.

I investigated the demographic cost of sexual reproduction within the context of natural variation in light availability using a field experiment, in which plants were assigned to either low, medium or high sexual reproductive effort. The demographic consequences were evaluated for both the plant and its subsequently produced clonal offspring, and light availability was used as a covariate in all analyses. There was no difference in parent plant survival, growth or future reproduction among reproductive effort treatments. In general, clonal offspring had similar growth and survival among treatment groups. However, clonal offspring size was affected by the treatments initially, but over time this affect disappeared. Light availability did not influence the demographic performance of plants or their clonal offspring in this experiment. These results do suggest that a trade-off does not exist between sexual and clonal reproductive modes in this species.

To understand the contribution of sexual and clonal reproduction to population dynamics in different light environments I established study plots in high and low light environments. In these plots I collected data on the vital rates of plants at two censuses per year, over 3 years. Here, I develop a framework to use these data to parameterize a new size-structured integral projection model in which the integral kernel contains two different types of recruitment. To show how this works, I utilized a single census interval as an example. The model presented here is time-invariant but future models will incorporate the natural seasonality. The population growth rate was faster in high light

than in low light and a life table response experiment revealed that this difference in population growth was primarily due to improved survival and growth at large sizes as well as increased clonal reproduction in high light. By removing reproductive modes from the model, I found that sexual reproduction contributes more to population growth than clonal reproduction. When only sexual reproduction is included in the model population growth rate is fastest in high light environments. By contrast, when only clonal reproduction is included in the model population growth rate is fastest low light.

Acknowledgements

I am very grateful to the members of my dissertation committee for helping me complete this work. None of this would have been possible without the constant encouragement I received from my advisor, Carol Horvitz. The writing was greatly improved by the careful editing of David Janos and Barbara Whitlock. Hans de Kroon encouraged me to place my results in a larger context. Guillermo Goldstein was a member of my dissertation committee up to the time of my defense and he provided insightful comments during all the stages of my project.

My labmates Lucero Sevillano, John Cozza, Robert McElderry and Carlos Garcia-Robledo gave me helpful comments in the planning and design phase and they improved my chapters by providing insightful suggestions. Additionally, Frans Juola and Robert McElderry, provided helpful comments on chapters of this dissertation as well. I appreciate the support of the staff in the Department of Biology, especially Beth Goad, Carolina Fernandez, Rosa Taveras, Rob Burgess and Francis Smith.

Data collection in the field could not have been completed without help from Tanya Hawley, Melati Kaye, John Chastain, Lindsey Bowerman, Isabelle Boittin, and Maga Gei. I received help with the isotope analyses in Chapter 2 from Pattrick Ellsworth and Amartya Saha. Jack Fisher allowed me to use space in his lab and mentored me in making the anatomical observations presented in Chapter 2. Plant material dissected for the anatomical observations presented in Chapter 2 was collected at Fairchild Botanical Garden. Jay Horn took the photographs presented in Figure 2.2. Erin Kuprewicz provided beautiful illustrations used throughout the dissertation.

In Costa Rica I was fortunate to receive support and friendship from a community of researchers in the Osa Penninsula, including Mike Boston, Reynaldo Aguilar Fernandez, Cathrine Bainbridge, Rayner Araya Marchena, Angela Braun, Jane Carlson, Cristina Lopez-Gallego, Charlie Foster, Larry Gilbert and Erika Deinert. I am grateful to the Ministerio de Ambiente y Energía in Costa Rica for granting permits (INV-ACOSA 013-39) for me to conduct my research. At Sirena Biological Station, Larry Gilbert and Erika Deinert provided logistical support. At La Selva Biological Station, Carlos Garcia-Robledo and Erin Kuprewicz provided logistical support and vital field supplies.

I was fortunate to receive funding from several sources. The initial three years of my graduate studies were funded by the James W. MacLamore Fellowship. In my fifth year I was supported as a Science Made Sensible Fellow by an NSF grant. Additionally, I received funds from the Curtis Plant Sciences Scholarship, Center for Latin American Studies, Organization for Tropical Studies and Heliconia Society International. I was supported during the writing and analysis phase by a University of Miami Cooper Fellowship awarded to Carol Horvitz.

My friends and family provided invaluable support during this process. Most importantly my wife, Tanya Hawley, spent countless hours helping me in the field, editing my chapters and generally preventing me from getting discouraged during the difficult phases of my project. My parents, Helen and Stephen Matlaga, always provided support and encouragement during my academic progress. Thanks to my brother and sister-in-law, Doug and Mari Matlaga, for listening to lengthy descriptions of my life in the field.

TABLE OF CONTENTS

List of Tables

Chapter I **page**

Chapter II

Chapter III

Chapter IV

List of Figures

Chapter I **page**

Chapter II

Chapter IV

Chapter V

Chapter I

Growth and survival across a gap-understory gradient: contrast in performance of sexually vs. clonally produced offspring[1]

SUMMARY

Sexually and clonally produced offspring may respond to environmental heterogeneity by growing and surviving at different rates. In forest understories the availability of light ranges from low in shaded closed canopy to high in tree fall gaps. We experimentally investigated the growth and survival of both types of offspring in three treatments, gap centers, gap edges, and in the shaded understory, over 16 months. We expected the demographic performance of both types of offspring to be highest in the centers of gaps and lowest in the shaded understory. However, we expected seedlings to be more sensitive to the gradient in light (larger difference in growth and survival between light levels) than clonal offspring because of their small size and lack of connection to maternal resources. Both offspring types grew fastest and obtained their largest sizes in gap centers. Contrary to our expectations, offspring types differed in which light conditions favored highest survival. Seedlings survived best in gap centers while clonal offspring had their highest survival in the shaded understory. In agreement with our hypothesis, survival and growth of seedlings were more sensitive to light availability, showing a large difference in growth and survival between light levels, compared to clonal offspring.

[1] Coauthor: Carol C. Horvitz

BACKGROUND

Many plants employ a mixed reproductive strategy where recruitment occurs by both sexual and clonal offspring. A longstanding explanation for the production of both reproductive modes in one life cycle is that sexual and clonal offspring are adapted for different ecological situations (Maynard Smith, 1978; Burt, 2000). Forest understories are environments where this mixed reproductive strategy is common and there is a gradient in the availability of light from low in shady closed canopy understory to high in tree fall gaps (Bierzychudek, 1982). It remains unclear how the demographic performance of sexual and clonal offspring changes along this gradient. Much attention has been given to characterizing the performance of sexual propagules (seeds and seedlings) at different points along the gradient in the availability of light; however, far less attention has focused on comparing the performance of sexual and clonal offspring (but see Greig, 1993).

In this dissertation clonal reproduction refers to the production of clonal propagules that have their own vital rates (rates of survival, growth and reproduction), distinct from those of their parents, even if they retain physiological connection to their parent for some time (Abrahamson, 1980). Additionally, we use the term offspring in the general sense, meaning a propagule that can be the product of either sexual or clonal processes.

It has long been recognized that clonal offspring can establish across a broader range of microsites and tolerate more extreme conditions than seedlings (Harper, 1977; Abrahamson, 1980). However, beyond this generalization little progress has been made

to improve our understanding of the mechanisms underlying patterns of establishment of sexual and clonal offspring across important abiotic gradients. For example, it is unclear if both types of offspring have the same 'optimal' location along a gradient or if they respond to temporal changes in abiotic conditions at a similar rate.

In the forest understory the availability of light is an important and dynamic resource gradient that may differentially affect the production of seeds and clonal offspring as well as their relative contribution to population-level recruitment (Abrahamson, 1975, 1980; Douglas, 1981; Bierzychudek, 1982; Cook, 1985; Kullman, 1992; Eriksson, 1993; Mandujano et al., 1998). Seedlings predominate in high light conditions in newly formed tree fall gaps, whereas clonal offspring become more abundant once the canopy has closed (Hughes et al., 1988; O'Dea et al., 1995; Kanno and Seiwa, 2004). Previous studies report patterns of standing abundance and do not address process; therefore they confound propagule production with performance. Additionally, how canopy closure influences the relative demographic performance of sexual and clonal offspring is poorly understood.

Sexual and clonal offspring begin life with contrasting anatomical and physiological attributes, which may contribute to their differential tolerance for abiotic conditions. Seedlings, which rely on few seed reserves and their ability to acquire new carbon from photosynthesis, may initially be at a disadvantage in resource poor environments compared to clonal offspring that receive resources from their parent (Hartnett and Bazzaz, 1983; Pitelka and Ashmun, 1985; Salzman and Parker, 1985). In addition, clonal offspring typically begin life at a more advanced stage than sexual offspring (i.e. Nishitani and Kimura, 1995). These differences may explain why clonal

offspring often have higher survival (Tukington et al., 1979; Howe and Snaydon, 1986), make up a greater proportion of recruits (Eriksson, 1985; 1989; Eckert, 2002), and contribute more to population dynamics (Grant and Grant, 1980; Cook, 1985; Huenneke and Marks, 1987; Eriksson, 1988, Eriksson and Bremer, 1993; Silvertown et al., 1993) than do sexual offspring.

Our study species, *Calathea marantifolia* Standley, occurs in Neotropical forests, where tree fall gaps with high light availability, are rare in space and time (Collins et al., 1985). Because light availability within tree fall gaps varies with gap size, shape, orientation, and time (Chazdon and Fetcher, 1984; Fetcher et al., 1985), the gradient of light availability from gap center to shaded understory also varies. High light availability in tree fall gaps typically increases photosynthetic capacity (Sims and Pearcy, 1991), and carbon gains (Pearcy, 1987). Plants in high light environments often have increased growth, survival (Chazdon et al., 1996) and sexual reproductive output (Kudoh et al., 1999). It is well documented that the growth and survival of seedlings increases with light availability (e.g. Sork, 1987; Bazzaz and Wayne, 1994; Balderrama and Chazdon, 2005).

The ability of a seedling to acclimate to a new light environment may be influenced by its initial light environment (Pompa and Bongers, 1991; Huante and Rincón, 1997; Huante et al., 1998). In our study species, *C. marantifolia*, previous physiological studies of photosynthetic capacity of leaves showed that both light saturation levels and rates of maximum net assimilation vary according to the light environment where that leaf was produced (P. Rundel, *personal communication*). Because of this, we investigate the influence of initial light environment on growth and

survival of offspring, as well as on the time needed for them to acclimate to a new light environment.

We propose that clonal offspring are demographically less sensitive to light availability than seedlings. We thus predict that survival and growth rates of clonal offspring will be more similar across light environments than survival and growth of seedlings. We tested this hypothesis for *Calathea marantifolia* (Marantaceae), an understory herb that regularly produces clonal offspring as well as seeds. In this species, like many others (Abrahamson, 1980; Cook, 1985) clonal offspring begin life at a larger size than seedlings and receive some resources from their parent through vascular connections (Chapter 2). We planted offspring into high (tree fall gap centers), medium (tree fall gap edges) and low (shady understory) light availability treatments, allowing us to compare the performance of clonal offspring and seedlings, across a gradient in the availability of light, analyzing: (1) cohort survivorship; (2) expected survival time; (3) final survival level; and (4) relative growth rates. Although many environmental variables may vary across the gradient from the centers of tree fall gaps to shaded understory (e.g. air and soil temperature and moisture availability), here we focus our interpretation to light availability and its influence on offspring demographic performance.

Overall, this dissertation examines the relative importance of sexual and clonal reproductive modes for population dynamics in *C. marantifolia*. In Chapter 2, we examine the extent and timing of clonal integration. In Chapter 3, we evaluate the potential trade-off between sexual and clonal reproduction. In Chapter 4, we construct an

integral projection model to understand how each type of reproduction contributes to population growth across the understory light gradient.

METHODS

STUDY SYSTEM—*Calathea marantifolia* (Marantaceae) is a Neotropical rhizomatous herb (Kennedy, 1978), described as a forest edge species (Cooley et al., 2004), although it occurs in deeper shade than several other *Calathea* species (Horvitz and Le Corff, 1993). Typically *C. marantifolia* is 0.7- 2.0 m in height and found in wet to semideciduous forests from central Ecuador to Honduras (Kennedy, 1978).

Calathea marantifolia has a relatively short sympodially branched rhizome (Kennedy, 1978). Excavations found no evidence of persistent connections to other aerial shoot systems via these rhizomes and therefore throughout this dissertation an individual is defined as an isolated aerial shoot system. An adult plant can have 1-5 aerial shoots, each with several basal and cauline leaves (Kennedy, 1978). In the populations within secondary forest at Sirena and La Selva Biological Stations adult plants typically have 1-2 aerial shoots with inflorescences and 1-2 without inflorescences (D. Matlaga, personnel observation). All the aerial shoots of an individual are unbranched and arise in a more or less compact clump from the rhizome (Cooley et al., 2004).

A reproductive episode of *C. marantifolia* begins with the production of a pedunculate inflorescence from the terminal node (Fig. 1.1A & F) of a shoot during the rainy season. After the infructescence has senesced, a shoot forms in the axil of the

subtending leaf (Fig. 1.1B). This shoot enlarges into a structure resembling a bulblet (a bud with thickened scales borne above ground; Harris and Harris, 2003), and later produces foliage leaves and roots while still attached to the parent plant (Fig. 1.1G-J). Between 1 and 10 months after the bulblet has formed, it contacts the ground and its roots penetrate the soil surface (Fig. 1.1D; Chapter 3). The connection to the parent can remain for several months (Fig. 1.1D; Chapter 3). Nearly all (> 98%) plants that produce an inflorescence subsequently produce a bulblet (Chapter 3). This pattern, in which a clonal offspring is produced after sexual reproduction and on the same aerial stem as the inflorescence, is found among many other members of the Zingiberales (Marantaceae- e.g. *Calathea donnell-smithii*; Costaceae- e.g. *Costus scaber*; Zingiberaceae- e.g. *Alpinia purpurata*).

Throughout the dissertation we refer to bulblets as clonal offspring regardless if they are connected to their parent. Harper (1977) argued a genetic perspective that offspring can only be produced sexually and that the production of clonal propagules is a type of growth, not reproduction. Instead we take a demographic perspective, similar to Abrahamson (1980), defining reproduction as the production of a unit with vital rates (e.g. growth, survival) that are separate from those of the parent. Using this approach the production of clonal bulblets is reproduction and bulblets are offspring because their probability of survival and growth differ from that of their parent plant (Chapters 2, 3 and 4).

The study population was located near Sirena Biological Station, Corcovado National Park (8º28’49’’N, 83 º35’22’’W), on the Pacific coast of Costa Rica. The region is described as tropical wet forest receiving >5 m of precipitation annually

(Hartshorn, 1983). Over 85% of the rain falls during May-November and the dry season extends from December-April (Sirena Biological Station, unpublished data). Our study sites were located in secondary forest, in areas which had been cattle pasture before the park was created in October 1975 (Phillips, 1989).

SITE AND OFFSPRING SELECTION—In June 2005 we located seven sites with recent tree fall gaps (>30 × 30 m) in the secondary forest. Of these, we randomly chose four sites, in which we experimentally planted offspring (seedlings and clonal offspring), assigning them randomly to one of three positions; center of the gap, edge of the gap, and adjacent shaded understory (>15 m from gap edge). We randomly chose two areas within 0.5 km of each site to serve as source areas for seedlings and clonal offspring for our experiment. In each source area, seedlings and clonal offspring were located at random, individually marked, and removed for transplantation to experimental sites. Prior to removal, we estimated the amount of light available in the source location for each individual, using the canopy scope technique (Brown et al., 2000). Because propagules were found at random they primarily came from the most common understory environment, shaded closed canopy and a minority were from more open tree fall gaps. We measured all leaf lengths and estimated leaf area of each offspring using a regression relationship between leaf lengths and area specific to *C. marantifolia* at Corcovado (Horvitz and Le Corff, 1993).

PLANTING PROTOCOL AND CENSUSES—We planted seedlings and young clonal offspring (still connected to their parent) into gap centers, gap edges and the shady understory in a block design (n = 4 blocks; site = block). We chose to move the parent plant with the clonal offspring together as a unit, without harming the parent-offspring

connection, because supplementation of the propagule from the parent is one of the main hypothesized advantages of clonal reproduction. We emphasize that the parent plants in the experiment are not cuttings. The entire plant was removed from the ground, including below-ground rhizome and above-ground shoot(s). Our intent was to use clonal and sexual offspring of comparable starting stage, therefore, we chose to use small seedlings (initial leaf area = 9.2 cm^2, SE $\pm$ 0.3) and young clonal offspring still connected to their parent plants (initial leaf area = 549.2 cm^2, SE $\pm$ 22.4; Fig. 1.1C). For each offspring type, this is the first stage where leaves are present.

Seedlings were uprooted from the ground in a plug of soil using a metal cylinder (7.5 cm diameter, 8 cm depth). Parent plants, with attached young clonal offspring (Fig. 1.1C), were excavated using a shovel. After the root-ball of the parent plant (approximately 40 × 40 × 35 cm) was removed from the ground, excess soil was shaken from the roots. Parent plants with attached clonal offspring were carried to the experimental site on a stretcher.

To standardize offspring density across light level treatments, seedlings and clonal offspring were planted in a grid design. In each light level treatment within a site (e.g. gap center, gap edge, or understory) a 3 m × 10 m grid was established, into which 35 seedlings and 20 parents with attached clonal offspring (Fig. 1.1C) were planted (4 sites × 3 light level treatments = 420 seedlings, 240 clonal offspring total). The sample size was not balanced because data from a separate study (Chapter 4) suggested that mortality of seedlings is higher than that of clonal offspring. Therefore, to be able to continue to estimate leaf area over the year-long study period we had to initially include more seedlings than clonal offspring in the experiment. Plants were removed from

source sites and planted into an experimental site on the same day. If it had not rained at least two days preceding the planting, the newly planted propagules were watered with 600 ml of water. This occurred only in site 3.

Survival was monitored and leaf lengths were measured on all individuals 90, 199, 310, 398 and 480 days after planting. Leaf area was estimated as described above.

TRANSPLANT SHOCK EXPERIMENT—To examine whether uprooting seedlings and parent plants from the soil influenced offspring performance, we located 100 seedlings and 60 parent plants with young clonal offspring, in randomly chosen locations near a 200 m transect in secondary forest. Plants were randomly assigned to one of two treatments, uprooting and replanting or no disturbance. Seedlings and parent plants were uprooted from the ground using the same methods as above, and planted back into their original hole. Offspring leaf area and survival were monitored as described above.

LIGHT AVAILABILITY—We quantified light availability in each treatment using hemispherical photographs taken on Aug 6-27, 2006, (398 days post-planting) at 20 locations within each grid. Photos were taken 30cm above the forest floor with a Nikon Coolpix 4500 digital camera and a Nikon FC-E8 fisheye converter lens (180º field of view). The camera was mounted on a tripod and leveled prior to photographing. Photographs were taken in the early morning, late afternoon or when it was evenly overcast (Pearcy, 1989; Rich, 1989). Photographs were oriented with north at the top of the image, allowing superposition of solar tracks. We used Gap Light Analyzer software (Frazer et al., 1999, 2000), which estimates direct site factor ('percent transmittance direct'), indirect or diffuse site factor ('percent transmittance diffuse') and the global site

factor ('percent transmittance total'). Direct (DSF) and indirect (ISF) site factors are defined as the proportion of direct and diffuse radiation received below the canopy as a fraction of that received above the canopy (Anderson, 1964; Rich, 1989). Global site factor (GSF), represents the total proportion of light reaching a site, and can calculated as

$$GSF = (ISF + DSF) / 2 \quad (1)$$

(Canham et al., 1990). Site factors can range from 1 (open sky) to 0 (complete obstruction).

DATA ANALYSIS—In the transplant shock experiment, we evaluated the effect of uprooting on offspring survival by comparing Kaplan-Meier cohort survivorship estimates (Fox, 2001; Levesque, 2007) of uprooted and undisturbed offspring. We compared the leaf area of uprooted and undisturbed offspring using a repeated-measures ANOVA. The main effects of the model were treatment (uprooted vs. undisturbed) and time. Analyses of leaf area throughout our study were performed on log of leaf area, base 10, to meet the assumptions of ANOVA.

We compared GSF scores among light level treatments (fixed factor) and sites (random factor) using a two-way ANOVA. Analyses were performed on GSF after it had been arcsine transformed, to conform to the assumptions of ANOVA. Pair-wise comparisons were performed among light level treatments pooled across sites using Tukey's post-hoc tests. In addition, we calculated a simple index to compare the gradient in GSF across sites; mean GSF in gap center was subtracted from the mean GSF in understory for each site.

To analyze the effects of light levels on survival trends of seedlings and clonal offspring we used a Kaplan-Meier survival analysis (Fox, 2001; Levesque, 2007). We

estimated cohort survivorship ('survival function'), mean survival time, and the probability of surviving to the end of the study (16 mos.), for each offspring type within each light level. Pair-wise comparisons of the survival functions of seedlings and clonal offspring among light levels were performed with a Log-Rank test (Krebs, 1999). The influence of experimental light level, site and initial light level on overall survival was analyzed using a logistic regression with all variables included in the model.

We used a repeated-measures ANOVA to analyze the effects of light level (fixed factor), site (random) and time (fixed) on offspring leaf area over the 16 month study period. Light availability in the source site (the site the offspring originated from) was used as a covariate in the model.

RESULTS

TRANSPLANT SHOCK EXPERIMENT—The act of uprooting and replanting *in situ* had no effect on survival, of either offspring type (Log-Rank tests of homogeneity of survival between treatments; Clonal offspring: $X^2 = 1.12$, d.f. = 1, $P = 0.290$; Seedlings: $X^2 = 1.33$, d.f. = 1, $P = 0.247$) or their leaf area (Clonal offspring: removal $F_{1,26} = 0.47$, $P = 0.497$; removal × time $F_{2,52} = 0.85$, $P = 0.433$; Seedlings: removal $F_{1,33} = 2.49$, $P = 0.124$; removal × time $F_{2,70} = 1.11$, $P = 0.336$).

LIGHT AVAILABILITY—One year post-planting, Global Site Factor (GSF) values were on average over 3 times greater at the centers of gaps than in the understory, and nearly twice as high at the edges of gaps than in the understory. Values for GSF ranged from 0.03 in the understory to 0.26 in the center of a gap. GSF differed

significantly among light level treatments and sites; in addition, there was a significant light level treatment × site interaction (Table 1.1). GSF was higher in the center of gaps than on their edges (Tukey's post-hoc $P < 0.0001$; Fig. 1.2), or in adjacent understory ($P < 0.0001$). GSF was also higher at the edges of gaps than in adjacent understory ($P < 0.0001$). The gradient in GSF, calculated as the mean GSF in gap center minus mean GSF understory, differed among sites; sites 1 and 2 had relatively steep gradients (0.17 and 0.18, respectively; Fig. 1.2) while sites 3 and 4 had lower gradients (0.12 and 0.10, respectively; Fig. 1.2).

OFFSPRING SURVIVAL—Survivorship of both clonal offspring and seedlings differed across light levels (Fig. 1.3; Log-Rank test of homogeneity of survival among light levels; Clonal offspring: $X^2 = 9.83$, d.f. = 2, $P < 0.007$; Seedlings: $X^2 = 119.16$, d.f. = 2, $P < 0.0001$). Over the duration of the study, survival of clonal offspring was lower in the centers of gaps than at their edges ($X^2 = 3.92$, $P < 0.048$) or understory environments ($X^2 = 9.14$, $P < 0.002$), while survival did not differ between gap edge and understory ($X^2 = 1.22$, $P < 0.269$; Fig. 1.3A). Survival of seedlings differed among all pair-wise comparisons of light levels (Fig. 1.3B). In contrast to clonal offspring, seedlings had higher survival at the centers of gaps than at their edges ($X^2 = 20.46$, $P < 0.0001$) or understory ($X^2 = 119.14$, $P < 0.001$), and survival was higher at gap edges than in the understory ($X^2 = 38.93$, $P < 0.0001$; Fig. 1.3B).

The probability of surviving the 16 month study period was influenced by light levels for both seedlings and clonal offspring, although offspring types differed in the direction of the overall trend and in the magnitude of the affect (Fig. 1.4). Clonal offspring showed a relatively modest difference between light levels and had their highest

probability of surviving in the understory (62%) and their lowest probability of surviving in gap centers (38%; Fig. 1.4). An opposite and more dramatic trend was observed for seedlings; the probability of surviving was highest in gap centers (68%) and lowest in the understory (8%; Fig. 1.4).

Estimated survival times for both offspring types were influenced by light levels, although seedlings showed a more dramatic response than clonal offspring (Fig. 1.4). On average, clonal offspring in the understory were expected to survive 37 days longer than those at the centers of gaps, but only 5 days longer than those at their edges (Fig. 1.4). Seedlings were expected to survive, on average, 147 days longer at the centers of gaps than in understory, and those at the edges of gaps were expected to survive 75 days longer than those in the understory.

Surviving to the end of the study was affected by light levels for both seedlings and vegetative offspring (Table 1.2). Vegetative offspring had a lower probability of surviving at the centers of gaps than in the understory (Tukey's post-hoc; $P < 0.0001$) or at gap edges ($P < 0.001$; Fig. 1.4). Survival of vegetative offspring was higher in the understory than at the edges of gaps, although this difference was only marginally significant ($0.05 < P < 0.10$; Fig. 1.4). Survival of seedlings was higher at the centers of gaps than at their edges ($P < 0.0001$) or the understory ($P < 0.0001$; Fig. 1.4). In addition, survival of seedlings was higher at the edges of gaps compared to the understory ($P < 0.0001$; Fig. 1.4). Light level at the location where plants were originally located before the experiment did not affect survival of either offspring type (Table 1.2).

OFFSPRING GROWTH—Light availability had a positive effect on clonal offspring and seedling growth (Table 1.2). Both propagule types attained larger leaf area

and grew faster at the center of gaps and at their edges than in the understory (Fig. 1.5). Clonal offspring and seedlings both showed a time × light level interaction (Table 1.2). At all light levels, clonal offspring lost leaf area between day 90 and day 400, but those in the understory lost the most leaf area (Fig. 1.5A). Leaf area of clonal offspring at the center of gaps and at their edges increased after day 400, while in the understory it did not change (Fig. 1.5A). Seedling leaf area increased at the center of gaps and at their edges throughout the 16 months, except between day 310 and day 400 (Fig. 1.5B). Light levels at the location where plants were originally located before the experiment did not affect leaf area of either offspring type (Table 1.2). Site affected the size of seedlings but not of clonal offspring. The only significant difference between sites was between 1 and 4. However, a time × site interaction affected the size of clonal offspring, indicating that the increase in leaf area over time was not uniform across sites, but seedlings size was not affected.

DISCUSSION

Results from our experiment agree with the longstanding notion that clonal offspring can establish across a broader range of microsites than sexual offspring (Harper, 1977; Abrahamson, 1980). Our findings extend the current understanding of sexual and clonal offspring demography in several important ways. Most importantly, our results suggest that the pattern of higher seedling abundance in new tree fall gaps and higher clonal offspring abundance in shaded understory (Abrahamson, 1980; Hughes et al., 1988; O'Dea et al., 1995; Kanno and Seiwa, 2004) may be the result of differential

rates of survival and growth of offspring. The relative proportion of recruits shifts from dominance by sexual offspring to clonal offspring as canopy gaps close (Abrahamson, 1980; Cook, 1985; Hughes et al., 1988). However, our study is the first to show that seedling survival is highest in tree-fall gap centers and clonal offspring survival is highest in the shaded understory. These results suggest that sexual and clonal offspring may have different optimal microsites for establishment. Results from this experiment do not allow us to speculate which of the differences between seedling and clonal offspring (i.e. size, integration with parent) are responsible for the difference in demographic performance between these two offspring types. Regardless of the mechanism responsible for the patterns we observed, our results clearly illustrate that clonal offspring differ less than seedlings in their growth and survival across light environments, and take more time to respond to new light treatments than seedlings. It is true that several microsite characteristics across the gradient in canopy cover may have influenced *C. marantifolia* offspring demography, but we believe that light availability is the most important factor, and therefore we focus our discussion to this variable.

Previous research has not addressed the response of sexual and clonal offspring to temporal variation in light availability. Moving seedlings between light environments to simulate temporal changes is known to affect their growth rates (Pompa and Bongers, 1991; Huante and Rincón, 1997; Huante et al., 1998); however, comparisons between seedlings and clonal offspring have not been made previously. We found that seedlings and clonal offspring differed dramatically in the rate and extent to which they acclimated to their new light environment. Seedling survival and growth responded to light levels faster and showed a large difference between levels compared to clonal offspring. Clonal

offspring, in all light levels, either maintained constant leaf area or experienced a reduction in leaf area during the first 400 days post-planting. Although clonal offspring maintained nearly constant leaf area, new leaves were produced and old leaves were shed during this time period (Matlaga, *personal observation*). Similarly, seedlings were also observed producing new leaves and shedding old leaves (Matlaga, *personal observation*). Apparently, the transition to independence is a developmental step that precedes augmentation in leaf area for clonal offspring, resulting in the observed time lag in growth.

We found that an offspring's light environment prior to planting did not influence its growth. This result is surprising because a previous study of *C. marantifolia* physiology found that the light level a leaf is born in, can 'set' the light-saturated photosynthetic capacity of the leaf (P. Rundel, *personnel communication*). In that study plants were grown under shadehouse conditions with three light levels (2, 10 and 50 % full daylight transmission). One possibility is that our lack of an effect of initial light environment on offspring demography is due to little variation in initial light availability, very few offspring originated in high light environments. Thus, there was a more dramatic difference in light levels in Rundel's experiment compared to ours.

Our experiment does not address the light-dependence of stages earlier than seedlings in the sexual reproductive cycle. We studied clonal offspring at the earliest possible stage, and for sexual offspring we studied already established seedlings, skipping the seed stage and the seed to seedling transition. Seeds of *C. marantifolia* can survive in the soil for up to one year (Horvitz et al., 2002). Horvitz et al. (2002) experimentally addressed the light-dependence of seed to seedling transition of *C.*

marantifolia. Light availability treatments in this experiment were comparable to the current study. Seedling emergence of *C. marantifolia* was not found to be light dependent even though it was in other species of *Calathea*.

In the current study we found higher seedling survival in high light environments, a result that is consistent with studies other species of *Calathea* as well as woody species. In a previous study of seedling survival of 5 Costa Rican *Calathea* species (including *C. marantifolia*) and one species in a related genus *Pleiostachya*, all species except one showed that the expected seedling survival time was higher in tree fall gaps than in shaded understory (Horvitz et al., 2002). Horvitz et al. (2002) who did not compare seedlings to clonal offspring and studied seedling survival from an earlier stage than the current study also found that *C. marantifolia* seedlings survived best in gaps and worst in the shaded understory. Tropical tree seedlings also show the increased survival with tree fall gap environments compared to the understory (Augspurger, 1984; Amézquita, 1998; Balderrama and Chazdon, 2005).

In all light levels the majority of clonal offspring mortality was seen at day 310. This spike in mortality occurred during a particularly severe dry season (Sirena Biological Station, unpublished data). In addition, during this time period over half of the clonal offspring were in the process of making the transition to independence from their parent plant. Results from a separate experiment show that severing the connection linking clonal offspring and parents, reduces the survival of clonal offspring (Chapter 2). Therefore, during the period when clonal offspring make the transition to independence they may be especially vulnerable to environmental stress, such as low water availability.

Because sexual and clonal reproduction in *C. marantifolia* is linked, with nearly all sexually reproducing individuals later producing clonal offspring, selection can not favor the production of one offspring type over another. Alternatively, selection may act on the allocation of resources between reproductive modes. The production of sexual offspring in *C. marantifolia* is more variable, and involves several risky steps compared to the production of clonal offspring. Flowers must be visited by pollinators that are large enough to engage the tripping mechanism (Kennedy, 1978). Once fruits have matured, approximately 40% of seeds survive to the seedling stage, although this transition is variable (Chapter 4). By contrast, over 90% of clonal offspring that are initiated are able to root in the soil and gain independence. However, in a separate study we manipulated sexual reproductive effort of mature plants and found that clonal offspring were produced with the same frequency, and were of the same size, regardless of seed production (Chapter 3). These results suggest that despite that the occurrence of sexual and clonal reproduction in *C. marantifolia* is linked there may not be a trade-off in reproductive output.

A model of *Calathea* metapopulation dynamics suggested that species may partition forest niches by occupying different locations along the gradient in the availability of light (Horvitz and Schemske, 1986). A survey of *Calathea*s with contrasting life histories showed that light partitioning did occur among species and those with clonal propagation occupied a wider range of light environments than those without (Horvitz, 1991). Our data provide an explanation for those results. Clonal offspring were less sensitive to the availability of light than seedlings. In gaps seedlings survived much better and grew much better than in the shaded understory. Clonal offspring during

the first 300 days (while still connected to parent plants), in both gaps and understory, survived very well and grew very little, with almost no difference in performance due to light availability. However, during the remaining 180 days (post independence), there were small differences due to light availability; those in gap centers had a somewhat higher probability of dying than those in the shaded understory and those that survived in gap centers grew a little, while those that survived in the shaded understory shrank, resulting in only small differences in size due to light at the end of the experiment. These results suggest that being attached to the parent provides buffering for clonal offspring with respect to survival, but that growth is associated with independence and increased risk.

Table 1.1. Analysis of variance for the effects of light availability treatments and site on global site factor (GSF). GSF values were arcsine transformed to conform with the assumptions of ANOVA.

Term	df	*F*	*P*
Light level	2	783.30	0.0001
Site	3	6.16	0.0001
Light level × Site	6	18.51	0.0001
Error	228		

Table 1.2. Estimated parameters for logistic regression of seedling and clonal offspring survival at the end of the 16 month study period.

Term	Vegetative offspring				Seedlings			
	Estimate	SE	Odds ratio	*P*	Estimate	SE	Odds ratio	*P*
Constant	1.06	0.58	2.9	0.066	-1.73	0.63	0.17	**0.006**
Light level	-0.48	0.16	0.61	**0.003**	1.53	0.16	4.6	**0.0001**
Site	-0.03	0.12	0.97	0.781	-0.23	0.10	0.79	**0.025**
Source light level	-0.37	1.84	0.68	0.997	0.196	0.63	1.21	0.757

Table 1.3. Repeated measures ANOVA of the effects of light level, site, and time on clonal offspring and seedling leaf area. Analysis was performed on log(leaf area).

	Clonal offspring				Seedlings			
Source of variation	df	SS	*F*	*P*	df	SS	*F*	*P*
Between subject effects								
Light level	2	4.83	4.80	**0.01**	2	23.88	21.28	**0.0001**
Site	3	2.25	1.49	0.220	3	3.20	1.901	0.132
Light level × site	6	4.17	1.42	0.215	6	11.30	3.35	**0.004**
Source light level (covariate)	1	0.40	0.79	0.374	1	0.71	1.27	0.261
Error	100	50.23			156	95.46		
Within subject effects of time								
Time	2.99	1.801	4.11	**0.007**	2.68	1.49	4.30	**0.007**
Time × Light level	5.99	11.74	13.40	**0.0001**	5.36	15.53	22.32	**0.0001**
Time × site	8.98	3.52	2.68	**0.005**	8.04	1.05	1.01	0.424
Time × Source light level	2.99	0.73	1.67	0.172	2.68	0.55	1.58	0.197
Error (time)	299.51	43.81			418.485	54.26		

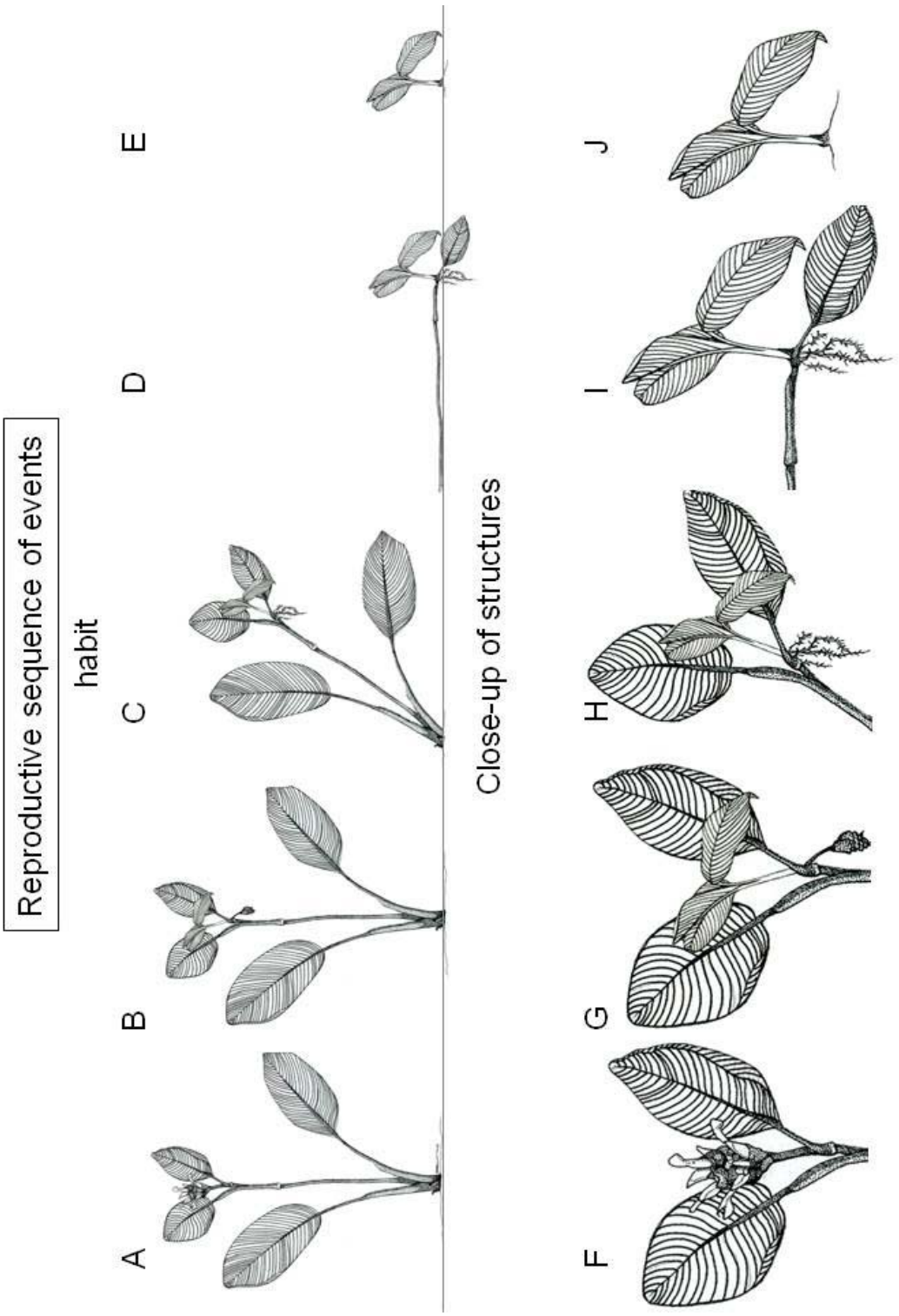
Reproductive sequence of events
habit
A
B
C
D
E
Close-up of structures
F
G
H
I
J

Figure 1.1. Reproductive sequence of events for a *Calathea marantifolia* shoot. A single pedunculate inflorescence is borne terminally during the wet season (A). The inflorescence has spirally arranged bracts and subtending the bracts flowers are borne in pairs (F). After the inflorescence has senesced, a clonal offspring is initiated from the same terminal node as the inflorescence (B). Initially the clonal offspring does not have roots (G). Over time the parent plant's shoot leans toward the ground (C). The clonal clonal offspring develops roots prior to contacting the soil (C). Upon contacting the ground the clonal offspring roots in the soil (D). After rooting the clonal offspring can retain the connection to the parent plant for up to several months (I). The connection to the parent is eventually broken (E) giving the clonal offspring its independent from the parent plant. Illustration by Erin Kuprewicz.

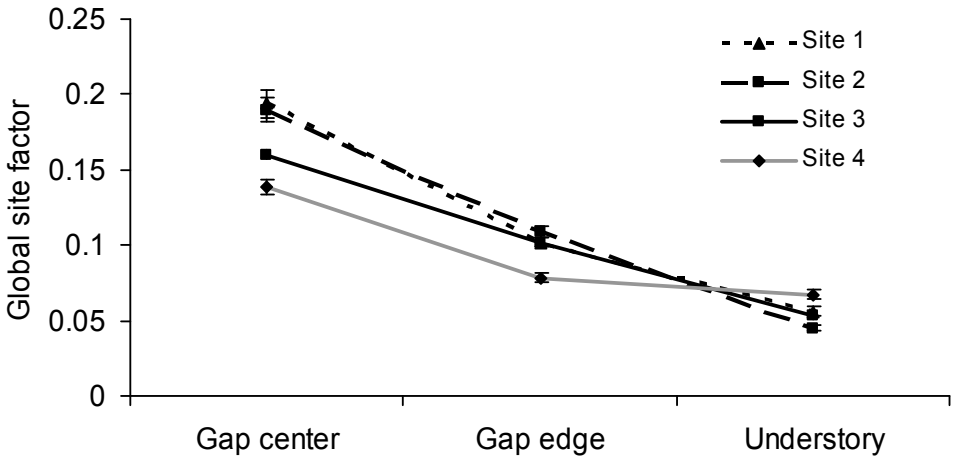

Figure 1.2. Mean (± 1SE) global site factor score at the centers of gaps, edges of gaps and shaded understory in four sites.

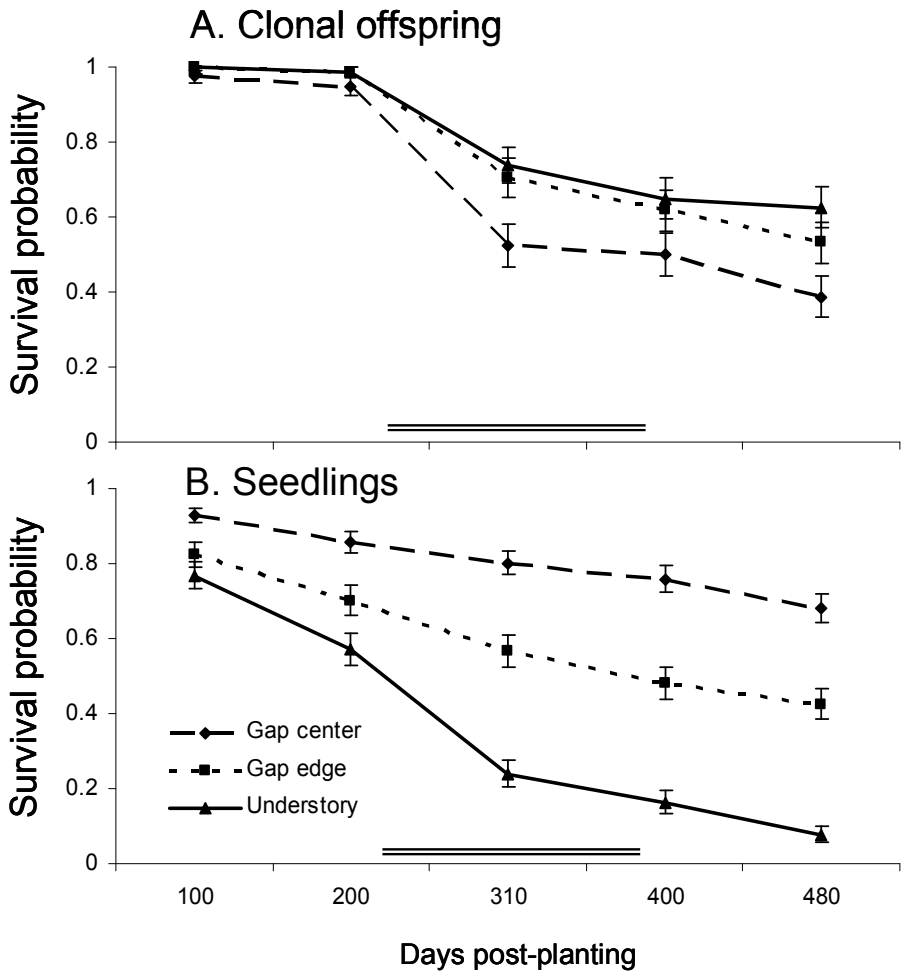

Figure 1.3. Survival probability of clonal offspring (A) and seedlings (B) in the centers of gaps, edges of gaps and shaded understory over the 16 month study period. Survival probabilities were estimated using Kaplan-Meier survival analysis. Double line along x-axis indicates dry season (months with less than 300 mm of cumulative rainfall; Sirena Biological Station, unpublished data).

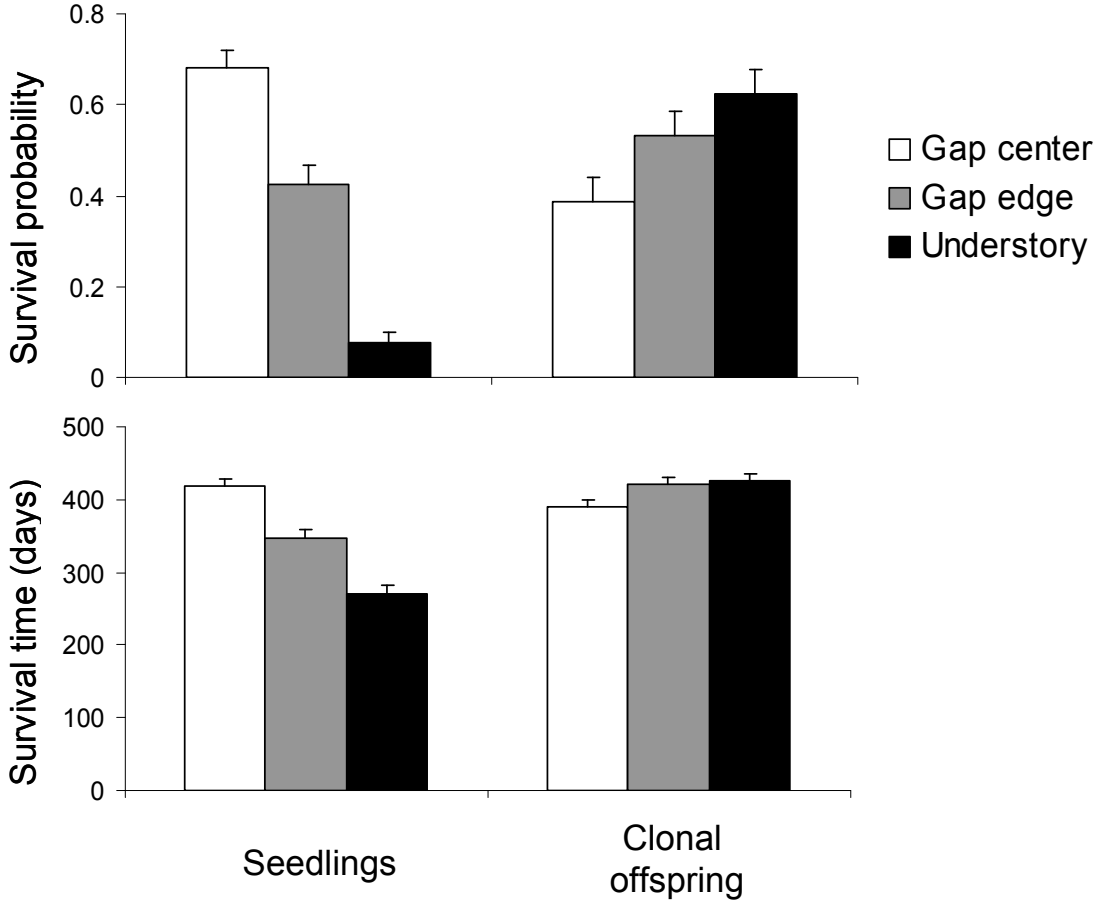

Figure 1.4. Mean (± 1 SE) survival probability at the end of the 16 month study period in gap center, gap edge and understory for clonal offspring and seedlings. Mean (± 1 SE) survival time (number of days, estimated by the Kaplan-Meier product-limit method) expected for clonal offspring and seedlings in the centers of gaps, edges of gaps and shaded understory.

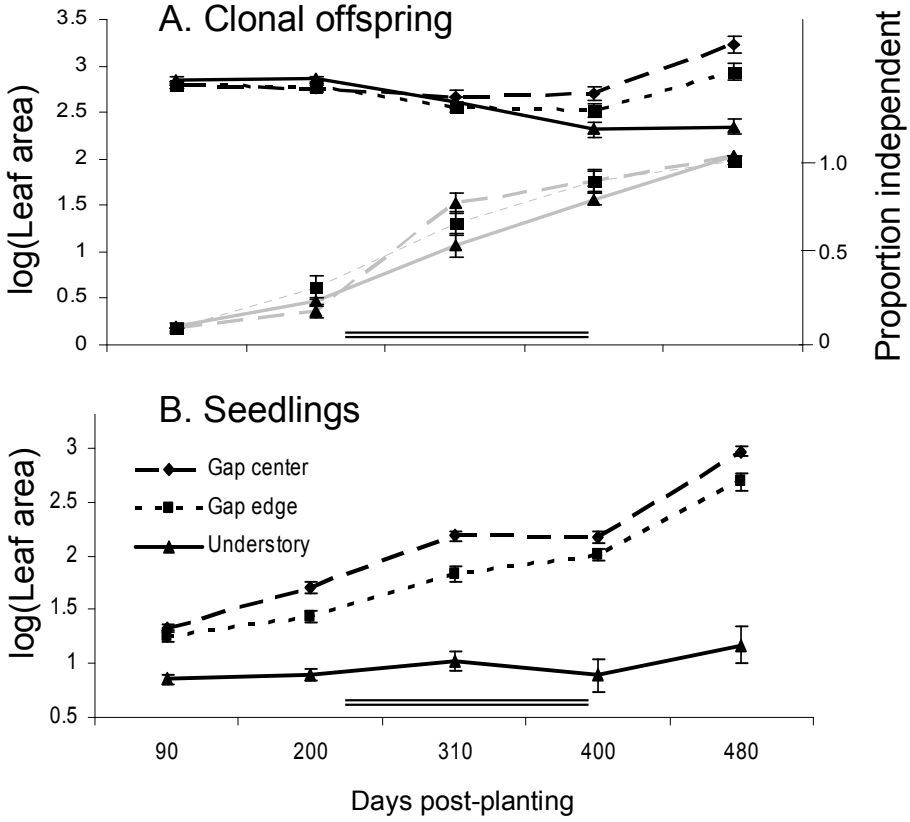

Figure 1.5. Mean (± 1SE) clonal offspring (A) and seedling (B) log(leaf area), measured as square centimeters, in gap center, gap edge, and understory. Proportion of living clonal offspring that achieved independence is shown below growth, with grey lines. Double line along x-axis indicates dry season (months with less than 300 mm of cumulative rainfall; Sirena Biological Station, unpublished data).

Chapter II

Ephemeral clonal integration: Evidence of diminished integration over time[2]

SUMMARY

A major advantage of clonal growth-forms is the intergenerational transfer of resources through vascular connections (clonal integration). Connections linking ramets can be persistent or ephemeral. For species with ephemeral connections it is unclear if the extent of clonal integration changes over time. We address this issue by tracking water movement using an isotopic label and by assessing the demographic performance of parent and offspring ramets over time in a severing experiment. Our study system was the understory herb *Calathea marantifolia* which has parent ramets that produce clonal bulbils (clonal offspring) that pass through distinct pre- and post-rooting stages. Little water was transported between parents and offspring, and the direction of movement was primarily from parent to pre-rooting offspring. Anatomical observations of inter-ramet connections showed that vascular bundles were twice as abundant in parent stems compared to inter-ramet connections. Severing inter-ramet connections reduced the growth of offspring ramets but not parents. Survival of pre-rooting offspring was reduced by 10% due to severing, but post-rooting offspring were not affected. Our results suggest that offspring ramets of *C. marantifolia* are weaned from their parent as they progress from pre- to post-rooting stages.

[2] Coauthor: Leonel da S. L. Sternberg

BACKGROUND

One of the principal differences between sexual and clonal reproductive strategies is the timing and amount of resources transferred from the parent to the reproductive offspring. Sexual offspring receive a relatively small, one-time investment of endosperm and cotyledon tissue. By contrast, clonally produced offspring can potentially receive a relatively large maternal investment over a long period through vascular connections (clonal integration). Across species, clonal plants form a continuum in terms of the spacing of ramets along vascular connections, with one end occupied by clumped growth-forms (phalanx) and the other by spreading (guerilla) growth-forms (Lovett-Doust, 1981; White, 1984). Phalanx growth-forms typically occupy late successional environments, while the guerilla strategy is found in early successional sites (Schmid and Bazzaz, 1987; Adachi et al., 1996). In tropical secondary forests and disturbed areas of primary forests, a type of guerilla growth-form with ephemeral vascular connections is common among members of the Zingiberales (Marantaceae- e.g. *Calathea donnell-smithii*; Costaceae- e.g. *Costus scaber*; Zingiberaceae- e.g. *Alpinia purpurata*). In this growth-form clonal bulbils are produced atop reproductive shoots. These shoots eventually fall to the ground where bulbils root directly and remain connected to their parent for some time before becoming independent. To our knowledge this type of guerilla growth-form has received no attention in the clonal plant literature, and the extent to which clonal bulbils are physiologically integrated with the parent ramet before and after they root in the soil is unknown.

One of the main advantages of the clonal life-history strategy is hypothesized to be the presence of vascular connections linking ramets. Some inter-ramet connections

are persistent, lasting longer than an individual ramet's lifespan, while others are ephemeral decaying shortly after a ramet is produced (Jónsdóttir and Watson, 1997; Tamm et al., 2002). Persistent connections can transport resources between ramets increasing the probability of ramet establishment (e.g. Hartnett and Bazzaz, 1983; Peltzer, 2002). For species with ephemeral connections it is not known if the extent of clonal integration, or its influence on ramet demography, decreases as connections age.

Physiological integration, resource sharing between interconnected ramets, may allow for transport of water and mineral nutrients through the xylem and carbohydrate transport through the phloem (Alpert and Mooney, 1986; Stuefer and Hutchings, 1994; Alpert, 1996; Wijesinghe and Hutchings, 1997). The directionality and intensity of resource transport depends on both source-sink dynamics and anatomical continuity between ramets (Pitelka and Ashmun, 1985) which can change over time (e.g. Marshall and Sagar, 1968). The directionality of resource movement in the majority of species and conditions studied, is from parent (or 'mother') ramets to offspring (or 'daughter') ramets (acropetal; Pitelka and Ashmun, 1985). Acropetal transport benefits offspring by increasing their growth and survival, often at a cost to their parent's growth or survival (Salzman and Parker, 1985; Pitelka and Ashmun, 1985; de Kroon and Schieving, 1990). The extent of integration is variable across species, with some having no integration (Price and Hutchings, 1992) while others are highly integrated (Hartnett and Bazzaz, 1985).

Clonal integration has been investigated by tracing the movement of resources between ramets, as well as through experimental manipulations that test for ramet interdependence in terms of growth or survival (Pitelka and Ashmun, 1985), but only

rarely are both methods combined (Jónsdóttir and Callaghan, 1989; de Kroon et al., 1996; 1998). Most commonly, isotopes such as ^{14}C, and ^{15}N, ^{32}P (reviewed in Pitelka and Ashmun, 1985; Marshal, 1990; Jónsdóttir and Watson, 1997) and more recently deuterium-labeled water (de Kroon et al., 1996 and 1998) are used as tracers in clonal plant studies. Isotope experiments quantify the transport of resources between labeled and recipient ramets at a specific point in time, but do not asses the demographic consequences of resource sharing. To evaluate the importance of resource integration for plant fitness, it is necessary to compare demographic performance (survival and growth) of ramets with intact and severed inter-ramet connections (Jónsdóttir and Watson, 1997).

We investigated the phenology of ephemeral physiological integration in the Neotropical understory herb *Calathea marantifolia* (Marantaceae). We investigated the degree of resource sharing, and its associated influence on ramet demography before and after offspring ramets root in the soil. Our hypothesis is that offspring ramets are more integrated with their parent prior to rooting compared to post-rooting, and therefore the demographic consequence of severing inter-ramet connections will be more severe prior to rooting. Specifically we addressed the following questions;

1) Does the connection linking parent and offspring ramets have the structural capacity for water transport?
2) Which direction is water transported; from parent to offspring or vice verse?
3) Does the proportion of translocated water decrease after offspring ramets root?
4) Does severing inter-ramet connections reduce the demographic performance (survival and growth) of parent and offspring ramets?

5) Are offspring ramets affected by severing their inter-ramet connections prior to rooting more than after they root?

METHODS

STUDY SYSTEM—Field experiments were conducted on *Calathea marantifolia* (Marantaceae) located in secondary forest and abandoned plantations at the La Selva Biological Station of the Organization for Tropical Studies (10º28' N, 83º59' W). La Selva is in the Atlantic lowlands of Costa Rica, and has primary and secondary forest classified as premontane wet tropical forest according to the Holdridge vegetation classification system (Hartshorn, 1983). We refer to offspring ramets that have not rooted in the soil as 'pre-rooting' and those that have as 'post-rooting.' Parents typically produce one offspring ramet per shoot, but may have several shoots at a time.

ANATOMICAL CHARACTERISTICS OF INTER-RAMET CONNECTIONS—Anatomical observations were carried out, and material was collected, at Fairchild Tropical Botanic Garden (Coral Gables, FL). We compared the appearance and quantity of vascular bundles between the stem connecting parent and offspring ramets (from here on 'connection') and the stem of the parent ramet (from here on 'parent stem;' Fig. 2.1). To investigate the abundance of the vascular bundles, we used cultivated plants from the garden. We collected 6 pre-rooting and 6 post-rooting offspring along with the connected parent ramet stem. Sections were made of the connection and parent stem free-hand using a single-edge razor blade. A concentrated HCl-phloroglucinol stain was used to visualize lignin (Ruzin, 1999). To determine the abundance of vascular bundles we

divided cross sections into eight equal pie-shaped pieces. We counted the number of vascular bundles for three randomly chosen pieces under a dissecting scope. The average number of bundles was calculated for each pie-shaped piece and was multiplied by eight to estimate the total number of vascular bundles per cross section. Photographs of the vascular anatomy were made using a Nikon Coolpix 4500 digital camera (Nikon Corp., Tokyo Japan).

INTER-RAMET WATER TRANSPORT—To examine reciprocal transport of water between parents and offspring (pre-rooting and post-rooting), we conducted a field experiment in natural populations. We traced the movement of deuterium enriched water between a labeled ramet (provided with enriched water) and a recipient (not provided with enriched water). We located 40 parent-offspring pairs with intact connections, 20 with offspring that had not yet rooted (Fig. 2.1A) and 20 with offspring that had already rooted (Fig. 2.1B). In each group, the parent-offspring pairs were randomly assigned to one of two treatments; (1) the parent was labeled and the offspring was the recipient, or (2) the offspring was labeled and the parent was the recipient. Thus, the fully crossed experiment included two factors: rooting stage of the offspring (unrooted or rooted) and which ramet was labeled (parent or offspring).

We provided labeled water to parents and offspring that had already rooted by dripping 700 ml of deuterium-enriched water 2 cm from the base of the ramet, over 6 hours (10:00 – 16:00) each day for five days (May 18-22, 2007). Water was quickly absorbed by the soil, and did not pool on the surface. Offspring that had not yet rooted were provided labeled water by spraying 10 ml of deuterium-enriched water on their exposed roots twice a day (10:00 and 13:00) each day for 5 days (May 18-22, 2007).

Several studies have shown that fractionation does not occur during water uptake by roots or during xylem transport (e.g. White et al., 1985). Because leaf water can lose label by equilibration with atmospheric humidity, we sampled petioles. Sections of leaf petiole (4 cm) were harvested from parents and offspring on May 22, 2007 between 16:00 – 17:00. A 5 ml sample of soil was taken at the base of the recipient ramet (unlabeled), to verify that enriched water had not moved through the soil. All samples were immediately placed in BD Vacutainer® 7ml Serum tubes (BD Franklin Lakes, NJ), sealed with parafilm, and stored at -18°C until processed.

Samples were taken to the Stable Isotope Laboratory, Department of Biology, University of Miami (Coral Gables, FL) for water extraction and determination of deuterium content. Water was removed from leaf petiole samples by squeezing for all samples except five for which distillation was used (Vendramini and Sternberg, 2007). For all soil samples, water was removed by distillation. Hydrogen isotope ratios are expressed as deviations in parts per thousand (‰) from the international standard vSMOW (Vienna-Standard Mean Ocean Water), by:

$$\delta D_{sample}(‰) = \left[\frac{D/H\ sample}{D/H\ vSMOW} - 1 \right] \times 1000 \qquad (1)$$

where D/H is the ratio of deuterium to hydrogen in the extracted and standard water(vSMOW). The precision of the analysis is $\pm$ 3‰. The δ D value of the water provided to the ramets was approximately 5000‰ and was produced by mixing one liter of local water with one milliliter of 99.8% D_2O.

Water samples were analyzed in a Multiflow system connected to an Isoprime mass spectrometer (GV, Manchester, UK). We used ~5 mg of platinum black powder (Sigma-Aldrich, St. Louis, MO, USA) to equilibrate hydrogen with water vapor for a 24 h period, and then analyzed the resulting equilibrated gas to derive the hydrogen isotope ratio of the water using a modification of Prosser and Scrimgeour (1995). Water aliquots of 0.5 mL (including internal laboratory standards) were placed each in 5.9 mL vials (Exetainer® vials; Labco, High Wycombe, UK) together with cuvettes containing platinum black catalyst and sealed with screw-caps with a pierceable rubber septum (Exetainer® cap; Labco). Isotope analysis of the equilibrated gas proceeded as in Vendramini and Sternberg (2007).

We evaluated the effectiveness of our labeling protocol by comparing δD values of labeled ramets in different groups using Kruskal Wallis tests.

To determine if recipient ramets had received enriched water through inter-ramet connections from the labeled ramet, we compared δD values of recipient ramets to background samples using a single sample comparison with a population mean (Sokal and Rohlf, 1995) and displayed the results graphically. Since we did not have samples from untreated ramets we used parent recipient ramets connected to pre-rooting offspring as background for analyses because their δD was on average the lowest (Table 2.1), did not have outliers, and was not significantly different from soil samples taken from the base of the parent ramet ($Z = 0.456$, $P = 0.742$). In addition, of the recipient ramets, δD values for parent recipient ramets connected to pre-rooting offspring were closest to mean δD found in precipitation in the month of May in the study region (δD =17. 6; Estrada Meteorological Station; IAEA/WMO, 2006).

For the recipient ramets that did receive enriched water, we calculated the proportion of water transported between labeled and recipient ramets as

$$\text{Proportion of water transported} = \frac{\delta D_{\text{recipient ramet(mean)}} - \delta D_{\text{backgroud(mean)}}}{\delta D_{\text{labeled ramet(mean)}} - \delta D_{\text{backgroud(mean)}}} \quad (2)$$

and the standard error of this index was calculated using the error propagation method of Taylor (1997).

DEMOGRAPHIC CONSEQUENCES OF PHYSIOLOGICAL INTEGRATION—To determine the demographic consequences of physiological integration, we conducted a field experiment in natural populations. During July 5-31, 2006 we haphazardly located parent-offspring pairs with offspring that had not yet rooted (n = 170) and with offspring that had already rooted (n = 170). Parent and offspring were individually marked, and all leaf lengths were measured. Area of each leaf was estimated from a previously-determined regression relationship between leaf length and area (Horvitz and LeCorff, 1993), and areas of all leaves per ramet were summed to calculate total leaf area. Parent-offspring pairs were randomly assigned to one of two treatments; severing or leaving the inter-ramet connection intact. Offspring that had not yet rooted were severed and reattached to the parent's reproductive shoot, 2cm below the original point of attachment, using two plastic cable ties. This allowed severed offspring to remain at the same height (and thus receive the same amount of understory light) as unsevered offspring, but prevented sap-flow. Offspring are attached to the terminal node of the parent's reproductive shoot, which is enclosed in the sheath of the axillant leaf. Therefore, to sever the connection, we needed to remove the parent's axillant leaf. To

standardize damage to the parent we removed the axillant leaf on parents in the non-severing treatment as well. Parent ramets in the severing treatment had any clonal offspring removed from their shoots 90, 150, 270, and 368 days after treatment began. Survival and growth of offspring was censused 90, 150, 270, and 368 days after treatment. Survival and growth of parents was censused one year after the treatment began.

No mortality of parent ramets was observed during the study period. We analyzed the effects of severing the inter-ramet connection on offspring survival using a Kaplan-Meier survival analysis (Fox, 2001; Levesque, 2007) to estimate cohort survivorship ('survival function'), mean survival time, and the survival probability at the end of the study. We analyzed the effects of severing the inter-ramet connection on the growth of offspring (change in leaf area) using a repeated-measures ANOVA. The main effects of the model were severing (yes or no), developmental stage of offspring (not yet rooted or already rooted) and time. The effect of severing on the growth of parents (change in leaf area) was evaluated with a t-test.

RESULTS

ANATOMICAL CHARACTERISTICS OF INTER-RAMET CONNECTIONS—We did not observe resin or other materials filling vessels in the inter-ramet connections or parent stem. There were significantly more vascular bundles in the stems of the parent (Fig. 2.2B) than in the tissue connecting parents to offspring (Fig. 2.2A; Man-Whitney $U = 5.50$, $P = 0.0001$; Parent stem 200.8 $\pm$ 12.1, Connection 90.4 $\pm$ 4.2; mean $\pm$ SE). We

observed a difference in the directionality of the vascular bundles between the parent stem and inter-ramet connection. All of the vascular bundles in the parent stem ran in parallel to one another (Fig. 2.2D). In contrast, vascular bundles in the inter-ramet connection did not run in parallel, and instead formed a plexus (Fig. 2.2C).

EFFECTIVENESS OF LABELING*:* ABUNDANCE OF DEUTERIUM IN LABELED RAMETS—The labeling protocol was effective, with labeled ramets showing abundant deuterium. The amount of deuterium in ramets that received enriched water by dripping was high and equal across groups including parents of rooted and unrooted recipient offspring and rooted labeled offspring (Table 2.1; $X^2 = 0.111$, d.f. = 2, $P = 0.946$). By comparison, ramets that received enriched water by spraying had a lower amount of deuterium, although they had much higher amounts of deuterium than recipient ramets (Table 2.1).

Soil samples taken from the base of the recipient ramets showed a very low abundance of deuterium ($\delta D = -31.8 \pm 8.8$, mean $\pm$ SE), indicating that deuterium did not move through the soil from labeled to unlabeled ramets.

INTER-RAMET WATER TRANSPORT: ABUNDANCE OF DEUTERIUM IN RECIPIENT RAMETS—Overall, we observed very little water transport between parents and offspring. Deuterium abundance was higher than background levels in few recipient ramets (Fig. 2.3), and the proportion of water translocated was below 5% for all treatments (Fig. 2.4). The abundance of deuterium in recipient ramets was unequal across treatments ($X^2 = 9.911$, d.f. = 3, $P = 0.019$). Translocated water moved, predominantly, from parent to offspring. Offspring that had not yet rooted had

significantly more deuterium, and a greater proportion of water transported, than those that had already rooted (Fig. 2.3 & 2.4; Mann-Whitney, $U = 15.00$, $P = 0.009$).

DEMOGRAPHIC CONSQUENCES OF PHYSIOLOGICAL INTEGRATION—Severing reduced the survival of offspring that had not yet rooted, but did not affect the survival of offspring that had already rooted (Fig. 2.5; Log-Rank test of homogeneity of survival between treatments; pre-rooting offspring $X^2 = 9.334$, d.f. = 1, $P = 0.002$; post-rooting offspring $X^2 = 4.04$, d.f. = 1, $P = 0.525$). On average, severing connections reduced the estimated days until death by 46 days for offspring that had not yet rooted. In contrast, offspring that had already rooted survived equally as long with intact or severed connections (Fig. 2.6A). Severing reduced the probability of surviving to the end of the year-long study by 10% for offspring that had not yet rooted, but rooted offspring were not affected (Fig. 2.6B).

Offspring growth was reduced by the act of severing connections, but growth of parents was not. The growth of offspring was influenced by whether or not they had already rooted, by whether or not the connections to their parents had been severed, and by a rooting × severing interaction (Table 2.2). Offspring who had not yet rooted lost leaf area over the study period, although the loss was more dramatic when connections to parents were severed (Fig. 2.7). Offspring who had already rooted with intact connections added a small amount of leaf area over the study period and those with severed connections maintained nearly constant leaf area (Fig. 2.7). The leaf area of parents was unaffected by severing, both initially (t = 0.152, d.f. = 333, $P = 0.879$) and one year later (t = 0.676, d.f. = 332, $P = 0.500$).

DISCUSSION

Our results show that water transport and the demographic consequences of clonal integration in *Calathea marantifolia* diminish before connections between parent and offspring are lost. Data from our isotope and severing experiments suggest that resource sharing in *C. marantifolia* is acropetal (moving from the parent to offspring), and that offspring receive fewer resources from their parent after they have rooted in the soil. Before rooting, offspring receive a small amount of water from their parents, and severing their connections reduces their demographic performance. Once offspring root in the soil, however, they receive no water from their parent, and severing their connection has little effect on their demography. It is surprising that parents showed no demographic cost of supporting offspring considering that offspring appear to utilize their resources, however several other studies have also found no cost associated with acropetal resource transfer (e.g. Stuefer and Hutchings, 1994; Van Kleunen and Stuefer, 1999).

Overall, we found that very little water is transported between parents and offspring. This lack of water translocation could be the result of several mechanisms. Initially, we suspected that a barrier to xylem flow may have been present in the inter-ramet connection linking parent and offspring, preventing water transport. However, in the connection we observed seemingly functional vascular bundles, with both xylem and phloem, similar to those in the parent's stem and no evidence of resin filled vesicles that could obstruct water movement. However, both the abundance and directionality of bundles was different between connections and parent stem. Compared to the parent

stem, inter-ramet connections had fewer vascular bundles and these bundles formed a plexus. The near absence of water translocation we observed may be related to a lack of sufficient vascular plumbing linking parent and offspring ramets.

An alternative explanation is that the low levels of water translocation were the result of source-sink dynamics. Water transport depends on the strengths of the water potential gradient and distances between sources (sites of high water potential) and sinks (low water potential; Pitelka and Ashmun, 1985). The gradient in water potential between ramets is created by transpiration at the leaf surface and water uptake by the roots (Pitelka and Ashmun, 1985). If the leaves of *C. marantifolia* offspring have low transpiration rates little water would be moved from the parent. Similarly, if the roots of offspring are equally efficient at water uptake as those of the parent and water availability is the same for parents and offspring, little water will be moved from the parent. Previous work has shown that when parents and offspring experience the same watering regime, water translocation levels are low (de Kroon et al., 1996). Therefore in our study, water transport between parents and rooted offspring may not have occurred because parents and offspring were rooted in soil with similarly high moisture content. Our isotope experiment was conducted during the rainy season with 56 mm of rain accumulating in the two weeks prior to our experiment, and an additional 75 mm fell during the experiment (*D.A. Clark* personal communication). Offspring that had not yet rooted, can only access water vapor from the air and water that drips onto their roots, explaining why they received more transported water from their parent than post-rooting offspring. These results are in agreement with previous work showing an increase in

water transport when offspring have access to less water than parents (de Kroon et al., 1996).

The directionality of water transport we observed was primarily from parent to offspring (acropetal). Our results showing that severing the inter-ramet connections reduces the demographic performance of offspring but not parents, also supports that resource transport is acropetal. Our results are in contrast to some clonal species with persistent connections, where parents experience a cost, typically a reduction in growth, associated with sharing resources with offspring (Salzman and Parker, 1985; Pitelka and Ashmun, 1985; de Kroon and Schieving, 1990). *Calathea marantifolia* may experience no, or little, cost of supporting offspring because of the dramatic size difference between parents and offspring. In terms of leaf area parents are nearly an order of magnitude larger than their offspring.

The demographic consequences of severing connections for offspring depended greatly on whether or not the offspring was rooted in the soil. Both the survival and growth of pre-rooting ramets were reduced by severing. However, the survival of post-rooting ramets was not affected and their growth showed only a slight reduction. These results are in agreement with results from our isotope experiment showing that pre-rooting offspring received translocated water from their parents but post-rooting offspring did not. Together these results suggest that offspring are gradually cut off from their parent's resources over time as inter-ramet connections age.

It is unclear how *C. marantifolia* offspring receive parental resources, which increases their demographic performance, in the virtual absence of water transport. Because the same transport system (xylem) translocates water and mineral nutrients, the

movement of these resources is positively correlated (i.e. Stuefer et al., 1996; de Kroon et al., 1998). We observed little water movement during our study and therefore it is possible that few mineral nutrients were transported as well. Photosynthates, which are transported in the phloem and not in the xylem are typically associated with increased growth (Evans, 1991). The increase in offspring growth and survival we observed when connections to the parent are not severed may be the result of offspring receiving photosynthates from their parent. Therefore, carbohydrate translocation may show a contrasting pattern to water translocation. Offspring ramets may receive a large proportion of their carbohydrates from the parent, in contrast to them receiving a small proportion of water. Additionally, resource transfer between parents and offspring may be seasonal with a more integration occurring during the dry season. Our isotope study took place during the rainy season while our severing experiment spanned wet and dry seasons. Further study is needed to understand which resources are shared between ramets and if integration is seasonal.

Table 2.1. Mean abundance of deuterium (± SE), expressed as δD, in parent and offspring ramets. Labeled ramets that received enriched water by spraying are in bold and labeled ramets that received enriched water by dripping are in plain faced type.

	Role of ramet			
Stage of offspring	Parent	Offspring	δD Parent	δD Offspring
Rooted	Labeled	Recipient	422 ± 82	-19 ± 2
Not rooted	Labeled	Recipient	399 ± 93	-2 ± 9
Rooted	Recipient	Labeled	-24 ± 6	381± 69
Not rooted	Recipient	Labeled	-21 ± 1	**129 ± 49**

Table 2.2. Repeated measures two-factor ANOVA for the effects of severing treatments (connection severed or intact), stage of offspring (pre- or post-rooting), and time on ramet size (log of leaf area).

Source of variation	df	SS	*F*	*P*
Between subject effects				
Stage of offspring	1	86.083	136.037	0.0001
Severing	1	25.737	40.672	0.0001
Severing × Stage of offspring	1	8.677	13.712	0.0001
Error	245	155.035		
Within subject effects				
Time	3	10.160	30.60	0.0001
Time × Stage of offspring	3	14.812	44.728	0.0001
Time × Severing	3	15.864	47.905	0.0001
Time × Stage of offspring × Sever	3	6.478	19.561	0.0001
Error	735	81.135		

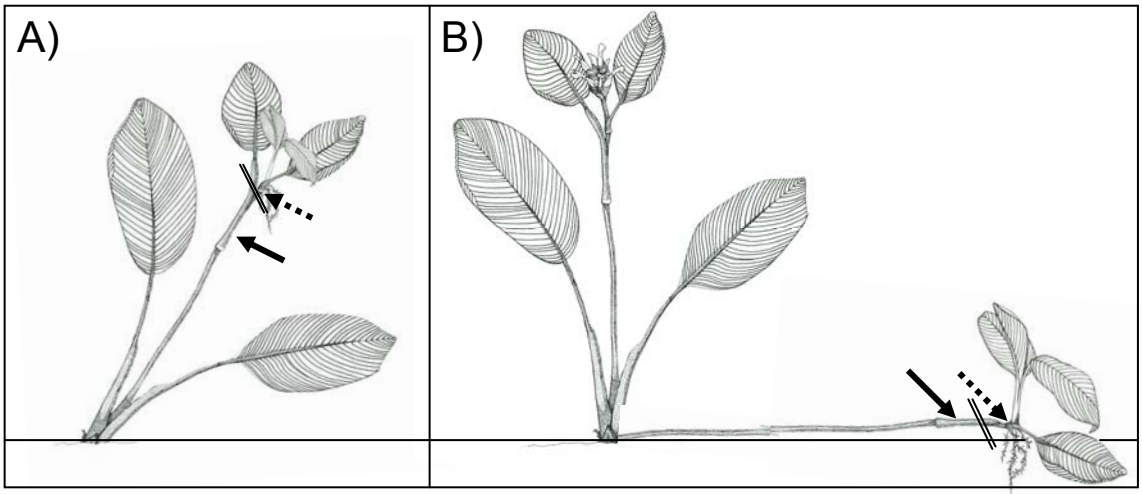

Figure 2.1. Severing treatments and points of anatomical comparison of *Calathea marantifolia*. Offspring that had not yet rooted (A) were severed (parallel lines) and reconnected to the parent shoot. Offspring that had already rooted (B) were severed at the same location (parallel lines) but not reconnected. Anatomical comparisons were made between the parent stem (solid arrow) and connection point between offspring and parent (dashed arrow). Illustration by Erin Kuprewicz.

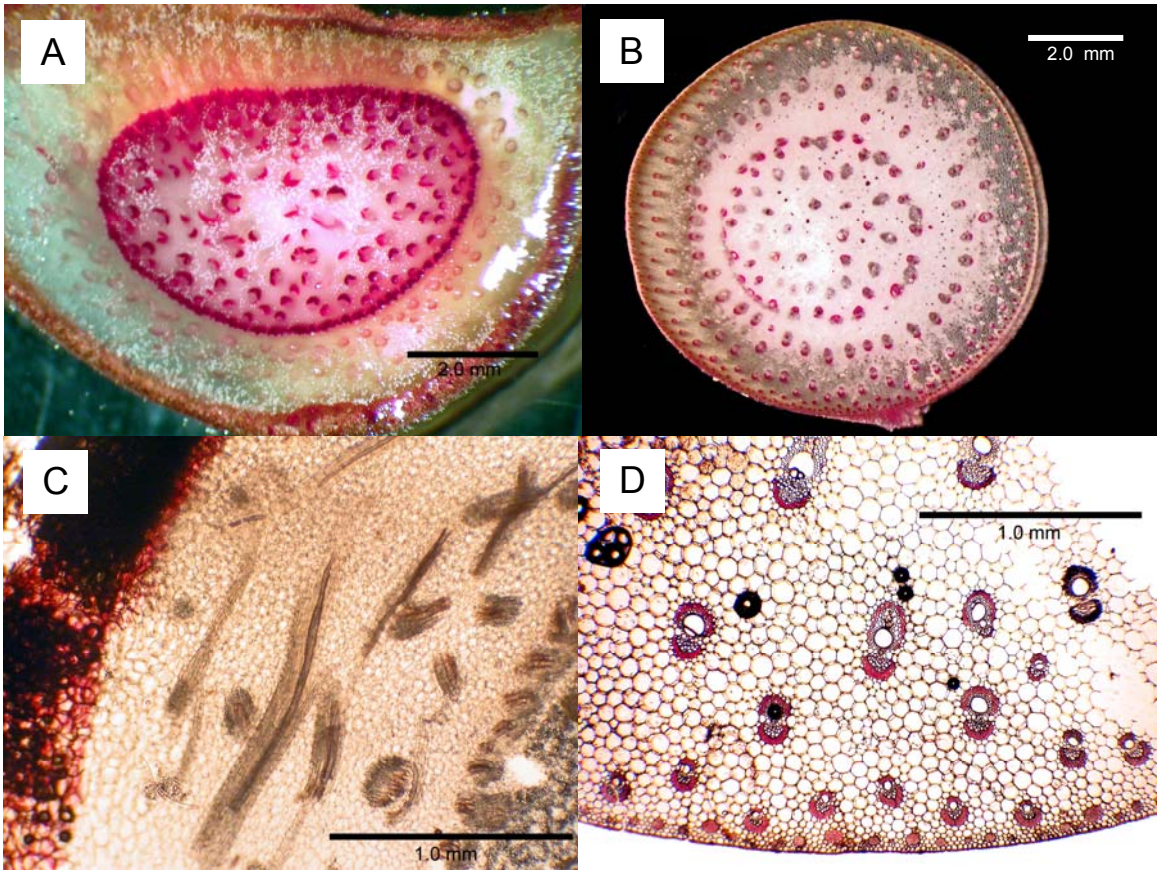

Figure 2.2. Vascular anatomy of the inter-ramet connection (A and C) and the parent stem (B and D) under different magnification. Photo by Jay Horn.

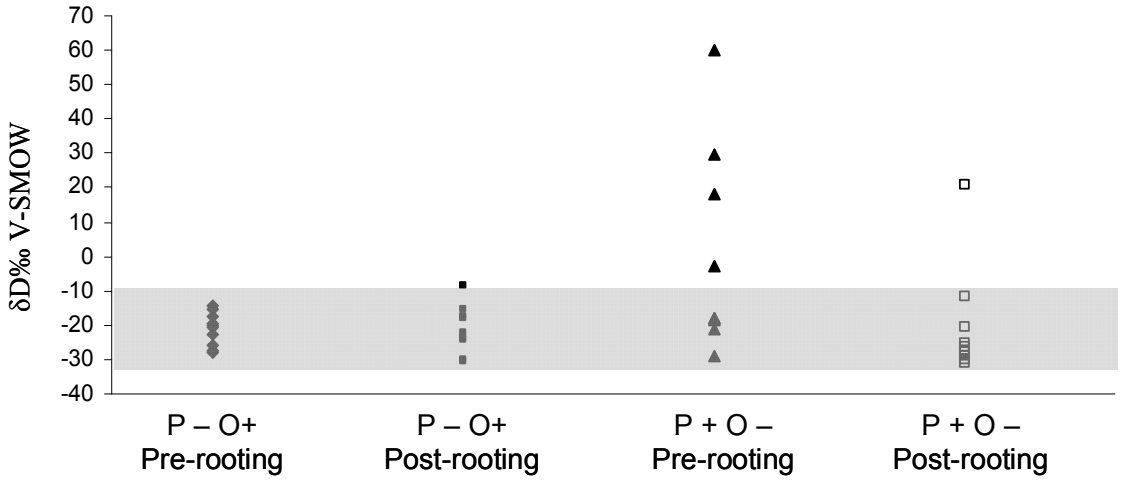

Figure 2.3. Deuterium (δD) values for recipient ramets (P = parent, O = offspring; + labeled ramet, - recipient ramet). Rooting status of the offspring ramet (pre- or post-rooting) noted below parent and offspring labeling. Ramets with petiole water having δD values within the grey area are not significantly different from background levels; those outside the grey area are significantly different at $P< 0.05$ according to a single-sample comparison with the background population (Sokal and Rohlf, 1995).

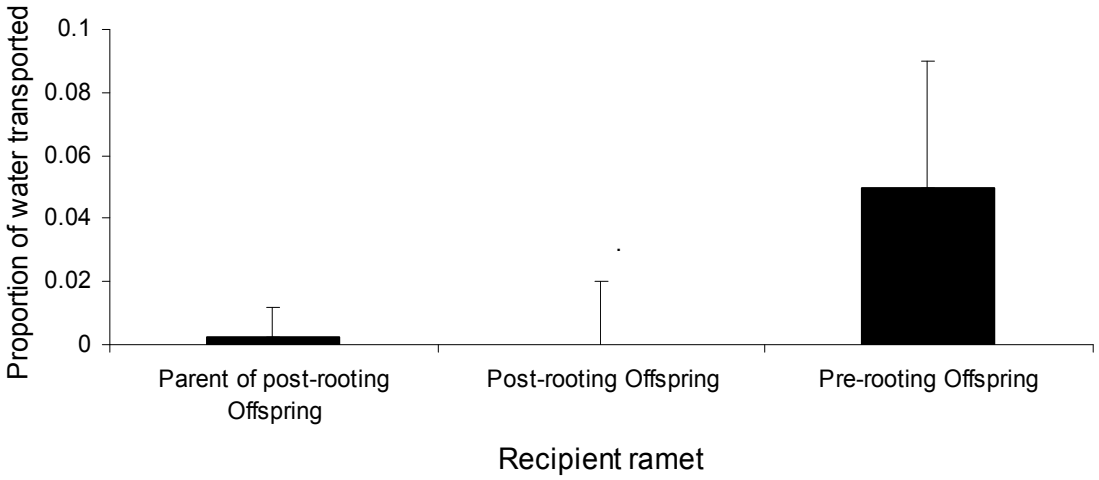

Figure 2.4. Mean proportion (± SE) of water transported from labeled to recipient ramets. Parent of pre-rooting offspring was used as background to allow for the calculation of the proportion of water transported in the other recipient ramets.

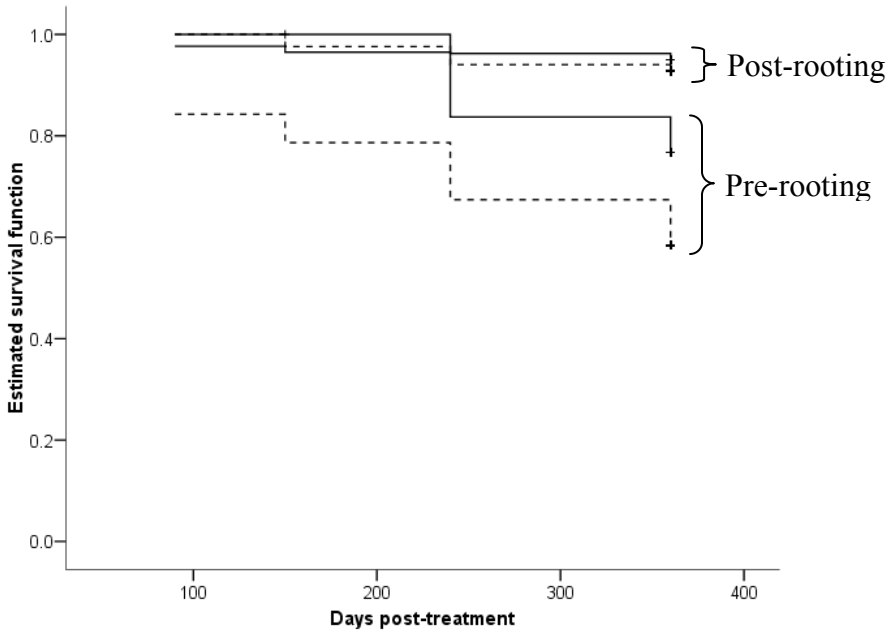

Figure 2.5. Cohort survivorship for offspring with intact (unbroken) and severed (dashed) connection to their parent, in their pre-rooting and post-rooting stages. Survival functions are Kaplan-Meier estimates.

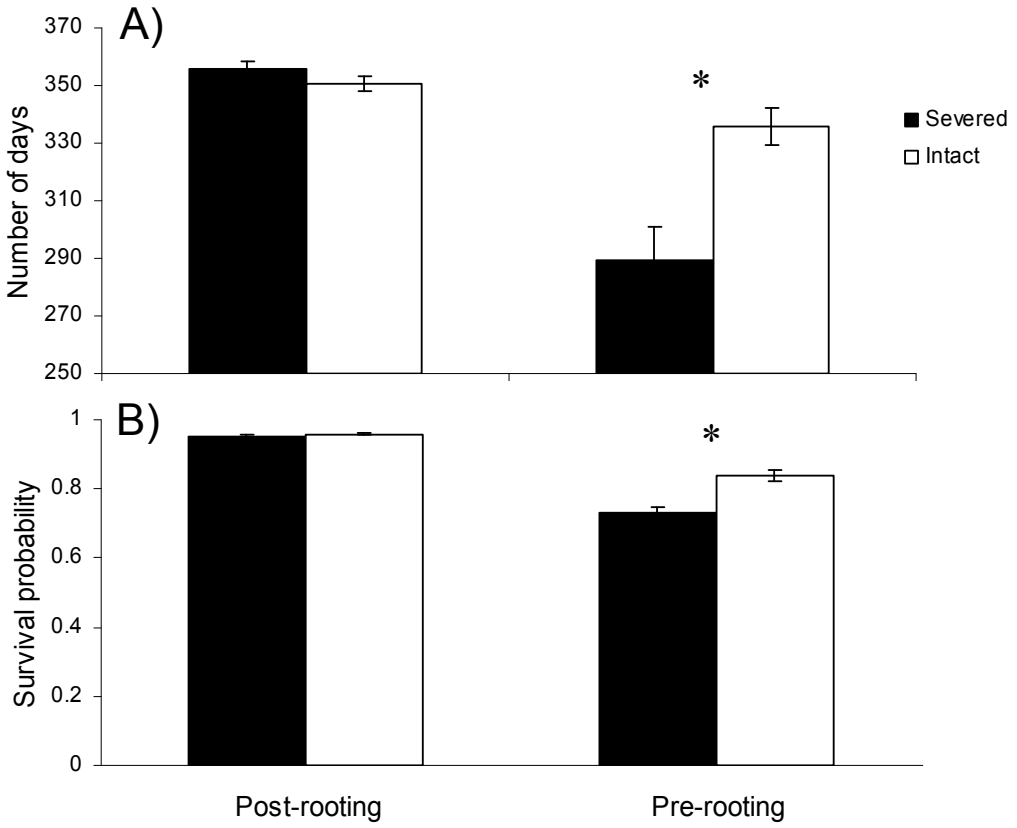

Figure 2.6. Mean (A) number of days until death (±1 SE), estimated by the product-limit method, Kaplan Meier and (B) cumulative mean probability of survival (±1 SE) at the end of the study for severed (connection to parent severed) and intact (connection to parent intact) offspring ramets. Significant differences, at the $p < 0.05$ by t-test are indicated with an asterisk (*).

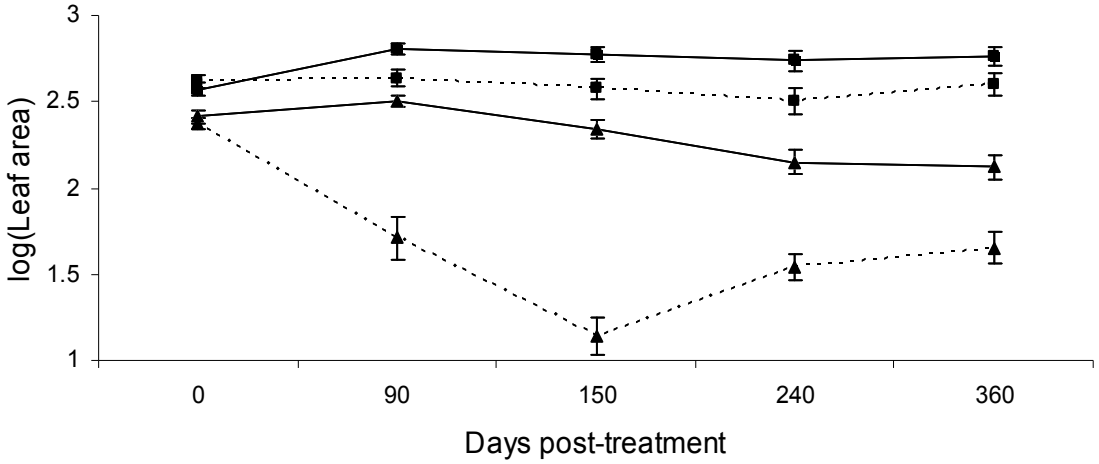

Figure 2.7. Mean (± SE) leaf area (log scale) of pre-rooting (triangles) and post-rooting (squares) offspring with severed (dashed) and intact (unbroken) connections to their parent measured as square centimeters.

Chapter III

Sex for free? No demographic cost of sexual reproduction[3]

SUMMARY

Life history theory predicts that there will be a trade-off between current reproduction and future growth, survival or reproduction and, therefore, reproducing may have a demographic cost. It remains unclear if clonal understory herbs experience a demographic cost associated with reproducing, or if larger costs are displayed by plants growing in areas where key resources are in short supply. We investigated this issue by manipulating the sexual reproductive effort of *Calathea marantifolia* plants and measured their subsequent demographic performance, as well as the performance of their clonal offspring. We analyzed the cost of reproduction within the context of natural variation in light availability by using Global Site Factor, an estimate of light availability, as a covariate in analyses of demographic performance. *C. marantifolia* plants were randomly assigned to low (removal of immature inflorescences), medium (inflorescences covered with mesh bags to prevent fruit production) and high (open pollination) sexual reproductive effort treatments (n = 93 per treatment). We measured the growth, survival and reproduction of plants and the growth and survival of their subsequently produced clonal offspring. We found that the large difference in sexual reproductive effort displayed among treatments did not result in a reduction of growth, survival or reproduction the following season. Clonal offspring produced after the treatments were applied were smaller in the high reproductive effort group but the difference in size

[3] Coauthor: Carol C. Horvitz

among treatment groups disappeared with time. Clonal offspring survival and developmental timing did not differ among treatments. Neither the demographic performance of plants nor of their clonal offspring was significantly influenced by light availability in this study, although low natural variability in light could be the cause of this result. Our results add to a large number of studies failing to demonstrate a demographic cost of reproduction in perennial understory plants.

BACKGROUND

In theory, allocating limited resources to reproduction should leave fewer resources available in the future for survival, growth, reproduction, and/or defense, and is thereby referred to as a 'cost' of reproduction (Reznick, 1985; Obeso, 2002). Therefore, increasing allocation to sexual reproduction may result in a measurable decrease to another competing function (Levins, 1968). The underlying physiological mechanism responsible for these trade-offs should lead to a demographic trade-off (Bell and Koufopanou, 1986). Quantifying how life history trade-offs influence fitness components is crucial to understanding the evolution of specific life history strategies.

A common strategy among perennial herbs in forest understories is to reproduce both sexually and clonally (Bierzychudek, 1982; Klimes et al., 1997; Aarssen, 2007). The growth and reproduction of perennial herbs is often limited by the availability of light (e.g. Chazdon, 1988; Fetcher et al., 1994) which is heterogeneously distributed over space and time (Anderson, 1964; Chazdon and Pearcy, 1991). It remains unclear to what extent investment in sexual reproduction influences the future demographic success of

both parent plants and their clonal offspring, and if these potential trade-offs are affected by heterogeneity in light.

The underlying mechanism responsible for a cost of reproduction is a physiological trade-off (Cody, 1966; Roff, 1992) the strength and timing of which can be measured using different currencies. Direct costs quantify immediate reductions in allocation (Newell, 1991; Ashman, 1992; Nicotra, 1999), typically in terms of biomass (Chapin, 1989; Obeso, 2002). To understand how costs of reproduction impact fitness the long-term or 'indirect cost' of reproduction should be quantified in terms of reductions in vital rates (e.g. growth, survival, and reproduction; Williams, 1966; Newell, 1991; Ashman, 1992).

The demographic cost of reproduction has primarily been quantified using two approaches. Correlation studies use natural variation in reproductive investment and measures of a potential cost (e.g. Shefferson and Simms, 2007), but may confound reproductive history and microhabitat with current reproductive investment. Experimental approaches assign plants to levels of reproductive investment, and thus have the advantage of keeping age and microhabitat of individuals similar (Stearns, 1989; Partridge, 1992; Obeso, 2002). Reproductive investment in plants can be manipulated by removing inflorescence buds in the immature stage (e.g. Horvitz and Schemske, 1988), preventing pollination by covering flowers (e.g. Saikkonen et al., 1998) and/or hand pollination to increase fruit-set (Calvo, 1993).

The majority of empirical studies have supported the predictions of life-history theory that reproduction has an associated cost in terms of future reduction in either survival, growth or reproduction (reviewed in Reznick, 1985; Roff, 2000). The trade-off

between sexual and clonal reproductive modes has received less attention than the trade-off between reproduction and growth of the parent plant (Bazzaz, 1997). It is thought that sexual and clonal reproductive modes compete for limited resources within a plant (e.g. Abrahamson, 1980; Cook, 1985; Eriksson, 1997). The majority of clonal herbs have sequential reproduction, with sexual reproduction occurring before clonal reproduction (Abrahamson, 1980). This situation has led to the hypothesis that clonal reproduction receives resources that are left-over from sexual reproduction because of low abundance or quality of pollinators (Abrahamson, 1980). This is supported by many studies which have reported a trade-off in allocation between sexual and clonal reproduction (Obeso, 2002), although the diversity of clonal growth-forms makes generalizations difficult. Increased allocation to sexual reproduction can result in a decrease in the size or number of clonally produced offspring (Law et al., 1983), rhizomes (Sohn and Policansky, 1977; Tobler et al., 2006), tubers (Westley, 1993; Mendez, 1999), axillary buds (Worley and Harder, 1996) rooted nodes (Prati and Smith, 2000), corms (Snow and Whigham, 1989), and rooted branches (Sutherland and Vickery, 1998). We studied the trade-off between reproductive modes in *Calathea marantifolia*, which has a form of clonal reproduction that has received no attention in the clonal plant literature, where clonal bulbils are produced above ground on reproductive shoots after fruiting has occurred. In this growth-form sexual and clonal reproduction are coupled in time (clonal reproduction occurring directly after sexual reproduction) and in proximity within the plant (both types of reproduction occurring on the same shoot).

The cost of reproduction can be influenced by plant size and by the availability of resources in the environment. Because the occurrence of reproduction and reproductive

effort are often size dependent in plant populations (Worley and Harder, 1996; Hemborg and Karlsson, 1998; Greer and McCarthy, 2000), it is necessary to consider size when investigating reproductive costs. Therefore, comparisons of demographic parameters between groups of plants with differing reproductive effort should include the relationship between demographic performance and plant size. Additionally, because the cost of reproduction is thought to result from limited resources being traded-off between competing functions, the availability of key resources may be an important covariate in analyses of the cost of reproduction. It has been proposed that costs of reproduction may only be observed when resource levels drop below a critical threshold (Reznick, 1985; Tuomi et al., 1997). This has been supported by studies showing a cost of reproduction in low fertility soils but no cost in high fertility soils (e.g. Biere, 1995). We investigate for a neotropical understory herb if the demographic cost of sexual reproduction is influenced by plant size, as well as natural variation in light availability. In this study we did not, however, experimentally control light; instead plants were found at random and therefore represented the most common environment, shaded understory.

To examine the hypothesis that increased sexual effort exacts a cost on future demographic performance of parent plants and their clonal offspring we experimentally manipulated sexual reproductive effort of *C. marantifolia* and measured the demographic performance in the following season. We address the following questions:

1) Does sexual reproductive effort influence future demographic performance for parent plants or their subsequently produced clonal offspring?
2) Does natural variation in the availability of light or plant size influence the cost of sexual reproduction?

METHODS

STUDY SYSTEM—The study population was located near Sirena Biological Station, Corcovado National Park (8º28'49''N, 83 º35'22''W), on the Pacific coast of Costa Rica. The region is described as tropical wet forest receiving >5 m of rain annually (Hartshorn, 1983). Over 85% of the rain falls May-November with a dry season extending from December - April (Sirena Biological Station *unpublished data*). Our study sites were located in secondary forest and were cattle pasture before the park was created in October 1975 (Phillips, 1989).

EXPERIMENTAL DESIGN—To investigate the effect of sexual reproductive effort on the demography of parent plants and clonal offspring, we conducted an experiment with one treatment factor (sexual reproductive effort) and three levels (low, medium and high sexual reproductive effort). Treatments were applied from August 15 to Sept 15, 2005 by locating groups of three *C. marantifolia* individuals with an immature inflorescence (prior to flower bud emergence) in the secondary forest surrounding Sirena Station. Individuals were randomly assigned to one of three treatments (n = 93 per treatment). In the low effort treatment, we removed the immature inflorescence. The bud stage of the inflorescence has floral meristems in each bract (up to 36 bracts) and because most flower buds are not formed at this point, this is a stage where resources for flower and fruit production have not been invested in the inflorescence. In the medium reproductive effort treatment, we covered the inflorescence with a mesh bag, preventing pollination and fruit production. In the high reproductive effort treatment, we did not

perform any manipulations and plants experienced natural levels of fruit-set by open pollination.

We were interested in knowing if natural variation in light availability affects the cost of variation and therefore the availability of light for each parent plant was quantified from photos taken on March 3-17, 2006 directly above each plant using a Nikon Coolpix 4500 digital camera and a Nikon FC-E8 fisheye converter lens (180º field of view). The camera was mounted on a tripod and leveled prior to photographing. Photographs were taken in the early morning or in late afternoon or when it was evenly overcast (Pearcy, 1989; Rich, 1989). Photographs were oriented with north at the top of the image, allowing superposition of solar tracks. We used Gap Light Analyzer software (Frazer et al., 1999, 2000) to estimate direct site factor ('percent transmittance direct'), indirect or diffuse site factor ('percent transmittance diffuse') and the global site factor ('percent transmittance total'). Direct (DSF) and indirect (ISF) site factors are defined as the proportion of direct and diffuse radiation received below the canopy as a fraction of that received above the canopy (Anderson, 1964; Rich, 1989). Global site factor (GSF), represents the total proportion of light reaching a site, and is estimated as

$$GSF = (ISF + DSF) / 2 \quad (1)$$

(Canham et al., 1990). Site factors can range from 1 (open sky) to 0 (complete obstruction).

DATA COLLECTION AND ANALYSES**:** SEXUAL REPRODUCTIVE EFFORT—After the treatments were applied, reproductive censuses were conducted approximately every 14 days for three months (9 censuses) to estimate sexual effort (number of flowers and immature fruits). It should be noted that *Calathea* inflorescences

produce two, or rarely four, flowers a day and each flower is present for one day only (Kennedy, 1978), thus our biweekly censuses of flowers does not represent total flower production. *Calathea* fruits require many days to mature (Kennedy, 1978). The effect of treatment on the total number of flowers and fruits observed was analyzed using a one-way Kruskal-Wallis non-parametric test. All statistical tests were performed using SPSS (Levesque, 2007). The relationship between the number of bracts and production of immature fruits for plants in the high sexual effort treatment was examined using linear regression.

PARENT PLANT DEMOGRAPHIC PERFORMANCE—Leaf area of the parent plant was estimated using leaf lengths and a regression relationship between leaf length and area specific to *C. marantifolia* at Corcovado (Horvitz and Le Corff, 1993). Total leaf area was estimated the first season when the treatments were applied and all shoots were individually marked so as to be able to identify new (i.e. unmarked) shoots the next season. The second season total and new leaf area (defined as the area of leaves produced between seasons, i.e. leaf area on unmarked shoots) were estimated when parent plants had immature inflorescences present, to allow comparison between seasons. Leaf area was analyzed using a one-way ANOVA. To examine if growth was influenced by our treatments, we compared the slope of the relationship between total leaf area the first and second seasons among treatments using an ANCOVA (Zar, 1999).

The survival of parent plants at the end of the second reproductive season was recorded, and we compared the percent survival among treatments using a contingency table analysis (Zar, 1999). We investigated how sexual effort influenced the timing of two important reproductive events; the initiation of clonal offspring production and

inflorescence production the second season. Censuses of each parent plant were conducted five times, roughly every 5.5 months between March 2006 and August 2007 to record when these events occurred. Using Kaplan-Meier survival analyses coupled with Log-Rank tests we estimated and compared the times until clonal offspring were initiated and until inflorescences were produced, among treatments (Fox, 2001; Levesque, 2007). In each season, inflorescence size was estimated by the number of bracts and analyzed using a one-way ANOVA.

Not all parent plants produced an inflorescence in the second season (plants reproductive in the second season: high effort $n = 58$, medium effort $n = 58$, low effort $n = 60$). We investigated whether the percent of plants that reproduced the second season depended upon reproductive effort the first season using a contingency table analysis (Zar, 1999). Additionally, to determine which factors affected whether plants reproduced in the second season we used a logistic regression. The binary dependent variable was the production of an inflorescence in the second season and the independent variables were reproductive effort treatment category, GSF, leaf area and the number of inflorescence bracts in the first season.

CLONAL OFFSPRING DEMOGRAPHIC PERFORMANCE—We recorded survival and estimated total leaf area, as described above, of clonal offspring approximately every 5.5 months between March 2006 and August 2007. We analyzed changes in clonal offspring leaf area using a repeated-measures ANOVA. The main effects of the model were sexual reproductive effort (low, medium and high), parent plant size, light availability (covariate) and time. We analyzed clonal offspring survival and

developmental phenology using a Kaplan-Meier survival analysis to calculate expected days until death, rooting and independence (Fox, 2001; Levesque, 2007).

RESULTS

SEXUAL REPRODUCTIVE EFFORT—Our treatments were successful in producing a gradient in reproductive effort, demonstrated by differences in the number of reproductive structures among treatment groups (Table 3.1). Plants in the low reproductive effort treatment did not produce flowers or fruits. The number of flowers observed on census dates did not differ between the medium and high sexual effort treatments (Z = -0.763, P = 0.445; Table 3.1). The number of fruits observed on census dates was much greater in the high sexual effort treatment compared to the medium sexual effort treatment (Z = -4.814, $P < 0.0001$; Table 3.1).

PARENT PLANT DEMOGRAPHIC PERFORMANCE—Irrespective of sexual reproductive effort in the first season, parent plants survived, grew and reproduced equally well the second season (Table 3.1). Plants had an equally high probability of surviving to the next flowering season (approximately 97-98%) among treatment groups (Table 3.1; X^2 = 0.816, d.f. = 3, P = 0.199). Total leaf area did not differ across treatment groups at the time the treatments were applied or the following season (Table 3.1). Plants grew at a similar rate among treatment groups; the amount of leaf area added between flowering seasons was the same (Table 3.1), as was the relationship between leaf area in the first and second seasons among treatments (Fig. 3.1; ANCOVA $F_{2,3}$ = 0.292, P = 0.747). Light availability (GSF) did not affect leaf area in either season (Linear regression: first P = 0.356 and second season P = 0.460).

In the year the treatments were applied, larger plants had inflorescences with more bracts than smaller plants (Fig. 3.2A). In plants in the high reproductive effort treatment, inflorescences with more bracts produced a greater number of immature fruits than inflorescences with fewer bracts (Fig. 3.2B; Linear regression $F_{2,103} = 200.1$, $P < 0.0001$, $r^2 = 0.765$). Approximately 300 days after the first season's inflorescence had senesced plants began to produce a new inflorescence (Table 3.1). Nearly 62% of plants produced an inflorescence; the percent of plants producing an inflorescence did not differ among treatment groups (Table 3.1; $X^2 = 2.19$, d.f. = 3, $P = 0.33$). None of the measured variables influenced the probability of reproducing the second season; the logistic regression model using reproductive effort treatment group, GSF, leaf area and the number of inflorescence bracts in the first season failed to significantly explain variation in the probability of producing an inflorescence (Overall model $X^2 = 8.88$, d.f. = 5, $P = 0.114$). For those plants that did reproduce, similar to the first season, larger plants had inflorescences with more bracts than smaller plants and this relationship was the same among treatment groups (ANCOVA $F_{2,3} = 0.492$, $P = 0.527$; Fig. 3.2C).

Between the reproductive seasons all parent plants except one produced a clonal offspring, taking on average 210 days to initiate the process and an additional 300 days to become independent from the offspring (Table 3.1). Neither the production nor timing of offspring production was different among treatment groups (Table 3.1).

CLONAL OFFSPRING DEMOGRAPHIC PERFORMANCE—The demographic performance of clonal offspring was similar across treatments groups for most measured variables except size, which was affected by a time × sexual effort interaction (Table 3.2). During the first nine months, when clonal offspring were still connected to their

parents, clonal offspring in the low effort treatment were considerably larger than offspring in the other treatment groups (Fig. 3.3). However, after 16 months post-treatment, clonal offspring leaf area became nearly identical across treatments (Fig. 3.3). Clonal offspring leaf area was significantly affected by the leaf area of the parent plant but parent leaf area did not interact significantly with reproductive effort treatments to influence offspring leaf area (Table 3.2).

There was no difference in the expected days until death among clonal offspring among treatment groups (Table 3.1). The probability that clonal offspring would survive the study period was unrelated to light availability (Logistic regression $P = 0.656$). The expected days until rooting were the same across treatment categories (Table 3.1). However, the expected days until independence from the parent plants was significantly lower for clonal offspring in the low sexual effort treatment compared to the other treatment groups (Table 3.1; Log-Rank test of homogeneity of time to rooting among treatment levels $X^2 = 5.3$, d.f. = 2, $P = 0.068$).

DISCUSSION

Our results indicate that, under the range of light environments studied, increased sexual reproduction of *Calathea marantifolia* does not reduce the demographic performance of parent plants or their clonal offspring the following year. The dramatic gradient in sexual reproductive effort we created, surprisingly did not alter the survival, growth or reproduction of parent plants the following season. Additionally, clonal offspring were produced at the same rate by plants in different treatment groups and

showed only a slight difference in their phenology of independence. Early-on, clonal offspring size was different among treatment groups, and in accordance with the predicted trade-off between reproductive modes, the largest offspring were produced from parents that invested little in sexual reproduction. Surprisingly, at the first census clonal offspring produced from parent plants with medium sexual reproductive effort were smaller than offspring produced on parent plants with high sexual effort, which is not consistent with a linear trade-off between sexual and clonal reproduction. We have not thought of an hypothesis that would explain this surprising result. The effect of sexual reproductive effort of parents on offspring size diminished over time, and therefore, offspring were of a similar size at the end of the study. Results of this study, and others, reporting no, or little, demographic cost to reproduction provide an opportunity to assess the conditions under which predictions of allocation theory are consistent with field observations.

Results from the majority of previous studies have been consistent with the predictions of life history theory, finding a trade-off between allocation to current reproduction and future survival, growth or reproduction (reviewed in Reznick, 1985; Tuomi et al., 1988; Obeso, 2002). Studies that have focused specifically on the trade-off between sexual and clonal reproductive modes predominantly have also found evidence of a trade-off (reviewed in introduction) but not always. In contrast, our experiment and several other studies have not found a demographic cost of sexual reproduction to the parent plant e.g. Kull, 1998; Horvitz and Schemske, 1988) or clonal offspring (Verburg and During, 1998; Cruz and Moreno, 2001).

We also compare results from our experiment to those of two studies investigating reproductive costs in the congeneric *Calathea ovandensis* (Horvitz and Schemske, 1988, 2001). Horvitz and Schemske (1988) experimentally created plants with either high (open pollination and removal of an herbivore of reproductive structures) or low reproductive effort (removal of immature inflorescences). Within the growing season *C. ovandensis* did display a cost of reproduction. Plants with high reproductive investment grew less and produced fewer inflorescences and fruits (Horvitz and Schemske, 1988), but the following year no demographic cost was observed, consistent with our results. Similarly, Horvitz and Schemske (2001) correlated natural variation in size and reproduction across five years and never found evidence reproductive plants had lower demographic performance compared to non-reproductive plants.

Several hypotheses have been proposed to explain why organisms may not experience reproductive costs. An evolutionary explanation is that trade-offs are traits shaped by natural selection, and under natural conditions there is strong selection against individuals that suffer a high reproductive cost (Jönsson and Tuomi, 1994) and therefore, plants may express a cost in terms of whichever currency has the smallest effect on fitness. This idea is consistent with results showing that the cost of reproduction is more often observed as a reduction in growth than survival (Reznick, 1985; Shefferson and Simms, 2007). In some species selection against a demographic cost, for either growth or survival, may be so strong that only an extremely small cost is expressed.

Alternatively, arguments based on the 'threshold hypothesis' (Tuomi et al., 1983) do not view reproductive costs as fixed, but instead as plastic responses to environmental variability. Because a cost of reproduction results from the trade-off of limited resources,

the threshold hypothesis argues a demographic cost may only be observed at times, or in locations, where the availability of key resources drop below a specific threshold (Tuomi et al., 1983). We quantified the natural variation in availability of light, which is a key resource influencing the demographic performance of understory herbs. We found that light availability (GSF) did not have a significant interaction with sexual effort in its effect on parent and clonal offspring demographic performance, indicating that across the observed range of light environments the cost of reproduction was not affected by variation in light. However, light was not explicitly controlled and the range of light environments encountered by choosing individuals at random in our study (GSF 0.11-0.03; median = 0.05) was relatively narrow compared to the total range of environments in which *C. marantifolia* maybe found at the study site (GSF 0.25-0.03).

Another explanation for the lack of a demographic cost of reproduction is that organisms may compensate for increased sexual effort by altering their resource intake, allowing them to mask the cost. Plants may alter their rates of photosynthesis or other metabolic functions to increase resource intake, compensating for increased investment in reproductive structures (Obeso, 2002). Plants could also draw resources from stored underground reserves to subsidize reproductive investment. Interestingly both our study species, *C. marantifolia*, and the *Calathea* species studied by Horvitz and Schemske produces underground tubers (approximately 6 cm × 3cm) attached to the roots that may store resources, which could be utilized to buffer reproductive costs. For species with underground reserves, demographic costs may be delayed (Ehrlen and Groenendael, 2001) and therefore only observable over the long-term.

A separate transplant experiment which investigated the effect of light heterogeneity on the demographic performance *C. marantifolia* offspring found that clonal offspring were much less sensitive to light availability than seedlings (Chapter 1). This experiment is consistent with the present study, in that light availability, within the moderately open to shaded understory environment (GSF 0.10-0.05), did not influence clonal offspring survival. However, these two experiments are not consistent in their results on clonal offspring growth. In the transplant experiment where light levels were experimentally manipulated, clonal offspring growth was positively affected by light availability. In our current study, we did not find that GSF influences clonal offspring growth. A changing light environment such as may be experienced during gap formation and was produced by our transplanting individuals, may trigger an increase in growth, which was not observed in the present study. A separate severing experiment investigated the demographic cost to parent plants for supporting clonal offspring (Chapter 2). It was found that clonal offspring do receive a demographic boost from being attached to their parent plant. However, similar to the current study, the help the parent plant provides for the clonal offspring was not found to reduce the parent plant's demographic performance.

The present study combined with our severing and transplant experiments examine the influence of light on the production and performance of sexual and clonal offspring in *C. marantifolia*. We found that sexual and clonal offspring have differing abilities to recruit across the light gradient, and therefore may play distinct roles in population dynamics across space and time. Additionally, the production of both offspring types is not associated with a demographic cost for the parent plant. Together

these results suggest a distinct advantage of this mixed reproductive strategy (broader range of recruitment sites) and no measureable cost.

Table 3.1. Effects of experimentally induced sexual reproductive effort on growth, survival and reproduction of the parent plant and growth and survival of subsequently produced clonal offspring. Description of the treatments is detailed in the methods. Bold indicates a significant difference among sexual effort categories, statistical test scores are reported in the text. Global site factor (GSF) is a measure of light availability, and its estimation is described in the methods.

Parameters of growth and reproduction	Sexual reproductive effort		
	Low	Medium	High
	Mean (SE)	Mean (SE)	Mean (SE)
Parent plant			
Sexual reproductive effort			
No. flowers	**0**	**20.4 (2.9)**	**17.9 (2.6)**
No. immature fruits	**0**	**2.0 (0.4)**	**46.0 (4.7)**
Light availability			
GSF	0.13 (0.003)	0.13 (0.004)	0.13 (0.003)
Leaf area			
First season (cm^2)	6242 (249)	6454 (245)	7103 (317)
Second season- total (cm^2)	7311 (348)	6978 (299)	6840 (362)
Second season- new (cm^2)	5372 (279)	4985 (245)	4964 (303)
Survival			
Percent survival	97	98	98
Subsequent reproduction			
Expected days until inflorescence production	299 (5)	307 (5)	313 (5)
Expected days until clonal offspring initiation	201 (10)	225 (10)	207 (9)
No. of inflorescence bracts	12.9 (0.7)	11.7 (0.7)	12.2 (0.8)
Percent of plants reproducing	61	63	61
Clonal offspring			
Developmental phenology			
Expected days until rooting	483 (13)	490 (14)	469 (13)
Expected days until independence	**487 (18)**	**532 (15)**	**511 (13)**
Survival			
Expected days until death	616 (8)	630 (7)	630 (7)
Percent survival at end of study	68	74	75
Leaf area			
Leaf area after 24 months (cm^2)	873 (170)	678 (84)	923 (131)

Table 3.2. Repeated measures two-factor ANOVA for the effects of sexual reproductive effort (low, medium and high) time, parent size (log of leaf area) and Global site factor (GSF) on clonal offspring size (log of leaf area) over the 24 month study period. GSF and size of parent plant were used as a covariates in the analysis.

Source of variation	df	SS	*F*	*P*
Between subject effects				
Sexual reproductive effort	2	1.05	1.343	0.265
GSF	1	0.08	0.217	0.642
Size of parent	1	9.97	25.45	**>0.0001**
Sexual reproductive effort × GSF	3	0.82	0.58	0.625
Sexual reproductive effort × Size of parent	2	1.63	2.12	0.124
Error	125	48.99		
Within subject effects				
Time	1	0.01	0.36	0.849
Time × Sexual reproductive effort	2	1.14	3.18	**0.045**
Time × GSF	1	0.238	1.332	0.251
Time × Parent size	1	0.07	0.06	0.926
Error	125	22.34		

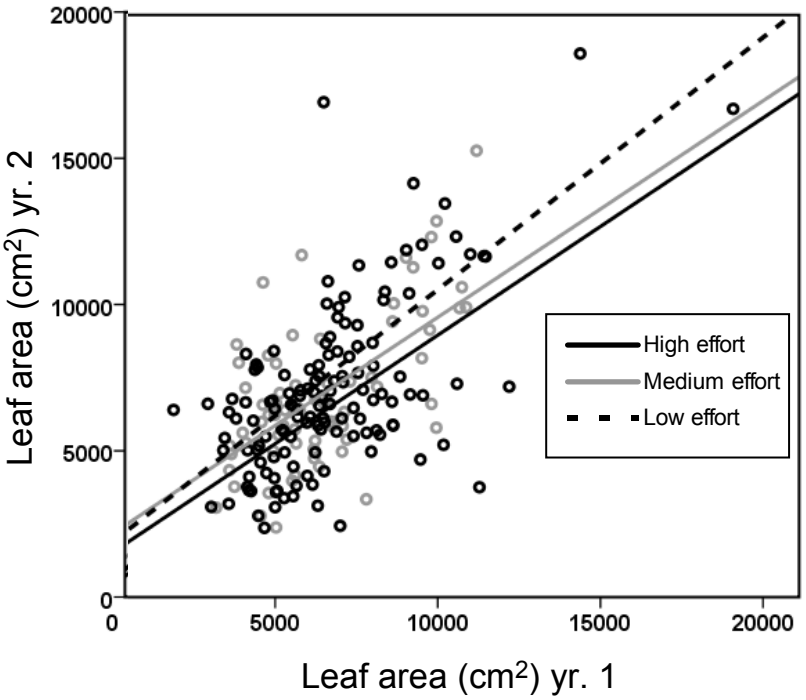

Figure 3.1. The relationship of size in year 2 (the year subsequent to the treatment) to size in year 1 (the year of the treatment), for each reproductive effort treatment. This relationship did not differ among treatment groups (see text).

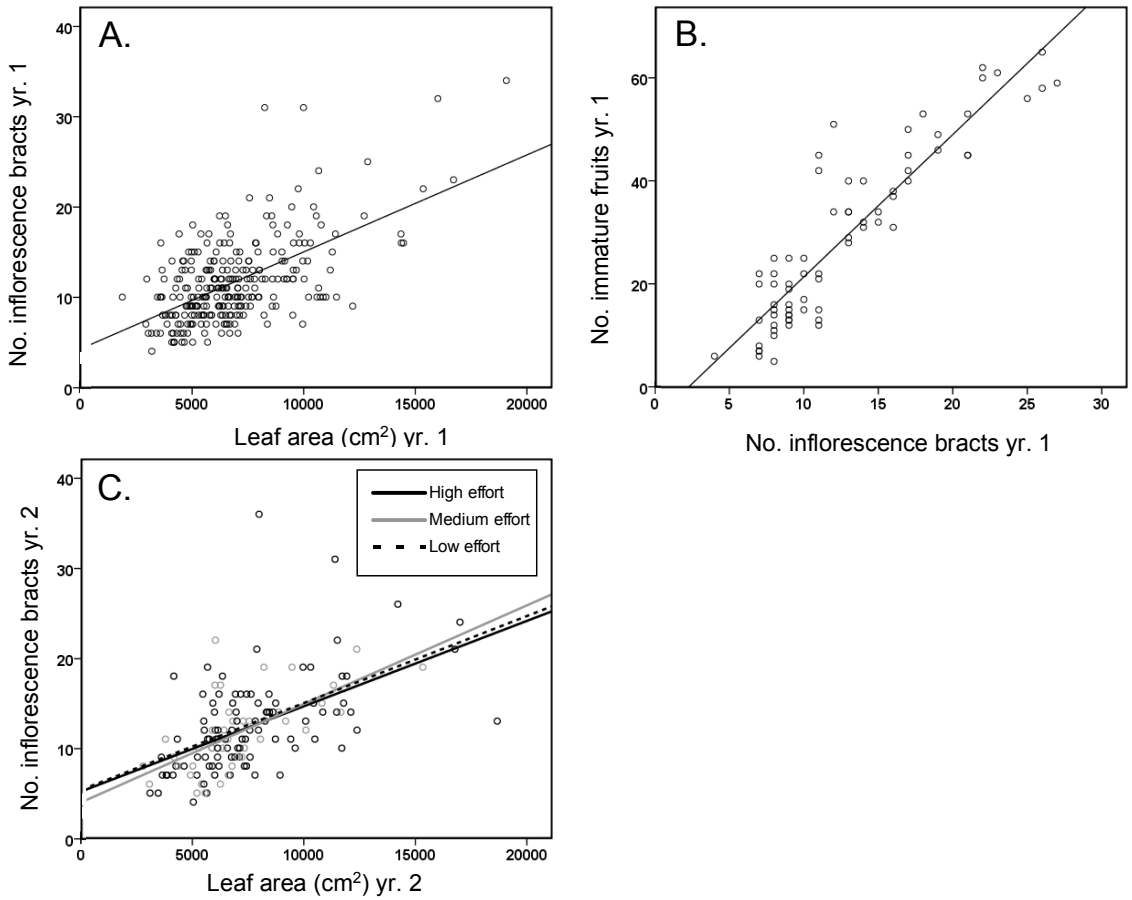

Figure 3.2. Size dependency of *C. marantifolia* reproduction. Relationship between plant leaf area and the number of inflorescence bracts in the first season when reproductive effort treatments were applied (A). Relationship between the number of inflorescence bracts and the total number of immature fruits observed on census dates for plants in the high reproductive effort treatment during the first season (B). Relationship between plant leaf area and the number of inflorescence bracts in the second season (C).

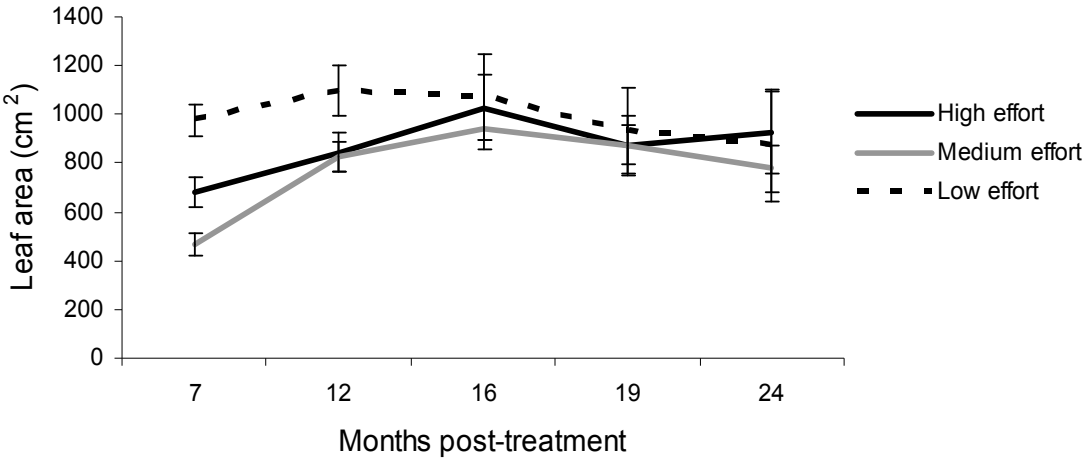

Figure 3.3. Mean leaf area (± SE) of clonal offspring produced from parent plants that experienced low, medium and high sexual reproductive effort the prior flowering season. Description of the treatments is detailed in the methods. The majority (>80%) of clonal offspring rooted and became independent in the interval between 12 and 16 months post-treatment.

Chapter IV

Developing an integral projection model to evaluate the contribution of sexual versus clonal reproduction to population dynamics[4]

SUMMARY

It is unclear whether the relative contribution of clonal and sexual reproduction to long term population dynamics varies with light availability within forest understories. In forest understories, many herbs produce both sexual and clonal offspring and are found across the light gradient from open canopy tree fall gaps to shaded closed canopy. In this chapter we show how an integral projection model of population dynamics provides a new way to evaluate the importance of clonal and sexual reproduction to population growth. We use data on the Neotropical understory herb *Calathea marantifolia* at different points along the light gradient to begin to examine the relative importance of the two reproductive modes in different environments to population growth. In a size-structured IPM, recruitment to various size classes is incorporated into the projection kernel as a layer added onto the survival-growth part of the dynamics. Here we extend this approach to two recruitment layers: one for sexually produced offspring and one for clonally produced offspring. This chapter constitutes the first step in model development. In a later paper we will expand this model to incorporate, first, seasonal variability and, second, inter-annual variability in demography to examine consequences for long run dynamics in a variable environment. Demographic data (growth, survival and reproduction) of individuals were recorded in eight plots (four high light and four low

[4] Coauthor: Carol C. Horvitz

light) in Corcovado National Park, Costa Rica from August 2004 until August 2007. In this paper we take a subset of these data, the first census interval (Aug. 2004 – Mar. 2005) to develop the basic integral projection model for our species. We also evaluate the contribution to variability in population dynamics between light and dark environments made by different sized individuals using a life table response experiment (LTRE) approach. Assuming a time-invariant model based on the first census, the rate of population growth (λ) was 8 % higher in high light ($\lambda = 1.33$) than in low light ($\lambda = 1.25$) per half year. LTRE analysis revealed that this difference in λ was primarily because of improved survival and growth of large sizes and to a lesser, extent increased clonal reproduction in high light. Removing sexual reproduction from the model reduced λ dramatically in both high (0.32 reduction in λ) and low light (0.21). Conversely, removing clonal reproduction from the model resulted in only a modest decrease in λ in both high (0.05) and low light (0.02). Overall, our results suggest that the population dynamics of *C. marantifolia* differs between light levels and that sexual reproduction contributes greatly to λ.

BACKGROUND

Plant populations within forest understories experience a gradient in the availability of light that may play an important role in their population dynamics. Light availability in the understory is highest where a gap in the canopy has been created by a tree fall and lowest where the canopy is closed. This gradient in light is known to affect the growth and survival of understory herbs (e.g. Barkham, 1980). The light gradient is

dynamic due to tree fall gaps and gap-phase regeneration but how the dynamic heterogeneity in light translates into an effect on population growth rate (λ) is not well understood. Because light has a positive effect on the vital rates of understory plants, λ also may be positively affected by light, but it is not known if individuals of all sizes and reproductive modes contribute equally to an increase in λ or if certain-sized individuals or a certain reproductive modes play a larger role than others. Understory herbs often have two distinct reproductive modes; sexual reproduction produces seeds and seedlings while vegetative reproduction produces clonal offspring typically as plantlets (e.g. Bierzychudek, 1982; Cook, 1985; Kanno and Seiwa, 2004). Each reproductive mode has the potential to respond differently to light availability thereby influencing λ differently. In species with these two reproductive modes, each type of offspring may be adapted for a distinct 'ecological situation' (Maynard Smith, 1978; Burt, 2000), thereby increasing the ecological niche breadth of the species as a whole. In understory herbs, sexual and clonal offspring may contribute differently to population growth at different points along the gradient of light availability.

Previous studies of clonal plants have found that sexual reproduction contributes far less than clonal reproduction to population growth (Bierzychudek, 1982; Eriksson, 1988, 1989; Nault and Gagnon, 1993; Silvertown et al., 1993; Dammon and Cain, 1998; Mandujano, 2001; but see Weppler et al., 2006). One explanation for this generalization may be that seeds have a large fitness payoff only in situations that are rare in time and space. In forest understories seedling recruitment is often infrequent and restricted to high light sites (Hartnett and Bazzaz, 1985; Hughes et al., 1988; O'Dea et al., 1995; Kanno and Seiwa, 2004). Conversely, clonal offspring frequently recruit even under

shaded understory conditions (De Steven, 1989; Eriksson, 1989). The understory light gradient can also differentially affect the production and performance of sexual and clonal offspring. High light levels have been found to increase the production of both types of offspring (Ashmun and Pitelka, 1984; De Steven, 1989; Cunningham, 1997; Svenning, 2000), while low light levels have been found to reduce seed production to near zero but not affect clonal offspring production (Abrahamson, 1980; Eriksson, 1997). The demographic performance (survival and growth) of clonal offspring is often less sensitive to resource availability than that of sexual offspring (Harper, 1977; Abrahamson, 1980; Cook, 1985; Chapter 1). Therefore, we propose that differences in population growth between high and low light conditions may be due to the differential production or demographic performance of sexual and clonal recruits.

Two approaches can be used to evaluate how demographic variables influence growth rates: prospective and retrospective analyses (Horvitz et al., 1996; Caswell, 2001 p. 258). Prospective approaches (e.g. sensitivity and elasticity analyses) evaluate how hypothetical changes in vital rates would change population growth. Retrospective approaches (e.g. life table response experiments- LTREs) decompose observed differences in population growth rate into contributions from individual demographic variables (Caswell, 2001 p. 258). We used both sensitivity and elasticity analyses and a LTRE to understand the differences in population growth of an understory herb growing in high and low light levels.

During the last two decades, questions about the contribution of life-cycle components to plant population dynamics have been addressed using projection matrix models, which are appropriate when vital rates (growth, survival and fecundity) vary

among discrete stages (Schemske et al., 1994; Caswell 2001). More recently, integral projection models (IPM) have provided an alternative, and are appropriate for species with vital rates that vary in response to a continuous variable (i.e. size) (Ellner and Rees, 2006). IPMs use a regression approach to estimate the functional dependence of survival, future size and reproduction on current size. These statistical results are combined to yield an integral kernel that projects a population forward in time in a manner analogous to a population projection matrix. Under similar assumptions to those needed in matrix models, an integral kernel model yields an asymptotic population growth rate (λ), and associated eigenvectors and state-dependent sensitivity and elasticity functions (Easterling, 1998; Easterling et al., 2000; Ellner and Rees, 2006). In contrast to matrix projection models, the population is not divided into discrete classes, and fewer parameters are estimated from the data. IPMs estimate the parameters (e.g., slopes and intercepts) of three regression relationships; the regression of survival, future size, and reproduction on current size (Easterling, 1998; Easterling et al., 2000).

Here we use an IPM to examine the importance of sexual and clonal reproduction for population dynamics in high and low light conditions. Specifically, we examine how the IPM can be used to answer the following question:

1) Do sexual and clonal reproductive modes contribute to population growth equally and do their contributions change across light environments?
2) Is the demographic quality, as measured by population growth rate, higher in tree fall gaps where light availability is high or in the shaded understory where light availability is low?

3) Which size-specific vital rates are responsible for differences in population growth between high and low light levels?

METHODS

STUDY SYSTEM—We studied the population biology of *Calathea marantifolia* in secondary forest surrounding Sirena Biological Station, Corcovado National Park (8º28'49''N, 83 º35'22''W), on the Pacific coast of Costa Rica. The region is described as tropical wet forest receiving >5 m of rain annually (Hartshorn, 1983). Over 85% of the rain falls from May-November with a dry season that extends from December-April (Sirena Biological Station, *unpublished data*). Our study sites were located in secondary forest that was cattle pasture prior to the park's creation in October 1975 (Phillips, 1989).

DATA COLLECTION—We recorded the survival, growth and reproduction of *C. marantifolia* in eight permanently marked plots from August 2004 until August 2007. The plots were chosen to represent the extremes of light availability for *C. marantifolia* in our study area. To select plot locations, 93 patches of *C. marantifolia* were located along established trails, and the canopy openness of each patch was estimated using the canopy scope technique (Brown et al. 2000). We used a stratified random sample of these choosing four patches in high light levels (0.3-0.4 canopy scope score) and four patches to represent low light levels (0.1-0.2 canopy scope score). The dimensions and overall area of each plot differed, but each was located in a relatively uniform light environment and contained approximately 100 *C. marantifolia* individuals. To increase sample sizes of reproductive plants and clonal offspring for estimation of their fates,

additional reproductive plants and clonal offspring were monitored directly outside each plot.

Within each plot, we marked individuals in August 2004 and followed their survival (biannual- March and August), growth (biannual- March and August), and reproduction (monthly during the 2004 reproductive season) until August 2007. Therefore, we collected data on three August-March census intervals and three March-August census intervals. The goal of the present chapter is to show how these data can be used to develop an IPM for a plant that has both clonal and sexual offspring. Here we use data from only the first census interval, August 2004 - March 2005. Subsequent papers will parameterize each census interval separately and then create the appropriate temporal sequences that include both seasonal and inter-annual variability to examine consequences for long run population growth.

Each plant was individually marked using an aluminum tag fixed to the ground at the plant's base using a flag with a metal stake. Canopy openness was estimated directly above each plant using the canopy scope technique during the August census (Brown et al., 2000). The average canopy scope score per plot was significantly higher in high light plots than in low light plots (Fig. 4.1; Mann-Whitney, $U = 31157$, $P > 0.0001$). Canopy scope score is significantly correlated with percent canopy openness, measured using fish-eye photography, within the secondary forest of Corcovado ($r^2 = 0.695$, $P = 0.0001$, *D.Matlaga*, unpublished data).

We estimated the proportion of seeds that become seedlings at each plot with seed box experiments (Horvitz et al., 2002). Fresh seeds from newly dehisced capsules were located and collected haphazardly from *C. marantifolia* in the secondary forest near

Sirena Biological Station. Because of the scarcity of ripe seeds at any one time it was not possible to stratify seed source by light environment. Fresh seeds were planted on the day they were collected in soil in wire mesh boxes (15cm × 15cm× 5cm; eight seeds per box; 10 boxes per plot) during August 2006. Wire mesh boxes were filled with local soil and placed in the ground so that the top of the box was flush with the surrounding soil surface. Seeds were placed just below the soil surface and the top of the box was fastened closed to prevent seed loss. Boxes were placed at random points around the edge of each plot. We quantified seedling emergence for each box in March 2007.

The proportion of seeds planted in August 2006 that had become seedlings by March 2007 was not significantly different between light levels (Mann-Whitney, U = 670, P = 0.585; respectively, the proportion emerging in high and low light were 0.26 ± 0.20 and 0.33 ± 0.25, [mean ± SD]), which is consistent with results from another study at the same field site (Horvitz et al., 2002). The overall mean was 0.31± 0.22, which was used to parameterize the relationship between size and the number of seedlings (see below).

THE MODEL—Integral projection models describe how populations structured by a continuous individual-level state variable change over a discrete time interval (Easterling et al., 2000; Ellner and Rees, 2006). Although all published models to date have used size as the state variable, any continuous variable that is predictive of demographic rates can be used.

The state of the population is described by the size distribution $n(y, t)$, which can be thought of as the number of size y individuals in the population at time t. More formally, $n(y, t)$ is the number density of individual size y at time t, defined by the

property that the number of individuals between size y and $y + dy$ at time t is given by $n(y,t)dy$. Typically $n(y,t)$ is a continuous function of y. In each time step individuals in the population may grow, survive and produce offspring. The expression $p(x,y)dy$ describes the probability that an individual of size x at time t is alive and in the size interval $(y, y + dy)$ at time $t + 1$. The number of seedlings produced is described as $f(x,y)dy$, at time $t + 1$ in the size interval $(y, y + dy)$ per size x individual alive at time t. In our model, we additionally define the number of clonal offspring of size y produced as $c(x,y)dy$, at time $t + 1$ in the size interval $(y, y + dy)$ per size x individual alive at time t. Thus, the complete integral projection model with clonal reproduction for the number of individuals of size y at time $t + 1$ is

$$n(y,t+1) = \int_{\Omega} [p(x,y) + f(x,y) + \mathrm{c}(x,y)]n(x,t)dx \qquad (1)$$

with the integration being over the set of all possible states Ω (sizes in our case). Integral projection models utilize projection kernels which are analogous to population projection matrices. The kernel for our model is described as $k(y,x) = p(x,y) + f(x,y) + c(x,y)$ and is a nonnegative surface representing all possible transitions from size x to y (Easterling et al., 2000; Ellner and Rees, 2006). Therefore the equation for the number of individuals of size y at time $t + 1$ is simplified to

$$n(y,t+1) = \int_{\Omega} K(y,x)n(x,t)dx \qquad (2)$$

with the integration being over the set of all possible sizes Ω. Similar to matrix models, integral projection models provide an asymptotic population growth rate with its associated stable stage and reproductive value distributions, and sensitivities and elasticities of population growth (Easterling, 1998; Easterling et al., 2000).

At present we have developed a model based on a single census interval: the first wet season. Our goal goal here is to workout the steps of construction for this species and show how it can be used to address our question. At our field site, the year encompasses distinct wet and dry seasons; our model has so far been parameterized for one wet season only. Therefore, the current version is a time-invariant model projecting population dynamics based on vital rates of this one census interval. The next version of the model will include a dry season interval and we will build a periodic model that combines both wet and dry seasons. Then we will consider temporal variation among years as well to eventually construct a stochastic population dynamics model.

PARAMETER ESTIMATION—To model the population dynamics of *C. marantifolia* we pooled data within the four high and four low light plots. For each of the two light environments, we performed regressions of leaf area in August 2004 versus survival in March 2005, fruit production over the reproductive season, and leaf area in March 2005. We used the natural log of leaf area as our measure of plant size for all parameter estimations.

The growth and survival function $p(x, y)$ was estimated as

$$p(x,y) = s(x) + g(x,y) \qquad (3)$$

The survival function $s(x)$ was estimated by logistic regression of survival from August 2004 to March 2005 on leaf area (Fig. 4.2a; Table 4.1). The model was fit using a logistic regression with the linear link $\log(s(x)/(1-s(x)) = a + bx$. Growth $g(x, y)$ was

modeled as a linear regression of log(leaf area at $t + 1$) as a function of log(leaf area at t) (Fig. 4.2b; Table 4.1). The model was $y = a + bx$. Variance in size at t+1 was obtained from the regression analysis and was modelled as being independent of size at t.

The sexual reproduction function $f(x, y)$ was estimated by first fitting the relationship between log(leaf area at t) and the number of fruits produced during the reproductive season using a generalized linear model with a Poisson fit (Fig. 4.2c; Table 4.1) with linear link $log(y) = a + bx$. We multiplied the number of fruits by the approximate number of seeds per fruit, (2.7, C. Horvitz, personal experience) and then by the mean seedling emergence per seed produced (0.31, as measured in wire-mesh boxes for seeds produced in August that had become live seedlings by the following March, see above results for the box experiment). This relationship provided the expected mean number of seedlings produced by reproductives of a given size. The size distribution of seedlings was obtained from empirical data on the mean and variance of seedlings in each environment, 75.6 ± 100.6, and 40.1 ± 101.2 in the high and low light, respectively.

The clonal reproduction function $c(x, y)$ was estimated by fitting the relationship between the log(leaf area at t) and the number of clonal offspring produced at time $t + 1$, using a generalized linear model with a Poisson fit (Fig. 4.2d; Table 4.1), with linear link $log(y) = a + bx$. This relationship provided the expected mean number of clonal offspring produced by reproductives of a given size. The size distribution of these offspring was obtained from empirical data on the mean and variance of clonal propagules in each environment, 626.7 ± 650.3, and 354.2 ± 296.6 in the high and low light, respectively.

NUMERICAL ESTIMATION OF THE KERNEL –Integral kernels cannot readily be solved directly, but they can be numerically estimated by creating a high dimensional matrix: subdividing the continuous size variable into small categories and then analyzing the dynamics of this matrix. Easterling et al. (2000) recommend trying different numbers of categories and looking for an asymptote in a desired parameter. Using population growth rate as our main parameter of interest, we found that 50 categories provided a stable solution. All the results we report here are for a 50 by 50 matrix used to numerically estimate the integral kernel.

SENSITIVITY ANALYSIS—To examine how different areas of the kernel influence population growth in high and low light levels we used sensitivity analysis. The formal definition of sensitivity and elasticity in integral projection models differs from that of matrix models, although it depends upon the stable stage distribution w and the reproductive value vector v, as in matrix models. Because fecundities, survivals and growth in integral projection models are represented by a surface rather than a matrix, sensitivity analysis of the model requires determining the sensitivity of population growth rate to changes in the kernel surface $k(y, x)$ over a small region centered over each point (y, x). Formally, we can think of a small disk centered at a particular point ($y = z_1$, $x = z_2$) and we can obtain the values of $w(z_2)$ and $v(z_1)$of these vectors at this point. Generally, the interpretation of both sensitivity and elasticity in integral projection models is similar to those for matrix models (Easterling et al., 2000). In integral projection models, however, sensitivity gives the rate of increase in λ as the kernel k is increased in a small disk centered at (z_1, z_2), scaled relative to the size of the disk (Easterling et al., 2000). The sensitivity of the growth rate λ to increasing the kernel in this small disk is:

$$s(z_1, z_2) = \frac{\partial \lambda}{\partial k(z_1, z_2)} = \frac{v(z_1)w(z_2)}{\langle w, v \rangle} \tag{4}$$

Where $s(z_1, z_2)$ is the sensitivity of λ to a small change in the $k(y, x)$ values near the point (z_1, z_2). The corresponding elasticity estimates are given by

$$e(z_1, z_2) = \frac{k(z_1, z_2)}{\lambda} \cdot \frac{v(z_1)w(z_2)}{\langle w, v \rangle} \tag{5}$$

(Easterling et al., 2000). In practice we estimated population growth rates, stable stage distributions, reproductive values, sensitivities and elasticities by standard matrix methods applied to the high dimensional matrix which we used to numerically estimate the integral kernel.

LTRE ANALYSIS—To identify which regions of the kernel surface were responsible for the difference in population growth between low and high light environments, we used a fixed-design LTRE (Caswell, 2001). We employed the standard matrix approach here, using the high dimensional matrix that numerically estimates our integral kernel. In this design the difference in λ between the high and low light, Δλ, is given by

$$\begin{aligned} \Delta\lambda &= \lambda^{h} - \lambda^{l} \\ &\approx \sum_{ij} \left(a_{ij}^{h} - a_{ij}^{l}\right) \times \left(\frac{\partial \lambda}{\partial a_{ij}} \right) \Bigg|_{(A^{i} + A^{l})/2} \end{aligned} \tag{6}$$

were $(a_{ij}^{h} - a_{ij}^{l})$ is the difference in a_{ij} between the high light matrix and the low light matrix, and $\frac{\partial \lambda}{\partial a_{ij}}$ is the sensitivity of λ to changes in a_{ij} evaluated at the mean value (i.e. the matrix midway between the two matrices being compared (Caswell, 2001 p. 260).

RESULTS

KERNELS AND KERNEL COMPONENTS—Leaf area at time *t* (August 2004) significantly predicted survival, leaf area, clonal offspring production and seedling production at time *t* +1 (March 2005) (Fig. 4.2a-d; Table 4.1). There was a positive relationship between plant size at time *t* and survival, size at time *t* +1, clonal offspring production, and seedling production in both high and low light (Fig. 4.2a-d; Table 4.1). The probability of surviving increased with size faster in low light, reaching an asymptote at log(size) ≈ 8, compared to high light which did not reach an asymptote at the largest sizes log(size) ≈ 10. Growth was faster in high light; the relationship between leaf area at time *t* and *t* +1 was steeper in high light than in low light (Fig. 4.2b). The steeper relationship between size and the number of clonal offspring produced in high light than low light resulted from many of the plants that produced multiple clonal offspring were from high light plots, including a very large individual that produced 6 clonal offspring (Fig. 4.2c). The fitted relationships between size and seedling production differed also differed between light levels, especially at large sizes (Fig. 4.2d). Many large plants (log(size) > 8) in high light plots produced few seedlings (0-15) which had the effect of decreasing the exponential nature of the fit, compared to low light (Fig. 4.2d).

The components of the kernel were overall similar between high and low light, although several differences were found. The kernel components are presented in a 3d plot, with the base in an *ij* arrangement (Fig. 4.3) which differs from an *xy* plot by having the 0,0 corner in the upper left rather than the lower left. Thus in each plot the axis on the right displays size at *t* and the axis on the left displays size at *t* +1. The main feature of the growth-survival function $p(x,y)$ is a ridge with a peak running just below the diagonal representing individuals that survive the time interval and increase in size (Fig. 4.3a & b). The sloping surfaces of the ridge result from the variance in size at time $t+1$ for a given size at time t. In both high and low light the ridge top increases in height towards the largest sizes at time *t*, although this increase is more rapid and reaches a greater height in low light, because survival is more sensitive to size in low light than in high light environments. The ridge is slightly lower in high light than in low light (Fig. 4.3a & b), because of higher growth in high light.

Both the sexual fecundity function $f(x,y)$ and the clonal fecundity function $c(x,y)$ are represented by isolated peaks along the far edge, therefore showing that reproduction is restricted mostly to large plants (Fig. 4.3c-f). The peak for the sexual fecundity function is near the far corner of the plot (Fig. 4.3c & d). The sexual fecundity function appears to show the largest difference between light levels of any of the kernel components but really it is only pronouncedly distinct. In low light the sexual fecundity peak is tall and there is less variability in recruit size demonstrated by a peak that is less broad compared to high light peak which is relatively short and broader (Fig. 4.3c & d). The clonal fecundity peak shows that mostly large plants produce clonal offspring, similar to the sexual fecundity function, but in contrast, clonal recruits are larger than

sexual recruits resulting in a peak closer to the right-hand corner of the plot (Fig. 4.3e & f). The broader base of the clonal fecundity peak in high light is the result of the phenomenon that, similar to sexual recruits, there is larger variability in recruit size in high light than in low light where the base of the peak is more narrow (Fig. 4.3e & f). Additionally, the clonal fecundity peak is taller in high light than in low light (Fig. 4.3e & f).

Putting the survival, clonal fecundity and sexual fecundity functions together gives the kernel $k(y,x)$ for *C. marantifolia*. The visual presentations of both high and low light kernels are dominated by the fecundity functions, since these numbers are >1 and the survival-growth surface represents numbers between 0 and 1 (Fig. 4.4a & b). The survival-growth component of the kernel is visible in the complete kernel as a faint ridge running below the diagonal (Fig. 4.4a & b).

SENSITIVITY AND ELASTICITY ANALYSES—The sensitivity surface, which represents the sensitivity of population growth to changes in the size-specific transitions of the kernel showed a single peak in the lower edge of the kernel with a peak at about log(size) = 4.5 for both low and high light levels (Fig. 4.5). This peak represents extremely rapid growth, which according to our data does not occur naturally in *C. marantifolia*. If it did, it would have the largest impact on population growth of any region of the kernel.

The elasticity surface for both high and low light was dominated by a ridge running below the diagonal which increased in height towards the right corner (Fig. 4.6a & b). Additionally, a smaller peak was found along the right-hand edge, the location of

which was different between high and low light (Fig. 4.6a & b). This smaller peak was located at smaller sizes along the $t + 1$ in low light than in high light (Fig. 4.6c & d).

POPULATION GROWTH—Population growth was 8% faster in high light, $\lambda = 1.33$, than in low light, $\lambda = 1.25$. In both light environments, sexual reproduction affected population growth to a much larger degree than clonal reproduction. By removing sexual reproduction from the model, population growth slowed greatly with a reduction in λ of 0.32 and 0.21 in high and low light, respectively. In contrast, by removing clonal reproduction from the model, population growth was only modestly slowed with a reduction in λ of 0.05 and 0.02 in high and low light, respectively. Removing sexual reproduction from the model for high light showed a larger reduction in λ compared to low light. When only sexual reproduction is included in the model population growth rate is faster in high light ($\lambda = 1.28$) than in low light ($\lambda = 1.23$). By contrast, when only clonal reproduction is included in the model population growth rate is faster low light ($\lambda = 1.04$) than in high light ($\lambda = 1.01$).

LTRE ANALYSIS—The LTRE analysis identified three primary differences in the kernels responsible for the $\Delta \lambda$ between high and low light environments (Fig. 4.7a & b). To help understand the complex surface of the contribution of regions of the kernel for $\Delta\lambda$, we also present the differences (high light – low light) for the functions p, f and c (Fig. 4.8).

The region of the kernel representing growth and survival has both areas with higher values in the low light matrix and areas with higher values in the high light matrix (Fig. 4.7a & b). In high light, the ridge of the p function is wider, with higher values than low light especially for the bottom of the ridge representing the fastest growth (Fig. 4.8a).

The right edge of the kernel has areas with higher values for the low light matrix (top portion) and areas with higher values for the high light matrix (mid to lower portion; Fig. 4.7). The entire peak of the *f* function is much taller in low light than in high light (Fig. 4.8b & Fig. 4.3d). Conversely, the *c* function is almost entirely taller in high light except for the top most section which is only slightly higher in low light (Fig. 4.8c). The higher λ observed in high light can be attributed to faster growth and higher clonal reproduction in that light environment.

DISCUSSION

In this chapter we have presented a framework showing how an integral projection model can be developed to answer our questions using field data from *Calathea marantifolia* in a single season. The results presented here are not a final answer to the questions posed in the introduction, but instead we have made a first step in developing the process that will ultimtely allow us to address these questions. The natural system where the field data were collected is highly seasonal and so far we have paramaterized our model with data from a single wet season time interval. Future work will explicitly include seasonal and interannual variation.

Using our integral projection model, we found that the population growth rate (λ) of *C. marantifolia* was higher in high light than in low light. Our study sites were located in secondary forest, so the difference in light availability between high and low conditions is relatively small compared to that of primary forest (Denslow, 1987). The tree fall gaps in our high light sites were relatively small compared to those created by

mature canopy trees in primary forest. In addition, our low light sites were not as deeply shaded as those found in the understory of mature forest under several layers of foliage. The low abundance of *C. marantifolia* in primary forest made it unfeasible to study the effects of the light gradient on demography there.

By removing one form of reproduction at a time from the model and observing the effect on λ, we found that sexual reproduction made a much larger contribution to λ than clonal reproduction. These findings are in contrast to those for the majority of clonal species, the population dynamics of which have been shown to be dominated by clonal reproduction while sexual reproduction plays a relatively minor role (Bierzychudek, 1982; Eriksson, 1988, 1989; Nault and Gagnon, 1993; Silvertown et al., 1993; Dammon and Cain, 1998; Mandujano, 2001). Nevertheless, Weppler et al. (2006) reported that λ of the alpine herb *Geum reptans* was equally sensitive to sexual and clonal modes of reproduction. Our results are surprising, considering that sexually produced seedlings of *C. marantifolia* recruit at a smaller size than do clonal offspring, similar to other species (Abrahamson, 1980; Caswell, 1985; Cook, 1985). Smaller size, in our model and in nature, translates to reduced rates of growth and a elevated probability of mortality. Therefore, based soley on recruit size we should expect sexual reproduction to contribute less to λ than clonal reproduction. Sexual and clonal reproductive modes differ in other regards than size of recruits, however, most importantly sexual offspring are more numerous than clonal offspring. Our field data show that large adult plants rarely produce more than one clonal offspring but regularly produce more than ten seedlings. This order-of-magnitude difference in propagule production between sexual

and clonal modes is great enough to eclipse the increase in mortality and decrease in growth associated with the small size of seedlings.

Our results that both types of reproduction influence λ more in high light than in low light is in contrast to results from a separate experiment investigating the influence of light on the demographic performance of offspring (Chapter 1). In that experiment, seedlings and clonal offspring were planted into gap centers, gap edges and the shaded understory. We found that seedlings survived best in tree fall gap centers and clonal offspring did so in shaded understory. Results from the IPM are in agreement with those from Chapter 1. When only sexual reproduction is included in the model population growth rate is faster in high light and when only clonal reproduction is included in the model population growth rate is faster low light.

We are interested in examining the trade-off between sexual and clonal reproduction at the population level. It has previously been argued that life-history trade-offs, such as the one between sexual and clonal reproduction, can be examined by the correlation between elasticity values (Silvertown et al., 1993). However, because in our study the areas of the elasticity surface that corresponded to sexual and clonal reproduction did not differ greatly between light conditions we can not evaluate their correlations. Additionally, Shea et al. (1994) point out that the correlation between elasticity values is not a reliable metric to evaluate life-history trade-offs. In a separate experiment, we investigated the trade-offs between sexual and clonal reproduction at the level of the individual and found only a small trade-off (a small, temporary reduction in clonal offspring size due to parent plant's sexual reproductive effort; Chapter 3). Our results showing that greater clonal offspring production occurred in high light and greater

seedling production occurred in low light is surprising. Despite that these two reproductive modes are linked in their phenology (clonal offspring are only produced after seeds) and in where on the plant they occur (terminal node of the same reproductive shoot) their influence on population dynamics may be somewhat independent.

Results from our LTRE indicate that the difference in λ between light levels was primarily the result of high growth and survival at large sizes as well as increased clonal offspring production in high light. Another difference illustrated in the LTRE analysis was greater seedling production in low light. The fitted relationships between size and both seedling and clonal offspring production appear to be heavily influenced by a few individuals. Because these fitted relationships determine $f(x,y)$ and $c(x,y)$ and therefore the kernel $k(x,y)$ it is possible that outliers may have a large impact on the entire model. This issue must be considered when we extend the current model to incorporate both wet and dry seasons in a the appropriate temporal sequence.

Table 4.1. Models and parameters describing the demography of *Calathea marantifolia* between August 2004 and March 2005. Numbers in parentheses are standard error of parameters, r^2 – coefficient of determination, X^2 – Chi squared value for the comparison of the null model to the model including log(size), all comparisons were highly significant.

Demographic process	Model
Survival	
High light	Logit(*surv*) = -1.41 (0.31) + 0.52 (0.05) *log* (*size t*); $P < 0.0001$, $X^2 = 93.83$
Low light	Logit(*surv*) = -1.44 (0.42) + 0.73 (0.08) *log* (*size t*); $P < 0.0001$, $X^2 = 90.94$
Growth	
High light	*log* (*size t*+1) = 0.12 (0.18) + 0.99 (0.02) *log* (*size t*), $\sigma = 0.99$; $P < 0.0001$, $r^2 = 0.750$
Low light	*log* (*size t*+1) = 1.00 (0.15) + 0.85 (0.02) *log* (*size t*), $\sigma = 0.89$; $P < 0.0001$, $r^2 = 0.724$
Fruit production	
High light	*fruits* = -6.33(0.42) + 1.03(0.04) *log* (*size t*); $P < 0.0001$, $X^2 = 448.33$
Low light	*fruits* = -11.72(0.89) + 1.62(0.10) *log* (*size t*); $P < 0.0001$, $X^2 = 292.88$
Clonal offspring production	
High light	*Clonal offspring* = -13.6(1.24) + 1.4(0.14) *log* (*size t*); $P < 0.0001$, $X^2 = 198.7$
Low light	*Clonal offspring* = -12.70(1.57) + 1.31(0.18) *log* (*size t*); $P < 0.0001$, $X^2 = 99.4$

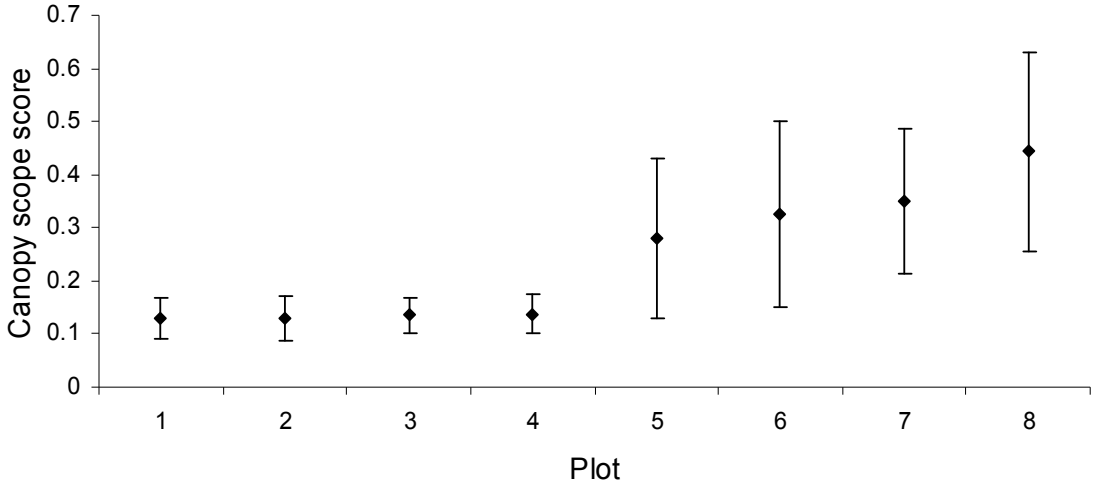

Figure 4.1. Mean canopy scope score (± st. dev.) for all plants in eight plots. Canopy scope score is correlated with canopy openness (methods). Canopy scope score was measured directly above each plant. Sample sizes per plot: 1 = 147, 2 = 155, 3 = 125, 4 = 138, 5 = 120, 6 = 150, 7 = 160, 8 = 154.

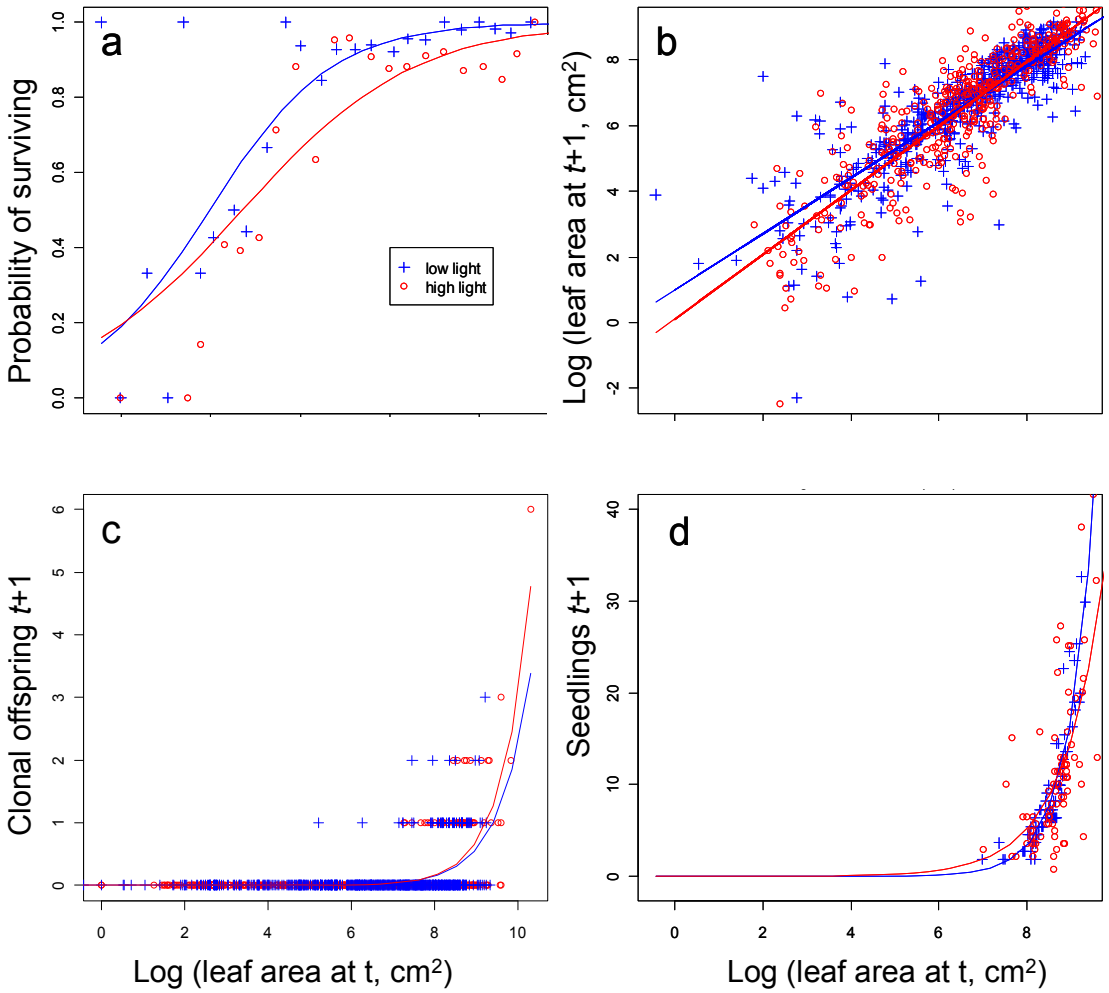

Figure 4.2. Fitted regression relationships for *C. marantifolia* in high and low light. Probability of surviving to *t*+1 as a function of size determined using a logistic regression (a). Size at *t* +1 (March 2005) as a function of size at *t* (August 2004) determined using a linear fit (b). Production of clonal offspring at *t*+1 as a function of size at *t* (c). Production of seedlings at *t*+1 as a function of size at *t* (d). Equations and statistics for fitted relationships are shown in Table 1.

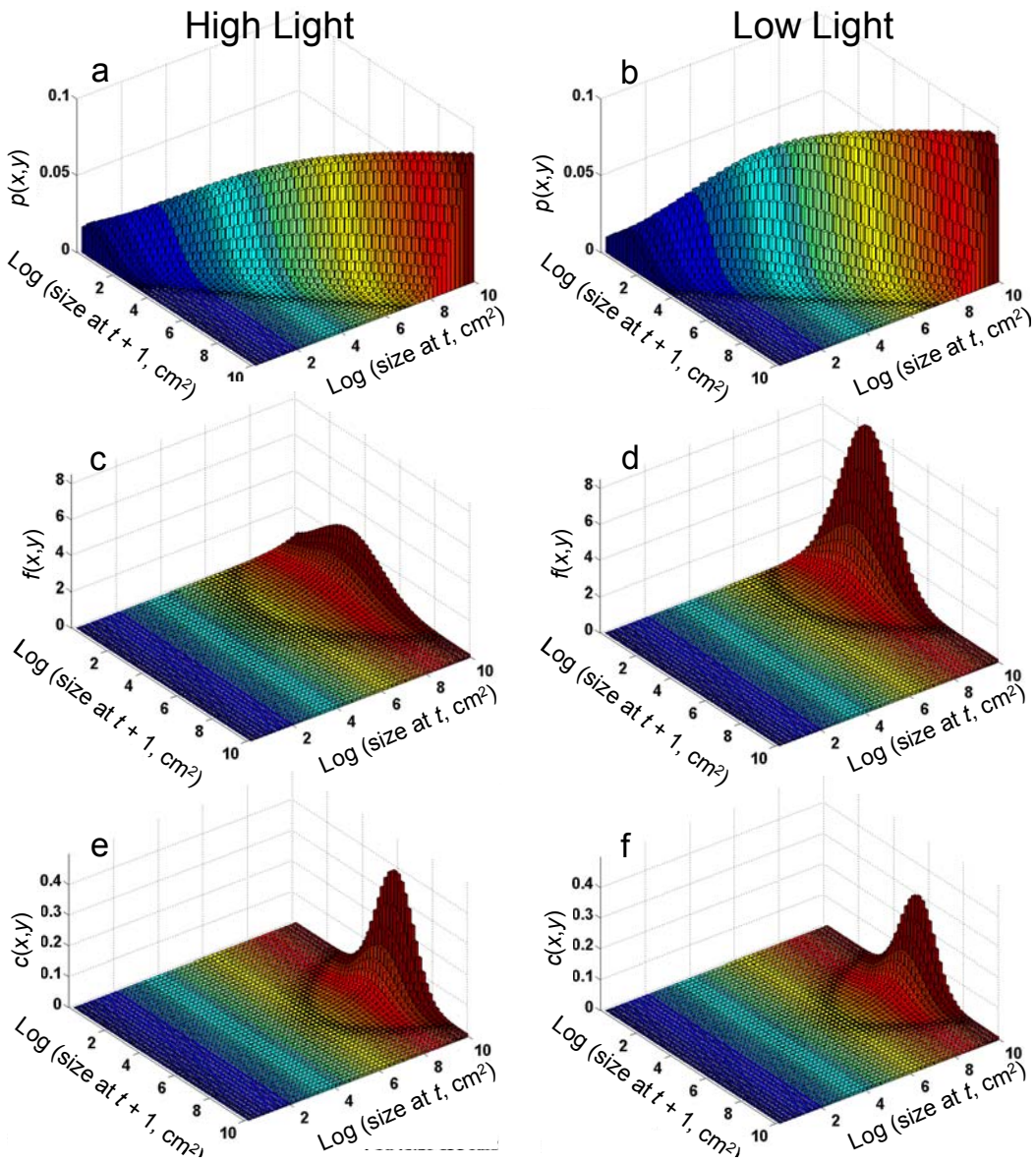

Figure 4.3. The components of the kernel for *Calathea marantifolia* in high and low light; the growth-survival function $p(x,y)$ (a & b), the sexual fecundity function $f(x,y)$ (c & d) and the clonal fecundity function $c(x,y)$ (e & f).

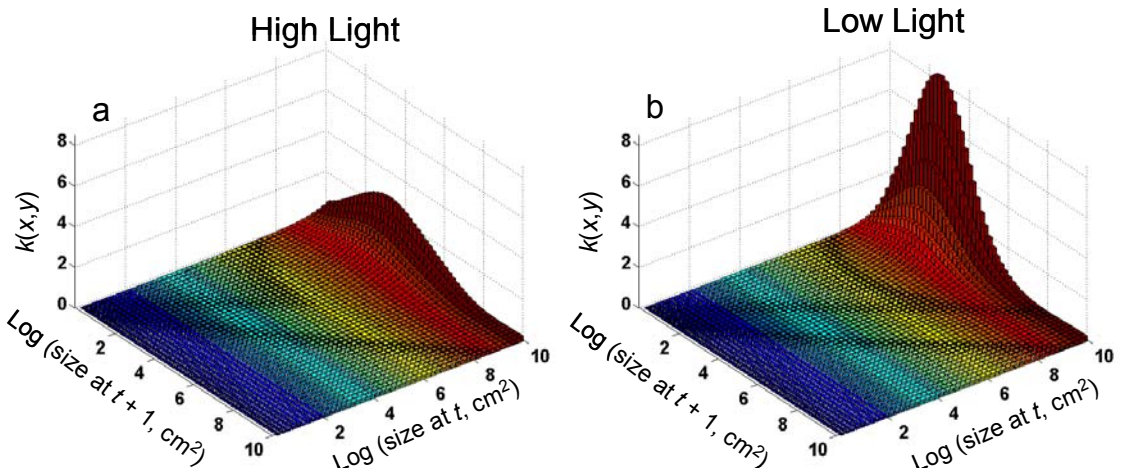

Figure 4.4. The fitted kernel *k* for *Calathea marantifolia* in high (a) and low light (b).

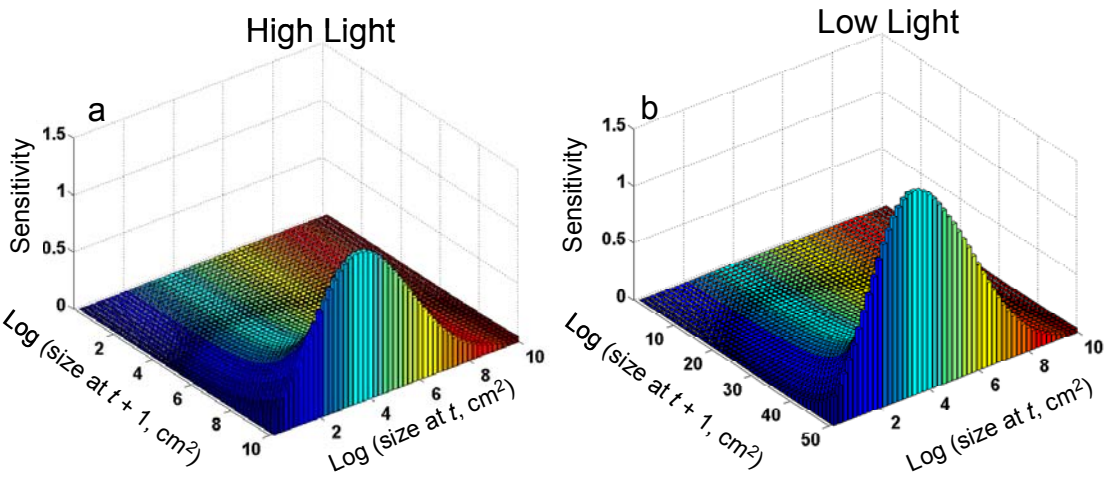

Figure 4.5. Sensitivity surface for the integral projection model fitted to the *Calathea marantifolia* data from high (*a*) and low (*b*) light environments.

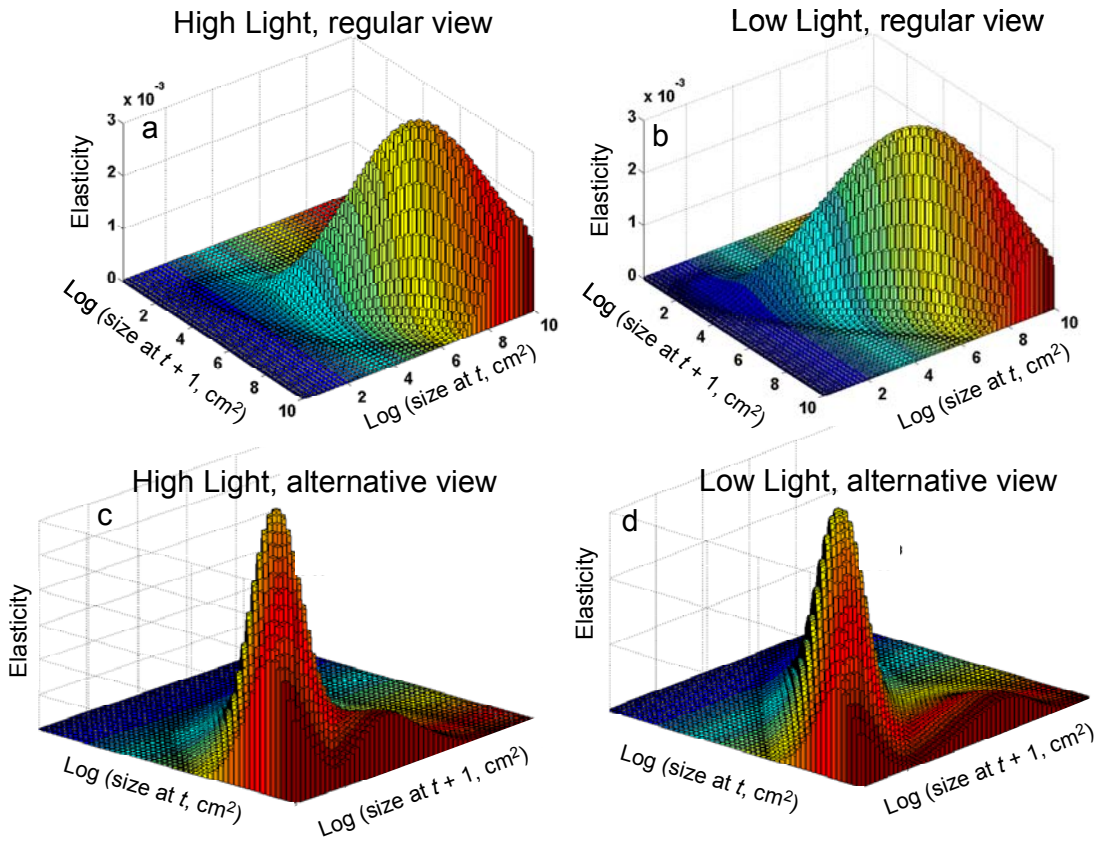

Figure 4.6. Elasticity surface for the integral projection model fitted for *Calathea marantifolia* in high and low light (a & b). The alternative views (c & d) show the portion of the elasticity surface that is hidden when viewed from the regular orientation.

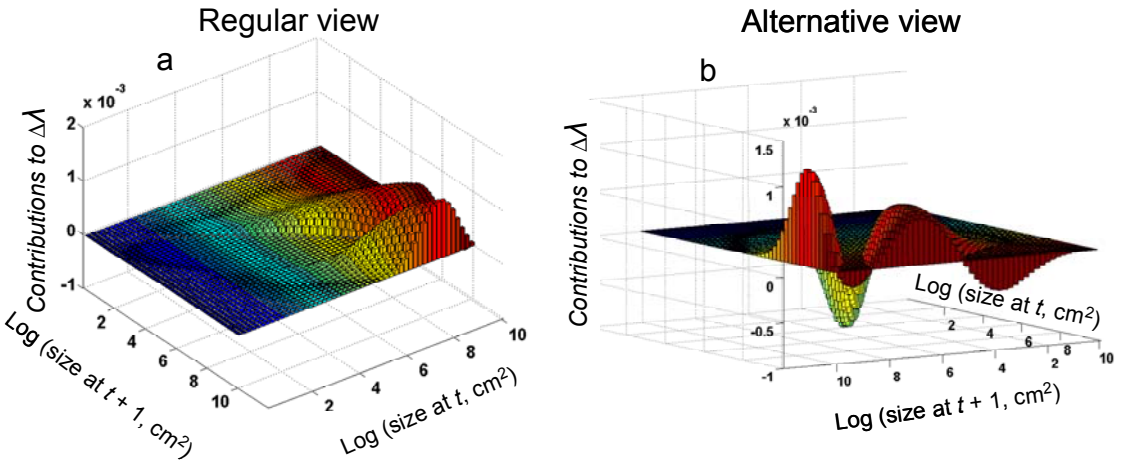

Figure 4.7. Surface showing the contribution of regions in the kernel towards the difference in population growth (λ) between high and low light levels from the regular view (a) and an alternative view (b). See methods for a description of the LTRE design.

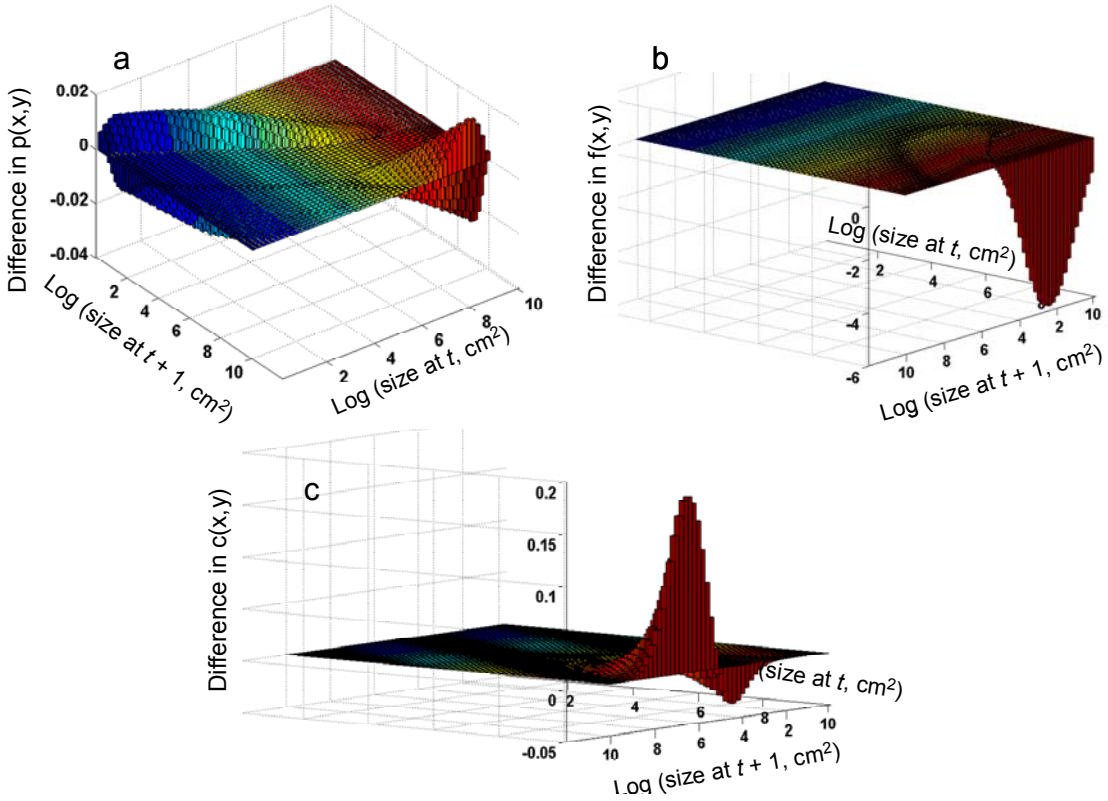

Figure 4.8. The difference (high light – low light) between the growth function *p* (a), the sexual fecundity function *f* (b), and the clonal fecundity function *c* (c). Figures b and c are presented at alternative views.

Chapter V

Synthesizing experimental and census data of *Calathea marantifolia* demography

In the previous chapters I presented results from several field experiments and one season of natural population census data that addressed specific questions about the importance of sexual and clonal reproduction in the demography of *Calathea marantifolia*. Some issues common to all the studies were the roles of light, plant size and parent-offspring connectedness in the comparative success of seedlings vs. clonal offspring and ultimately in their relative contribution to population dynamics. Here I provide a synthesis across studies with the goal of placing the findings of each individual study into a larger context that illuminates the biology of this species as a whole.

GROWTH AND SURVIVAL OF OFFSPRING—The experimental treatments aimed at addressing the light-dependency of offspring performance (Chapter 1), the extent of clonal integration between offspring and parent (Chapter 2) and the demographic cost of sexual reproduction (Chapter 3) showed a much larger effect on the size of offspring than on their survival. The experiment that compared the success of clonal offspring to seedlings in different light environments (Chapter 1) revealed that light availability among gap centers, gap edges and shaded understory influenced the growth and survival of these offspring types but to different degrees. Seedlings showed a 46-fold difference in their size across the light treatments but only a 9-fold difference in their probability of surviving. Similarly, clonal offspring showed a 5-fold difference in leaf area across light treatments but only 1.8-fold difference in their probability of surviving. In the experiment investigating clonal integration and its influence on the

demography of clonal offspring and their parent plant (Chapter 2), I found that severing the connection between offspring and parents resulted in reduced demographic performance of clonal offspring. Offspring that had not yet rooted experienced a dramatic reduction in their leaf area (up to a 7-fold reduction) due to severing, but their probability of surviving was only reduced by 10%. Offspring that had already rooted, suffered a 1.3-fold reduction in leaf area but no reduction in their probability of surviving.

In the third experiment, I manipulated sexual reproductive effort of plants and measured the demographic effect on their subsequently produced clonal offspring (Chapter 3). Initially clonal offspring displayed a 2-fold difference in leaf area across treatments but this difference disappeared over time, and clonal offspring had an equally high probability of surviving across the treatment groups.

Results from these experiments suggest that a threshold may exist in the proportional change in leaf area needed to influence survival among clonal offspring in this species. When clonal offspring suffered a 1.3-fold (Chapter 2) and 2-fold (Chapter 3) reduction in leaf area their survival was unaffected. However, when clonal offspring leaf area was reduced by 5-fold (Chapter 1) and above (i.e. 7-fold reduction; Chapter 2) survival was significantly reduced. Therefore, for clonal offspring the threshold of the proportion of leaf area that can be lost before survival is influenced may lie between a 2- and 5-fold reduction. I do not have sufficient data on seedlings to suggest if a similar threshold may exist. However, in the experiment investigating the light-dependency of offspring performance (Chapter 1) I did monitor seedlings leaf area and survival over time. I found that by the time of the first census seedlings show a 3-fold difference in

their leaf area and a significant difference in their survival across the light treatments. Therefore, the threshold of the proportion of leaf area that can be lost before survival is influenced may be similar for seedlings and clonal offspring.

In my experiments I created treatments with larger variability among groups than is found naturally to understand how these extremes influenced offspring demographic performance. Here I provide some context for how the variability among treatments compares to the variation found naturally. In my experiment examining the light-dependency of offspring performance I chose to span the extremes of the natural light availability gradient. Therefore the extremes of the light gradient are better represented than if offspring were planted in locations at random. A detailed discussion of light availability in each experiment is below. In the reproductive trade-off experiment (Chapter 3) I manipulated the reproductive effort of plants producing a much larger range of fruit-set than I observed naturally in the demography plots. In the demography plots I observed very few plants that lost their immature inflorescences to herbivores or damage, which would approximate my severing of the immature inflorescence bud. Natural fruit-set was relatively high and varied little between the three years and among the individuals I observed. In my experiment investigating clonal integration between parent and offspring (Chapter 2) I severed connection between parent and offspring either prior to offspring rooting or after offspring rooting. Under the natural conditions I observed in the demography plots, very few clonal offspring lose their connection to parents prior to rooting. Therefore my treatment of severing the connection for pre-rooting offspring represents an extreme of the natural variation. However, offspring often have their connection to parents broken shortly after rooting since the unprotected connection is

lying on the ground and is vulnerable to trampling, and therefore my treatment of severing the connection for post-rooting offspring is well within the natural variation. Overall, my treatments were successful in representing the variation occurs in the field, including the extremes which are rare under natural conductions.

DEMOGRAPHIC COST OF PRODUCING OFFSPRING—Results from the severing and cost of reproduction experiments (Chapters 2 & 3) suggest that reproductive plants do not experience a demographic cost associated with producing either sexual or clonal offspring. In the severing experiment, results showed that plants supplement the growth and survival of their clonal offspring, but there was no evidence that plants paid a cost for providing this supplementation; removing clonal offspring from parent plants did not increase their demographic performance. In the cost of reproduction experiment I dramatically manipulated the sexual reproductive effort of plants and measured the effect on their future growth, survival and reproduction. I found that future demographic performance was not affected by large differences in reproductive effort.

Overall, the production of sexual offspring and the supplementation of clonal offspring did not result in a demographic cost for the above-ground portion of the plant in next season. My results do not rule out that a cost may be displayed in the below-ground portions of the plant (i.e. under ground storage structures) or on a longer time scale than a single year.

INFLUENCE OF LIGHT ON DEMOGRAPHY—In the light-dependency experiment (Chapter 1), the cost of reproduction experiment (Chapter 3) and in the population demography study (Chapter 4) I examined the influence of light availability on several aspects of *C. marantifolia* demography. The strategy of sampling individuals

across light environments differed among these studies and therefore the range of light levels represented in the samples also differed. The first step in integrating these results is to place all estimates of light availability in the same metric. Light availability was quantified as Global site factor in the light-dependency experiment (Chapter 1) and the cost of reproduction experiment (Chapter 3). In the population demography study (Chapter 4) I quantified light availability using the canopy scope technique (Brown et al. 2000) due to the large sample size.

Correlating canopy scope scores and Global site factor—To determine the relationship between canopy scope score and Global site factor (GSF) within the secondary forest surrounding Sirena Station I used a stratified random sample. First I located *C. marantifolia* plants across the gradient of light availability in May of 2006. I explicitly choose plants that were in low, medium and high light levels (20 plants each) based on my experience at this site. I randomly choose 13 plants out of the 20 in each light level. At each of the 39 plants I determined the canopy scope score and took a hemispherical photo directly above the plant. Photographs and estimates of GSF were carried out as described in Chapters 1 and 3. The relationship between canopy scope score and GSF was estimated using linear regression.

Canopy scope score was significantly correlated with GSF (Fig. 5.1). This relationship is consistent with the results of Brown et al. (2000) showing that canopy scope score is significantly correlated with percent canopy openness. Brown et al. (2000) reported that due to physical limitations of the canopy scope method, its accuracy is reduced in environments where light levels are very high (30-100 % openness). This is

not a problem here or in the results presented in Chapter 4 because canopy openness does not reach this threshold.

Comparing light availability—In the experiment investigating the relative light-dependency of seedlings and clonal offspring (Chapter 1) I intentionally chose the extremes of the light availability gradient and clonal offspring and seedlings experienced the largest range of light levels of all the experiments (Fig. 5.2). In contrast, when I choose reproductive plants at random in natural populations for the cost of reproduction experiment (Chapter 3) the range of light environments was narrower, and the light levels are similar to those in darker two of the treatments in the transplant experiment (the understory and gap-edge levels). The demography plots were chosen using a stratified random sample and were intended to represent the two ends of the light availability continuum (Chapter 4). However, plants in high light plots span a relatively large range of GSF values, primarily due to two factors (Fig. 5.2). First, there was greater spatial heterogeneity in light availability in the high light sites than in the low light. Second, due to shading there was a large difference in the amount of light available to tall and short plants in high light, but in low light the difference between the amount of light available to tall and short plants was relatively small. In the demography plots light availability was quantified for individuals of all sizes. In contrast, in the transplant experiment light availability was only quantified at the height experienced by seedlings and clonal offspring, and in the cost of reproduction experiment light was only quantified directly above adult plants.

Influence of light on growth—The growth of both seedlings and clonal offspring differed among light levels of the transplant experiment. In contrast, in the cost of

reproduction experiment, clonal offspring growth was not affected by light availability. This contrast in the light-dependency of clonal offspring growth may have resulted from two factors. First, in the cost of reproduction experiment GSF was estimated directly above the parent plant and therefore the actual GSF value at the height of the clonal offspring may have been considerably lower. Second, the majority of individuals in the cost of reproduction experiment were located in low light environments (few with > 0.08 GSF). There was only a slight difference in the regression relationship between size at time *t* and size at time *t* + 1 among high and low light plots, indicating that growth was similar across light levels. However, the slope of the relationship was slightly steeper in high light, showing that growth was greater in high light than in low light.

Influence of light on survival—Survival of clonal offspring and seedlings differed among light levels in the transplant experiment, but light availability did not influence the survival of clonal offspring in the cost of reproduction experiment. The most likely reasons are the same ones I proposed to explain the difference in the effects of light on growth, mentioned above. The relationship between size and the probability of surviving was steeper in low light demography plots than in high light plots, illustrating that the benefit of increased size on survival was more pronounced in low light. Results from the demography plots do not contrast with those from the transplant experiment. The transplant experiment was conducted with seedlings and clonal offspring which are small (< 650 cm^2). There was little difference between high and low light plots in survival of plants of similar size.

The transplant experiment revealed that seedlings survived best in high light while clonal offspring survived best in low light. These results are consistent with

estimates of population growth rate determined using the integral projection model. When a version of the model was run in which the only kind of reproduction was sexual reproduction, population growth rate was faster in high light than in low light. In contrast, when a version of model was run in which the only kind of reproduction was clonal reproduction population growth rate was faster in low light than in high light. Further analyses are needed to determine if the faster growth rate in these environments is due to differential offspring survival.

Overall, I found that clonal offspring size is influenced by the availability of light and being connected to the parent plant, but the sexual reproductive effort of the parent did not have a large influence. In comparison, the size of seedlings is more sensitive to light levels than clonal offspring. Survival of clonal offspring appears to be buffered by changes in leaf area and my results suggest that there is a threshold in the proportion of leaf area that is lost before survival is reduced. Surprisingly, I found no evidence that plants suffer a demographic cost to producing sexual offspring or supporting their connected clonal offspring. By combining an experiment examining demographic performance of offspring with a model parameterized with demographic data of all plant sizes, I was able to make the link that when only one type of reproduction is considered in the model population growth is fastest in the light environments where that type of offspring survives best. This work is novel in correlating the demographic performance of offspring types with their influence on population growth across the environmental gradient of light availability.

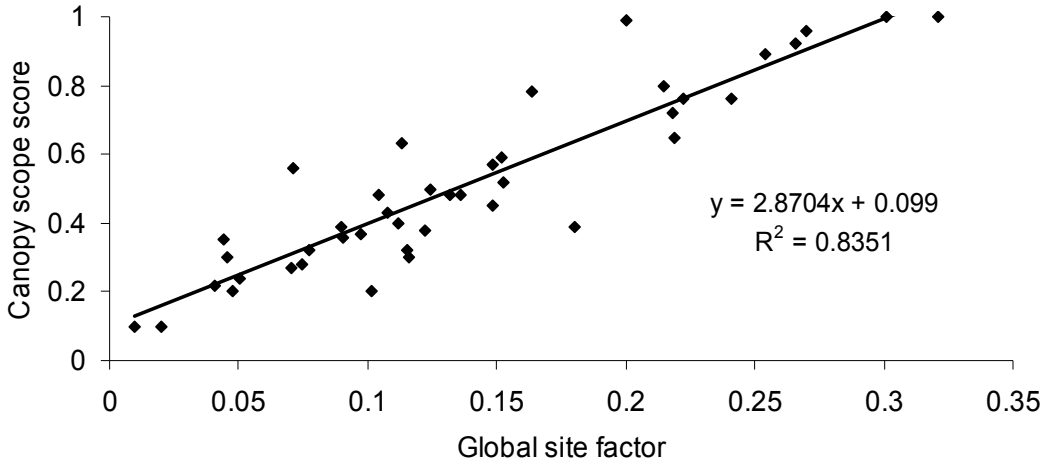

Figure 5.1. Relationship between canopy scope score and global site factor in 39 locations in the understory at Sirena Biological Station, Corcovado National Park (Costa Rica).

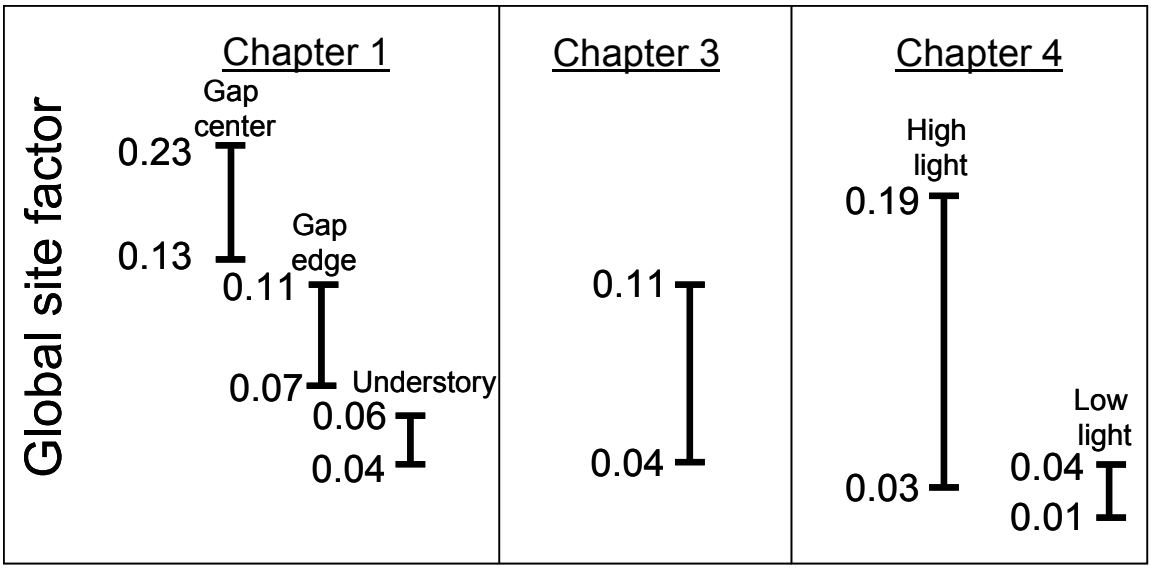

Figure 5.2. Range of Global site factor values for *C. marantifolia* plants in chapters 1, 3, and 4.

Literature cited

Aarssen, L. W. 2007. Death without sex- the 'problem of the small' and selection for reproductive economy in flowering plants. Evolutionary Ecology 22: 279-298.

Abrahamson, W. G. 1979. A comment on vegetative and seed reproduction in plants. Evolution 33: 517-519.

Abrahamson, W. C. 1975. Reproduction of *Rupus hispidus* L. in different habitats. American Midland Naturalist 93: 471-478.

Abrahamson, W. C. 1980. Demography and vegetative reproduction. *In* O. T. Sobrig [ed.], Demography and evolution in plant populations, 89-106. University of California Press, Berkeley, California, USA.

Abrahamson, W. G. and H. Caswell. 1982. On the comparative allocation of biomass, energy and nutrients in plants. Ecology 63:982-991.

Ackerman, J. D. and A. M. Montalvo. 1990. Short- and long- term limitations to fruit production in a tropical orchid. Ecology 71: 263-272.

Adachi, N., I. Terashima, and M. Takahashi. 1996. Central die-back of monoclonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. Annals of Botany 77: 477–486.

Alpert, P., and H. A. Mooney. 1986. Resource sharing among ramets in the clonal herb *Fragaria chiloensis*. Oecologia 70: 227-233.

Alpert, P. 1996. Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. Journal of Ecology 84: 395-406.

Amézquita, P. 1998. Light environment affects seedling performance in *Psychotria aubletiana* (Rubiaceae), a tropical understory shrub. Biotropica 30: 126-129.

Anderson, M. C. 1964. Studies of woodland light climate. I. The photographic computation of light conditions. Journal of Ecology 52: 27-41.

Ashman, T. L. 1992. Indirect costs of seed production within and between seasons in a gynodioecious species. Oecologia 92: 266-272.

Ashmun, J. W., and L. F. Pitelka. 1984. Light-induced variation in the growth and dynamics of transplanted ramets of the understory herb, *Aster acuminatus*. Oecologia 64:255-262.

Augspurger, C. K. 1984. Light requirements of neotropical tree seedlings: a comparative study of growth and survival. Journal of Ecology 72: 777-795.

Balderrama, S. I. V., and R. L. Chazdon. 2005. Light-dependent seedling survival and growth of tour tree species in Costa Rican second-growth rain forests. Journal of Tropical Ecology 21: 383-395.

Barkham, J. P. 1980. Population dynamics of the wild daffodil (*Narcissus pseudonarcissus*). I. Clonal growth, seed reproduction, mortality and the effects of density. Journal of Ecology 68:607-633.

Bazzaz, F. A., and D. D. Ackerly. 1992. Reproductive allocation and reproductive allocation in plants. *In* M., Fenner [ed.], Seeds: the ecology of regeneration in plant communities, 1-26. C.A.B. International, Oxton, U.K..

Bazzaz, F. A., and P. M. Wayne. 1994. Coping with environmental heterogeneity: The physiological ecology of tree seedling regeneration across the gap-understory continuum. *In* M. M. Caldwell and R. W. Pearcy [eds.], Exploitation of environmental heterogeneity by plants: Ecophysiological processes above- and belowground, 349-385. Academic Press, New York, New York, USA.

Bazzaz, F. A. 1997. Allocation of resources in plants: state of the science and critical questions. *In* F.A. Bazzaz and J. Grace [eds.], Plant resource allocation, 1-37. Academic Press, San Diego, California, U.S.A.

Bell, G. and V. Koufopanou. 1986. The cost of reproduction. Oxford Surveys in Evolutionary Biology 3: 83-131.

Bierzychudek, P. 1982. Life histories and demography of shade-tolerant temperate forest herbs: a review. New Phytologist 90: 757-776.

Biere, A. 1995. Genotypic and plastic variation in plant size: effects on fecundity and allocation patterns in *Lychnis flos-cuculi* along a gradient of natural soil fertility. Journal of Ecology 83: 629-642.

Brown, N., S. Jennings, P. Wheeler, and J. Nabe-Nielsen. 2000. An improved method for the rapid assessment of forest understorey light environments. Journal of Applied Ecology 37: 1044-1053.

Burt, A. 2000. Sex, recombination, and the efficacy of selection- was Weismann right? Evolution 54: 337-351.

Calvo, R. N. 1990. Four-year growth and reproduction of *Cylopogon cranichoides* (Orchidaceae) in south Florida. American Journal of Botany. 77: 736-741.

Calvo, R. N. 1993. Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. Ecology 74: 1033-1042.

Canham, C. D., J. S. Denslow, W. J. Platt, J. R. Runkle, T. A. Spies, and P. S. White. 1990. Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. Canadian Journal of Forest Research 20: 620-631.

Caswell, H. 1985. The evolutionary demography of clonal propagation. *In* J.B.C. Jackson, L.W. Buss, and R.E. Cook [eds.], Population biology and evolution of clonal organisms,187-224. Yale University Press, New Haven, CT, USA.

Caswell, H. 2001. Matrix population models. Sinauer Associates, Sunderland, Massachusetts, USA.

Chapin, F. S. 1989. The cost of tundra plant structures: evaluation of concepts and currencies. American Naturalist 133:1-19.

Chazdon, R. L., and N. Fetcher. 1984. Light environments of tropical forests. *In* E. Medina, H. A. Mooney, and C. Vasquez-Yanes [eds.], Physiological ecology of plants in the wet tropics, 27-36. Dr. W. Junk, Boston, Massachusetts, USA.

Chazdon, R. L. 1988. Sunflecks and their importance to forest understory plants. Advances in Ecological Research 18: 1-63.

Chazdon, R. L., R. W. Pearcy, D. W. Lee, and D. Fetcher. 1996. Photosynthetic responses of tropical forest plants to contrasting light environments. *In* S. S. Mulkey, R. L. Chazdon, and A. P. Smith [eds.], Tropical forest plant ecophysiology, 5-55. Chapman and Hall, New York, New York, USA.

Cody, M. L. 1966. A general theory of clutch size. Evolution 20: 174-184.

Colling S, K. P. Dunne, S. T. A. Pickett, and P. S. White. 1985. Responses of forest herbs to canopy gaps. *In* S. T. A. Pickett and P. S. White [eds.], The ecology of natural disturbance and patch dynamics, 217-234. Academic Press Inc., New York, New York, USA.

Cook, R. E. 1985. Growth and development in clonal plant populations. *In* J. B. C. Jackson, L. W. Buss, and R. E. Cook [eds.], Population biology and evolution of clonal organisms, 259-296. Yale University Press, New Haven, Connecticut, USA.

Cooley, A. M., A. Reich, and P. Rundel. 2004. Leaf support biomechanics of Neotropical understory herbs. American Journal of Botany 9: 573-581.

Cruz, A., and J. M. Moreno. 2001. No allocation trade-offs between flowering and sprouting in the lignotuberous, Mediterranean shrub *Erica australis*. Acta Oecologica 22: 121-127.

Cunningham, S. A. 1997. The effect of light environment, leaf area, and stored carbohydrates on inflorescence production by a rainforest understory palm. Oecologia 111: 36-44.

Dammon, H., and M. Cain. 1998. Population growth and viability analyses of the clonal woodland herb, *Asarum canadense*. Journal of Ecology 86: 13-26.

De Kroon, H., B. Fransen, J. W. A. Rheenen, A. Van Rheenen, A. Van Dijk, and R. Kreulen. 1996. High levels of inter-ramet water transport in two rhizomatous *Carex* species, as quantified by deuterium labeling. Oecologia 106: 73-84.

De Kroon, H., E. Van Der Zalm, J. W. A. Van Rheenen, A. Van Dijk, and R. Kreulen. 1998. The interaction between water and nitrogen transport in a rhizomatous sedge (*Carex flacca*). Oecologia 116: 38-49.

Denslow, J. S. 1987. Tropical rainforest gaps and tree species diversity. Annual Review of Ecology and Systematics 18: 431-451.

De Kroon, H., and F. Schieving. 1990. Resource partitioning in relation to clonal growth strategy. *In* J. van Groenendael and H. de Kroon [eds.], Clonal growth in plants: regulation and function, 113-130. SPB Academic Publishing, The Hague, The Netherlands.

De Steven, D. 1989. Genet and ramet demography of *Oevocarpus mapora* ssp. *maropa*, a clonal palm of the Panamanian tropical moist forest. Journal of Ecology 77: 579-596.

Douglas, D. A. 1981. The balance between vegetative and sexual reproduction of *Mimulus primuloides* (Scrophulariaceae) at different altitudes in California. Journal of Ecology 89: 339-350.

Easterling, M. R. 1998. The integral projection model: theory, analysis and application. PhD. dissertation, North Carolina State University.

Easterling, M. R. S. P. Ellner, and P. M. Dixon. 2000. Size-specific sensitivity: applying a new structured population model. Ecology 81: 694-708.

Eckert, C. G. 2002. The loss of sex in clonal plants. Evolutionary Ecology 15: 501-520.

Ehrlén, J. 1999. Modeling and measuring plant life histories. *In* T. Vuorisalo and P. Mutikainen [eds.], Life history evolution in plants, 27-61. Kluwer Academic Publishers, The Netherlands.

Ehrlén, J., and J. Van Groenendael. 2001. Storage and the delayed costs of reproduction in the understory perennial *Lathyrus vernus*. Journal of Ecology 89: 237-246.

Ellner, S. P. and M. Rees. 2006. Integral projection models for species with complex demography. American Naturalist 167: 410-428.

Eriksson, O. 1985. Reproduction and clonal growth in *Potentilla anserine* L. (Rosaceae): the relation between growth form and dry weight allocation. Oecologia 66: 378-380.

Eriksson, O. 1988. Ramet behaviour and population growth in the clonal herb *Potentilla anserine*. Journal of Ecology 76: 522-536.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos 55: 231-238.

Eriksson, O. 1993. Dynamics of genets in clonal plants. Trends in Ecology and Evolution 8: 313-316.

Eriksson, O., and B. Bremer. 1993. Genet dynamics of the clonal plant *Rubus saxatilis*. Journal of Ecology 81: 533-542.

Eriksson, O. 1997. Clonal life histories and the evolution of seed recruitment. *In* H. de Kroon, and J. van Groenendael [eds.], The ecology and evolution of clonal plants, 211-226. Backhuys Publishers, Leiden, The Netherlands.

Evans, J. P. 1991. The effect of resource integration on fitness related traits in a clonal dune perennial, *Hydrocotyle bonariensis*. Oecologia 86: 268-275.

Fetcher, N., S. F. Oberbauer, and B. R. Strain. 1985. Vegetation effects on microclimate in lowland tropical forest in Costa Rica. International Journal of Biometeorology 29: 145-155.

Fetcher, N., S. F. Oberbauer, and R.L. Chazdon. 1994. Physiological ecology of plants at La Selva. *In* L. McDade, K.S. Bawa, H. Hespenheide and G. Hartshorn [eds.], La Selva: Ecology and Natural History of a Neotropical Rainforest, 128-141. University of Chicago, Chicago, Illinois, USA.

Fox, J. F. 1995. Shoot demographic responses to manipulation of reproductive allocation by bud removal in a willow. Oikos 72: 283-287.

Fox, G. A. 2001. Failure-time Analysis- Studying times to events and rates at which events occur. *In* S. M. Scheiner and J. Gerevitch [eds.], Design and Analysis of Ecological Experiments, 235-266. Oxford University Press, New York, New York, USA.

Frazer, G. W., C. D. Canham, and K. P. Lertzmanan. 1999. Gap Light Analyzer (GLA), version 2.0 imaging software to extract canopy structure and gap light transmission indices from true-color fisheye photographs. Copyright 1999: Simon

Frazer University, Burnaby, BC, and the Institute of Ecosystem Studies, Millbrook, New York, USA.

Frazer, G. W., C. D. Canham, and K. P. Lertzman. 2000. Gap Light Analyzer (GLA), Version 2.0: image-processing software to analyze true-color, hemispherical canopy photographs. Bulletin of the Ecological Society of America 81: 191-197.

Garcia, M. B. and J. Ehrlén. 2002. Reproductive allocation and herbivory timing in a perennial herb: fitness components at the individual and population levels. American Journal of Botany 89: 1295-1302.

Geber, M. A. 1990. The cost of meristem limitation in *Polygonum arenastrum*: negative correlations between fecundity and growth. Evolution 44: 799-819.

Grant, V., and K. A. Grant. 1980. Clonal microspecies of hybrid origin in the *Opuntia lindheimeri* group. Botanical Gazette 141: 101-106.

Greer, G. K. and B. C. McCarthy. 2000. Patterns of growth and reproduction in natural populations of the fern *Polystichum acrostichoides*. American Fern Journal 90: 60-76.

Greig, N. 1993. Regeneration mode in Neotropical *Piper*: habitat and species comparisons. Ecology 74: 2125-2135.

Harris, J. G., and M. H. Harris. 2003. Plant identification terminology. Spring Lake Publishing, Spring Lake, Utah.

Harper, J. L. 1977. Population biology of plants. Academic Press, London, UK.

Hartnett, D. C., and F. A. Bazzaz. 1983. Physiological integration among intraclonal ramets in *Solidago canadensis*. Ecology 64: 779-788.

Hartnett, D. C., and F. A. Bazzaz. 1985. The integration of neighborhood effects by clonal genets of *Solidago Canadensis*. Journal of Ecology 73: 415-427.

Hartshorn, G. S. 1983. Plants: Introduction. *In* D. H. Janzen [ed.], Costa Rican natural history, 118-157. University of Chicago Press, Chicago, Illinois, USA.

Hemborg, A. M. and P. S. Karlsson. 1998. Altitudinal variation in size effects on plant reproductive effort and somatic costs of reproduction. Ecoscience 5: 517-525.

Horvitz, C. C., and D. W. Schemske. 1986. Seed dispersal and environmental heterogeneity in a Neotropical herb: a model of population and patch dynamics. *In* A. E. Estrada and T. Fleming [eds.], Frugivores and Seed dispersal, 169-186. Dr. W. Junk, Dordrecht, Netherlands.

Horvitz, C. C., and D. W. Schemske. 1988. Demographic cost of reproduction in a Neotropical herb: An experimental field study. Ecology 69: 1741-1745.

Horvitz, C. C. 1991. Light environments, stage structure, and dispersal syndromes of Costa Rican Marantaceae. *In* C. R. Huxley and D. F. Cutler [eds.], Ant-Plant Interactions, 463-485. Oxford University Press, New York, New York, USA.

Horvitz, C. C., and J. Le Corff. 1993. Spatial scale and dispersion pattern of ant and bird dispersed herbs in two tropical lowland rain forests. Vegetatio 107/108: 351-362.

Horvitz, C., D. W. Schemske, and H. Caswell. 1996. The relative importance of lifehistory stages to population growth: prospective and retrospective analyses. *In* S. Tuljapurkar and H. Caswell [eds.], Structured population models in marine, terrestrial and freshwater systems, 247-271. Chapman and Hall, New York, New York, USA.

Horvitz, C. C., M. A. Pizo, B. B. Bello, J. Le Corff, and R. Dirzo. 2002. Are plant species that need gaps for recruitment more attractive to seed-dispersing birds and ants than other species? *In* D. J. Levey, W. R. Silva, and M. Galetti [eds.], Seed dispersal and frugivory: ecology, evolution and conservation, 145-159. CABI Publishing, Wallingford, UK.

Howe, C. D., and R. W. Snaydon. 1986. Factors affecting the performance of seedlings and ramets of invading grasses in an established Ryegrass sward. Journal of Applied Ecology 23: 139-146.

Huante, P., and E. Rincón. 1997. Responses to light changes in tropical deciduous woody seedlings with contrasting growth rates. Oecologia 113: 53-66.

Huante, P., E. Rincón, and F. S. Chapin III. 1998. Foraging for nutrients, responses to changes in light, and competition in tropical deciduous tree seedlings. Oecologia 117: 209-216.

Huenneke, L. F. and P. L. Marks. 1987. Stem dynamics of the shrub *Alnus incana* sp. *rugosa*: transition matrix models. Ecology 68: 1234-1242.

Hughes, J. W., T. J. Fahey, and F. H. Bormann. 1988. Population persistence and reproductive ecology of a forest herb: *Aster acuminatus*. American Journal of Botany 75: 1057-1064.

IAEA/WMO. 2006. Global Network of Isotopes in Precipitation. The GNIP Database. Website http://isohis.iaea.org [accessed 24 September 2008].

Jongejans, E., H. de Kroon, and F. Berendse. 2006. The interplay between shifts in biomass allocation and costs of reproduction in four grassland perennials under simulated successional change. Oecologia 147: 369-378.

Jónsdóttir, I. S., and T. V. Callaghan. 1989. Localized defoliation stress and the movement of ^{14}C-photoassimilates between tillers of *Carex bigelowii*. Oikos 59: 39-49.

Jónsdóttir, I. S., and M. A. Watson. 1997. Extensive physiological integration: An adaptive trait in resource-poor environments? *In* H. de Kroon and J. van Groenendael [eds.], The ecology and evolution of clonal plants, 109-136. Backhuys Publishers, Leiden, The Netherlands.

Jönsson, K. I. and J. Tuomi. 1994. Costs of reproduction in a historical perspective. Trends in Ecology and Evolution 9: 304-306.

Jurik, T. W. 1985. Differential costs of sexual and vegetative reproduction in wild strawberry populations. Oecologia 66: 394-403.

Kanno, H. and K. Seiwa. 2004. Sexual vs. vegetative reproduction in relation to forest dynamics in the understory shrub, *Hydrangea paniculata* (Saxifragaceae). Plant Ecology 170: 43-53.

Kennedy, S. 1978. Systematics and pollination of the "Closed-flowered" species of Calathea (Marantaceae). University of California Publications in Botany, Vol. 71. University of California Press, California, USA.

Krebs, C. 1999. Ecological Methodology. Addison-Wesley Educational Publishers, Inc., Menlo Park, California, USA.

Klimes, L., J. Klimesová, R. Hendriks, and J. van Groenendael. 1997. Clonal plant architecture: a comparative analysis of form and function. *In* H. de Kroon, and J. van Groenendael [eds.], The ecology and evolution of clonal plants, 1-29. Backhuys Publishers, Leiden, The Netherlands.

Kudoh, H., H. Shibaike, H. Takasu, D. F. Whigham, and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. Journal of Ecology 87: 244-257.

Kull, T. 1998. Fruit-set and recruitment in populations of *Cypripedium calceolus* L. in Estonia. Botanical Journal of the Linnean Society 126: 27-38.

Kullman, L. 1992. The ecological status of grey alder (*Alnus incana* (L.) Moench) in the upper subalpine birch forests of the central scades. New Phytologist 120: 445-451.

Kwano, S., T. Takada, S. Makayama, and A. Hiratsuka. 1987. Demographic differentiation and life-history evolution in temperate woodland plants. *In* K.M. Urbanska [ed.], Differentiation patterns in higher plants, 153-181. Academic Press, New York, USA.

Law, R., R. E. D. Cook, and R. J. Manlove. 1983. The ecology of flower and bulbil production in *Polygonum viviparum*. Nordic Journal of Botany 3: 559-565.

Levesque, R. 2007. SPSS Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago, Illinois, USA.

Levins, R. 1968. Evolution in changing environments. Princeton, NJ, USA, Princeton University Press.

Loehle, C. 1987. Partitioning of reproductive allocation in clonal plants: a benefit cost model. Oikos 49: 199-208.

Lovett-Doust, L. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). Journal of Ecology 69: 743–755.

Mandujano, M. Del C., C. Montana, I. Mendez, and J. Golubov. 1998. The relative contributions of sexual reproduction and clonal propagation in *Opuntia rastrera* from two habitats in the Chihuahuan desert. Journal of Ecology 86: 911-921.

Mandujano, M. C., C. Montana, M. Franco, J. Golubov, and A. Flores-Martinez. 2001. Integration of demographic annual variability in a clonal desert cactus. Ecology 82: 344-359.

Marshall, C., and G. R. Sagar. 1968. The distribution of assimilates in *Lolium multiflorum* Lam. Following differential defoliation. Annals of Botany 32: 715-719.

Marshall, C. 1990. Source-sink relations of interconnected ramets. *In* J. van Groenendael and H. de Kroon [eds.], Clonal growth in plants: regulation and function, 23-42. SPB Academic Publishing, The Hague, The Netherlands.

Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge, UK.

Mendez, M. 1999. Effects of sexual reproduction on growth and vegetative propagation in the perennial geophyte *Arum italicum* (Araceae). Plant Biology 1: 115-120.

Nault, A., and D. Gagnon. 1993. Ramet demography of *Allium tricoccum*, a spring ephemeral, perennial forest herb. Journal of Ecology 81: 101-119.

Newell, E. A. 1991. Direct and delayed costs of reproduction in *Aesculus californica*. Journal of Ecology 79: 365-378.

Nicotra, A. B. 1999. Reproductive allocation and the long-term costs of reproduction in *Sipanura grandiflora*, a dioecious Neotropical shrub. Journal of Ecology 87: 138-149.

Nishitani, A., and M. Kimura. 1995. Contrasting demographic characteristics of seeds and vegetative propagules in an understory herb *Syneilesis palmate* (Compositae). Plant Species Biology 10: 1-10.

Obeso, J. R. 2002. The costs of reproduction in plants. New Phytologist 155: 321-348.

O'Dea, M. E., J. C. Zasada, and J. C. Tappeiner III. 1995. Vine Maple clone growth and reproduction in managed coastal Oregon Douglas-fir forests. Ecological Applications 5: 63-73.

Partridge, L. 1992. Measuring reproductive costs. Trends in Ecology and Evolution 7: 99-100.

Pitelka, L. F., and J. W. Ashmun. 1985. Physiology and integration of ramets in clonal plants. *In* J. B. C. Jackson, L. W. Buss, and R. E. Cook [eds.], Population biology and evolution of clonal organisms, 399-436. Yale University Press, New Haven, Connecticut, USA.

Pearcy, R. W. 1987. Photosynthetic gas exchange responses of Australian tropical forest trees in canopy, gap and understory microenvironments. Functional Ecology 1: 169-178.

Pearcy, R. W. 1989. Radiation and light measurements. *In* R. W. Pearcy, J. Ehleringer, H. A. Mooney, and P. W. Rundel [eds.], Plant Physiological Ecology: Field Methods and Instrumentation, 95-116. Chapman and Hall, New York, New York, USA.

Peltzer, D. A. 2002. Does clonal integration improve competitive ability? A test using Aspen (*Populus tremuloides* [Salicaceae]) invasion into a prairie. American Journal of Botany 89: 494-499.

Phillips, P. L. 1989. The relationship of successional and primary tropical forest to color infrared photography. Thesis. University of Texas at Austin, Austin Texas, USA.

Pitelka, L. F., D. S. Stanton, and M. O. Peckenham. 1980. Effects of light and density on resource allocation in a forest herb, *Aster acuminatus* (Compositae). American Journal of Botany 67: 942-948.

Pitelka, L. F., and J. W. Ashmun. 1985. Physiology and integration of ramets in clonal plants. *In* J. B. C. Jackson, L. W. Buss, and R. E. Cook [eds.], Population biology and evolution of clonal organisms, 399-436. Yale University Press, New Haven, Connecticut, USA.

Pompa, J., and F. Bongers. 1991. Acclimation of seedlings of three Mexican tropical rain forest tree species to a change in light availability. Journal of Tropical Ecology 7: 85-97.

Prati, D. and B. Smith. 2000. Genetic differentiation of life-history traits within populations of the clonal plant *Ranunculus reptans*. Oikos 90: 442-456.

Price, E. A. C., and M. J. Hutchings. 1992. Studies of growth in the clonal herb *Glechoma hederacea*. II. The effect of selective defoliation. Journal of Ecology 80: 39-47.

Primack, R. B., S. L. Miao and K. R. Becker. 1994. Costs of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*): defoliation, increased fruit production, and fire. American Journal of Botany 81: 1083-1090.

Prosser, S. J., and C. M. Scrimgeour. 1995. High-precision determination of $^{2}H/^{1}H$ in H_2 and H_2O by continuous-flow isotope ratio mass spectrometry. Analytical Chemistry 67: 1992-1997.

Reekie, E. G. and F. A. Bazzaz. 1987. Reproductive allocation in plants. III. Effect of reproduction of vegetative activity. American Naturalist 129: 907-919.

Reznick, D. 1985. Cost of reproduction: en evaluation of the empirical evidence. Oikos 44: 257-267.

Rich, P. M. 1989. A manual for analysis of hemispherical canopy photography. Los Alamos National Laboratory, LA-11733-M, Los Almos, New Mexico.

Roff, D.A. 1992. The evolution of life histories. Chapman and Hall, New York, USA.

Roff, D. A. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. Journal of Evolutionary Biology 13: 434-445.

Ruzin, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, New York, USA.

Rydgren, K. and R. H. Økland. 2003. Short-term costs of sexual reproduction in the clonal moss *Hylocomium splendens*. 106: 212-220.

Saikkonen, K., S. Koivunen, T. Vuorisab, P. Mutikainen. 1998. Interactive effects of pollination and heavy metals on resource allocation in *Potentilla anserina* L. Ecology 79: 1620-1629.

Salzman, A. G., and M. A. Parker. 1985. Neighbors ameliorate local salinity stress for a rhizomatous plant in a heterogeneous environment. Oecologia 65: 273-277.

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584 – 606

Schmid, B., and F. A. Bazzaz. 1987. Clonal integration and population structure in perennials: effects of severing rhizome connection. Ecology 68: 2013–2022.

Shea, K., M. Rees, and S. N. Wood. 1994. Trade-offs, elasticities and the comparative method. Journal of Ecology 82: 951-957.

Shefferson, R. P., and E. L. Simms. 2007. Cost and benefits of fruiting to future reproduction in two dormancy-prone orchids. Journal of Ecology 95: 865-875.

Silvertown, J., M. Franco, I. Pisanty, and A. Mendoza. 1993. Comparative plant demography- relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. Journal of Ecology 81: 465-476.

Silvertown, J. W., and J. Lovett Doust. 1993. Introduction to Plant Population Biology. Blackwell Science, Oxford.

Sims, D. A., and R. W. Pearcy. 1991. Photosynthesis and respiration in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. American Journal of Botany 79: 449-455.

Snow, A. A. and D. F. Whigham. 1989. Costs of flower production in *Tipularia discolor* (Orchidaceae). Ecology 70: 1286-1293.

Sohn, J. and D. Policansky. 1977. The cost of reproduction in mayapple *Podophyllum peltatum* (Berberidaceae). Ecology 58: 1366-1374.

Sokal R. R., and F. J. Rolf. 1995. Biometry: The principles and practice of statistics in biology research. W.H. Freeman, New York.

Sork, V. 1987. Effects of predation and light on seedling establishment in *Gustavia superba*. Ecology 68: 1341-1350.

Sterns, S. C. 1989. Trade-offs in life-history evolution. Functional Ecology 3: 259-268.

Stuefer, J. F., and M. J. Hutchings. 1994. Environmental heterogeneity and clonal growth: a study of the capacity for reciprocal transport in *Glechoma hederacea* L. Oecologia 100: 302-308.

Stuefer, J. F., H. De Kroon, H. J. During. 1996. Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. Functional Ecology 10: 328-334.

Sutherland, S. and R. K. Vickery. 1988. Trade-offs between sexual and asexual reproduction in the genus *Mimulus*. Oecologia 76: 330-335.

Svenning, J. 2000. Growth strategies of clonal palms (Arecaceae) in a neotropical rainforest, Yasuni Ecuador. Australian Journal of Botany 48: 167-178.

Svensson, B. M., B. A. Carlsson, P. S. Karlsson, and O. Nordell. 1993. Comparative long-term demography of three species of *Pinguicula*. Journal of Ecology 81: 635-645.

Tamm, A., K. Kull, and M. Summul. 2002. Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. Evolutionary Ecology 15: 383-401.

Taylor, J. R. 1997. Error analysis. University Science Books, Sausalito, California, USA.

Tobler, M.A., P. A. Van Zandt, K. H. Masenstein, and S. Mopper. 2006. Growth and reproduction of a clonal plant in response to salinity and florivory. Wetlands 26: 803-812.

Tuomi, J., T. Hakala, and E. Haukioja. 1983. Alternative concepts of reproductive allocation, cost of reproduction, and selection in life-history evolution. American Zoologist 23: 25-34.

Turkington, R., M. A. Cahn, A. Vardy, and J. L. Harper. 1979. The growth, distribution of neighbour relationships of *Trifolium repens* in a permanent pasture. III. The establishment and growth of *Trifolium repens* in natural and perturbed sites. Journal of Ecology 67: 231-243.

Van Kleunen, M. and J. F. Stuefer. 1999. Quantifying the effects of reciprocal assimilate and water translocation in a clonal plant by the use of steam-girdling. Oikos 85: 135-145.

Venecz, J. I., and L. W. Aarssen. 1998. Effects of shoot apex removal in *Lythrum salicaria* (Lythraceae): Assessing the costs of reproduction and apical dominance. Annals of Botany Fennici 35: 101-111.

Vendramini, P. F. and L. Da Sternberg. 2007. A faster plant stem-water extraction method. Rapid Communications in Mass Spectrometry 21: 164-168.

Verburg, R. W. and H. J. During. 1998. Vegetative propagation and sexual reproduction in the woodland understorey pseudo-annual *Circaea lutetiana* L.. Plant Ecology 134: 211-224.

Weiner, J. 1988. The influence of competition on plant reproduction. *In* J. Lovett-Doust and L. Lovett-Doust [eds.], Reproductive plant ecology: Patterns and strategies, 228-245. Oxford University Press, Oxford, UK.

Weppler, T., P. Stoll, and J. Stöcklin. 2006. The relative importance of sexual and clonal reproduction for population growth in the long-lived alpine plant *Geum reptans*. Journal of Ecology 94: 869-879.

Westley, L. C. 1993. The effect of inflorescence bud removal on tuber production in *Helianthus tuberosus* L. Ecology 74: 2136-2144.

White, J. W. C., E. R. Cook, J. R. Lawrence, and W. S. Broecker. 1985. The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. Geochimica et Cosmochimica Acta 49: 237-246.

White, E. G. 1984. A multispecies simulation model of grassland producers and consumers: producers. Ecological Modeling 24: 241–262.

Wijesinghe, D. A., and M. J. Hutchings. 1997. The effects of spatial scale on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. Journal of Ecology 85: 17-28.

Williams, G. C. 1966. Natural selection, the costs of reproduction, and the refinement of Lack's principle. American Naturalist 100: 687-690.

Worley, A. C. and L. D. Harder. 1996. Size-dependent resource allocation and costs of reproduction in *Pinguicula vulgaris* (Lentibulariaceae). Journal of Ecology 84: 195-206.

Wyka, T. and C. Galen. 2000. Current and future costs of reproduction in *Oxytropis sericea*, a perennial plant from the Colorado Rocky Mountains, USA. Arctic, Antarctic and Alpine Research 32: 438-448.

Zar, J. H. 1999. Biostatistical analysis. Forth Edition, Prentice Hall, New Jersey.