The invasive tropical shrub Clidemia hirta (Melastomataceae) in its native and introduced ranges: tests of hypotheses of invasion

Saara Jennie DeWalt

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THE INVASIVE TROPICAL SHRUB CLIDEMIA HIRTA
(MELASTOMATACEAE) IN ITS NATIVE AND INTRODUCED RANGES:
TESTS OF HYPOTHESES OF INVASION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
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requirements for the degree of
Doctor of Philosophy

in

The Department of Biological Sciences

by
Saara Jennie DeWalt
A.B., Brown University, 1994
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Abstract

Exotic pest plants often grow to greater stature, become more abundant, and display increased shade tolerance in their introduced ranges than in their native ranges. These differences have been hypothesized to result from genetic shifts in biomass allocation, growth, or photosynthesis between genotypes in native and introduced ranges or from plastic, phenotypic responses to different environmental conditions, such as lower herbivore or fungal pest loads in areas of introduction. I used the tropical shrub *Clidemia hirta* (Melastomataceae) as a model exotic pest plant to test these two non-mutually exclusive hypotheses of invasion. *Clidemia hirta* invades forest understory and is more abundant in much of its introduced range in parts of Oceania, Asia, and Africa than in its native range in Central and South America, where it does not occur in forest understory. Contrary to predictions, I found less genetic variation, as detected with allozymes, within and among native, Costa Rican populations than introduced, Hawaiian populations of *C. hirta*. Hawaiian and Costa Rican populations also were markedly dissimilar genetically (Nei’s $I = 0.64$), but there were few ecologically important differences in biomass allocation, growth, or photosynthetic parameters between Costa Rican and Hawaiian genotypes grown under high or low light in a common garden experiment. The absence of *C. hirta* from forest understory in its native range likely results, at least in part, from the strong pressures of insect herbivores and pathogens (natural enemies). A natural enemy exclusion study conducted in the field showed that insect herbivore and fungal pathogen damage was substantially greater on Costa Rican than Hawaiian plants and that these natural enemies caused substantial mortality of *C. hirta* planted into forest understory in Costa Rica but not Hawaii. These results coupled with demographic data collected over three years in two Hawaiian populations suggest that biological control could cause a decline in *C. hirta* population growth rates in Hawaiian forests. For now the expanded habitat distribution and vigor of *C. hirta* in its introduced range seems to result from an ecological response to enemy release rather than a genetic shift in resource acquisition, allocation, or growth.
Chapter 1

Introduction

Introductions of flora and fauna to provinces outside their home range have contributed to homogenization of the world’s biota. Biological invasions are natural ecological processes and the movement of plants and animals across geographic barriers always has occurred (Sauer 1988); however humans have greatly accelerated the rate of introductions and moved organisms across barriers that likely would not have been spanned naturally. Out of those introduced, many species have established, become naturalized, and invaded communities and ecosystems to the detriment of native species (Gordon 1998, Holway 1998, Parker et al. 1999, Mack et al. 2000, Alvarez and Cushman 2002). The environmental damage and control costs of non-native, invasive organisms in the United States alone are estimated at $137 billion per year (Pimentel et al. 2000). Non-native plant invasions, in particular, can change ecosystem-level processes and reduce biological diversity at many trophic levels (Vitousek et al. 1987, Richardson et al. 1989, Vitousek and Walker 1989, Vitousek 1992, Maron and Connors 1996). In this dissertation, I use one of these disruptive non-native, invasive species to test hypotheses of invasion and to compare genetic aspects of the species and attributes of the environments between where the species is native and where it is introduced.

TERMINOLOGY

Many terms are used to describe biological invasions. Considerable debate has arisen over which terms should be used to convey accurately the ecological and biogeographical aspects of invasions (Rejmánek 1995, Daehler 2001, Davis and Thompson 2001, Rejmánek et al. 2002). I will use terms advocated by Richardson et al. (2000). “Invasion” will be defined as a species’ expansion into and colonization of an area it has not previously inhabited. Therefore, invasions may involve human-mediated introduction of species or natural expansions of species, such as those following glaciation or land formation. The term “non-native, invasive species” indicates a species that has been introduced outside of its native geographical range, become naturalized, and proliferated. Williamson (1996b) highlighted different levels of invasion success: introduced, established, and pests. Introduced species are those that are found outside
control or captivity in potentially self-sustaining populations. However, many introduced plants fail to establish or, in other words, fail to create self-sustaining populations. Of the species that do establish, some have harmful economic or environmental effects. These species are called “pests.” Here, invasive plants that have been introduced outside their native range by humans and have a strong negative impact, either economically or environmentally, will be called “exotic pests” or “weeds” (Richardson et al. 2000, Rejmánek et al. 2002). Thus, the term “invasive” does not imply that the species has a harmful effect, only that it has spread and proliferated.

INVASIBILITY AND INVASIVENESS

Attributes of communities have been examined to determine which biotic and abiotic characteristics of recipient environments affect invasibility. The result of these studies has been recognition that few communities are resistant to plant invasions (Lodge 1993, Rejmánek 1999). Even seemingly resistant communities, such as species-rich tropical rain forests, have been found to be susceptible (Usher 1991, Rejmánek 1996). Oceanic island forests have been more heavily invaded (Elton 1958, Lonsdale 1999), but invasions in mainland forests also have occurred (e.g., Sheil 1994, Sheil et al. 2000, Peters 2001).

Species’ attributes also have garnered much attention, primarily because of the potential power to predict invasiveness from morphological, growth, reproductive, or photosynthetic traits. Characteristics of invaders of agricultural and natural areas differ to a large degree (Roy 1990, Daehler 1998). Agricultural weeds tend to have some of the traits that Baker (1965, 1974) defined as aspects of an “ideal weed”: ability to reproduce sexually and asexually, rapid growth, short pre-reproductive period, high phenotypic plasticity and tolerance to different environmental conditions, and continuous and high seed production. Abiotically pollinated species are common invaders of both natural and agricultural areas (Daehler 1998). Largely woody angiosperm families are over represented in non-native, invasive species lists of natural areas (Daehler 1998). Among North American woody angiosperms, invasiveness elsewhere and facility for vegetative propagation were the best predictors of invasiveness in North America (Reichard and Hamilton 1997). For the most part, the species’ traits used in these predictive models are measured on plants in the areas of introduction.

Yet, there is evidence that conspecifics often differ in key ways in their native and introduced ranges. Plants may become more abundant (Fowler et al. 1996, Williamson and
Fitter 1996a), have faster growth rates (Blossey and Nötzold 1995, Fowler et al. 1996), grow to
greater stature (Pritchard 1960, Blossey and Nötzold 1995), or have higher seed production
(Noble 1989, Paynter et al. 1996) where they have been introduced than where they are native.
In addition, some introduced species invade habitats in which they do not occur in their native
range. Of a list of 42 non-native, woody invaders of tropical forests (Rejmánek 1996), at least
eight species are woody pioneer plants that are restricted to open habitats in their native ranges
but invade closed tropical forests where they have been introduced (Table 1.1). This
phenomenon likely occurs for more species, but information on habitat distributions of species in
their native range often is scant.

Several non-mutually exclusive hypotheses have been proposed to account for the
proliferation and habitat expansion of species in their introduced compared to their native range.
These hypotheses include a release from herbivory or fungal pathogens (Elton 1958, Crawley
1987, Blossey and Nötzold 1995, Keane and Crawley 2002), increased resource availability
(Denslow 2003), less dispersal or recruitment limitation (Tilman 1997), and appearance of more
vigorous genotypes (Blossey and Nötzold 1995, Blossey and Kamil 1996) in the areas of
introduction. These hypotheses all propose a limitation to abundance or habitat distribution in
the area of origin and then a change, shift, or release in the area of introduction. The first three
hypotheses suggest that the changes involve a plastic response to differences in environmental
conditions, while the last hypothesis suggests that genotypes in the native and introduced ranges
differ genetically. Many studies have examined genetic and environmental factors that affect
non-native species in their introduced range (e.g., Waloff and Richards 1977, Noble 1989, Novak
et al. 1991, D'Antonio 1993), but few studies have examined the effects of these factors on
species in their native ranges.

The goals of this dissertation were to examine some of the ecological and genetic
characteristics of one woody tropical invasive species, Clidemia hirta var. hirta (L.) D. Don
(Melastomataceae), to test two proposed hypotheses of invasion, and to provide management
recommendations based on these results. This species is limited to partially to completely open
habitats where native, but invades mainland and island tropical forest understory where it has
been introduced (Table 1.1). I focused on testing hypotheses that might explain the differences
in habitat distribution and abundance. Certainly for all exotic species, suitable climate and
adequate resource availability are prerequisites for invasion. However, I do not focus on these
Table 1.1. Non-native, invasive woody species of old-growth (OG) tropical forests that are found primarily in open areas in their native range. Invasive range is from Rejmánek (1996). References or personal communications refer to the source of information on the habitat in the native range.

<table>
<thead>
<tr>
<th>Species (Family)</th>
<th>Native Range</th>
<th>Native Range</th>
<th>Introduced Range</th>
<th>Introduced Range</th>
<th>Reference or Personal Communication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clidemia hirta</em> (Melastomataceae)</td>
<td>Central and South America</td>
<td>Open, disturbed areas</td>
<td>Hawaii, Asia, Africa</td>
<td>Open areas, OG forest</td>
<td>Wester and Wood (1977)</td>
</tr>
<tr>
<td><em>Cryptostegia grandiflora</em> (Asclepiadaceae)</td>
<td>Madagascar</td>
<td>Open, disturbed areas</td>
<td>Northern Australia, Puerto Rico</td>
<td>Open areas, OG forest</td>
<td>L. Civeyrel pers. comm.</td>
</tr>
<tr>
<td><em>Dioscorea alata</em> (Dioscoreaceae)</td>
<td>Asia</td>
<td>Gaps, forest edges</td>
<td>Tanzania</td>
<td>OG forest</td>
<td>R. Ganesan pers. comm.</td>
</tr>
<tr>
<td><em>Duranta erecta</em> (Verbenaceae)</td>
<td>Central and South America</td>
<td>Open, dry, coastal areas</td>
<td>Tanzania, Uganda, Madagascar</td>
<td>OG forest</td>
<td>P. Acevedo pers. comm.</td>
</tr>
<tr>
<td><em>Litsea glutinosa</em> (Lauraceae)</td>
<td>Asia</td>
<td>Gaps, forest edges</td>
<td>Tanzania, Madagascar Mauritius</td>
<td>OG forest</td>
<td>R. Ganesan pers. comm.</td>
</tr>
<tr>
<td><em>Maesopsis eminii</em> (Rhamnaceae)</td>
<td>Africa</td>
<td>Gaps, grasslands</td>
<td>Tanzania, Puerto Rico, Rwanda, Fiji, India</td>
<td>Open areas, OG forest</td>
<td>Bingelli et al. (1998)</td>
</tr>
<tr>
<td><em>Miconia calvescens</em> (Melastomataceae)</td>
<td>Central and South America</td>
<td>Gaps, steep slopes</td>
<td>Tahiti, Hawaii</td>
<td>Open areas, OG forest</td>
<td>Bingelli et al. (1998)</td>
</tr>
<tr>
<td><em>Thunbergia grandiflora</em> (Acanthaceae)</td>
<td>India</td>
<td>Gaps, forest edges</td>
<td>Singapore, Australia</td>
<td>OG forest</td>
<td>R. Ganesan pers. comm.</td>
</tr>
</tbody>
</table>

aspects of invasion. Instead, I conducted field, laboratory, and greenhouse experiments to determine whether or not the observed differences in the abundance and habitat distribution of this species in its native and invasive ranges result from *environmental* differences in natural enemy loads (enemy release hypothesis) and/or *genetic* differences that affect competitive ability or shade tolerance (genetic shift hypothesis). The observed differences could result from many other factors, but I focused on the often cited, yet infrequently tested, enemy release and genetic shift hypotheses. These also are testable hypotheses. Below I provide background information on *Clidemia hirta* and detail the structure of the dissertation.
**STUDY ORGANISM**

*Clidemia hirta* is a densely branching, slightly woody (suffrutescent) shrub that grows to a height of 1 – 3 m depending on environmental conditions. The species is native to lowlands of Central and South America and the Caribbean Islands where it occurs in mesic to wet environments from sea level to 1500 m elevation (Figure 1.1). In its native range, *Clidemia hirta* is found in naturally and anthropogenically disturbed areas such as pastures, riversides, roadsides, and tree plantations but not in old-growth forests (Wester and Wood 1977). *Clidemia hirta* is now found worldwide in climatic conditions similar to its native range (Figure 1.1). It is recognized as an aggressive, disruptive invader of open and forested areas of the Hawaiian archipelago, American Samoa, Fiji, Mauritius, Seychelles, Southeast Asia (Peninsular Malaysia, Singapore and Borneo), Sri Lanka, and Tanzania (Lever 1937, Wester and Wood 1977, Gerlach 1993, Sheil 1994, Strahm 1999, Singhakumara et al. 2000, Peters 2001, Teo et al. 2003).

*Clidemia hirta* was first reported on the island of Oahu, Hawaii, in 1941 (Anonymous 1954) and spread in the 1970s and 1980s to the islands of Kauai, Maui, Molokai, Lanai, and Hawaii (Smith 1992). No native Melastomataceae are found on the Hawaiian Islands, but 14 species in this

![Figure 1.1 Geographical distribution of Clidemia hirta in areas where it is native (circles) and introduced (squares).](image)
family have been introduced and become naturalized in Hawaii (Wester 1992). *Clidemia hirta* is on the Hawaii State Noxious Weed list (Division of Plant Industry 1992).

The introduction of *C. hirta* around the world likely resulted from seed contamination of nursery stock of coffee plants. The species is thought to have been brought to Fiji and Java between 1880 and 1886 as a contaminant of coffee plants from Guyana (Simmonds 1933); how it later spread to other areas is not known. Unlike ornamental or crop plants, *C. hirta* appears not to have been selected for horticultural or agricultural characteristics. The fruits are pulpy, dark-blue berries that are produced year-round. Fruit may contain between 200 and 900 seeds, each about 0.5 mm in diameter. The seeds are primarily bird-dispersed, but they have also been found in feces of Indian mongoose (*Herpestes auropunctatus*) and rats (*Rattus rattus*) in Hawaii (A. Medeiros, *unpublished data*).

The breeding system of *C. hirta* may include both sexual reproduction and apomixis. Self-pollination, on the other hand, is highly unlikely given the spatial separation between anthers and stigma in the flowers (Renner 1989). In addition, the anthers of *Clidemia* species, and most species in the Melastomataceae, are poricidal and pollen escapes the anther only via a vector (Renner 1989). Bees that collect pollen from Melastomataceae have been found to vibrate around 420 Hz or higher, frequencies high enough to suggest that low-frequency vibrations produced by wind likely could not release pollen from the anthers (Renner 1989). Agamospermy (a form of apomixis) has been documented in *C. hirta* within its native range (Renner 1989). Thus, at least some of the seeds in the native range are produced asexually and are genetically identical to the parent.

**DISSERTATION STRUCTURE**

I chose to study *Clidemia hirta* in one part of its native range – Costa Rica – and one part of its introduced range – Hawaii. The source of Hawaiian populations of *Clidemia hirta* was, and is still, unknown. Thus, my comparisons between the ecology and genetics of this species in the two areas make no assumptions that source populations are located in Costa Rica or even Central America.

In Chapter 2, I begin an evaluation of how genetic differences between native and introduced areas may contribute to the success of *C. hirta* in Hawaii. The first step of this process was to determine how similar Costa Rican and Hawaiian populations are in their genetic
relatedness and levels of genetic variation within and among populations. I compared allozyme-detectable variation in 20 populations each of Costa Rica and Hawaii. In Hawaii, I sampled populations on four of the main islands to try to elucidate the introduction history.

Chapter 3 follows from this research by using a subset of these populations to test the genetic shift hypothesis. This hypothesis states that genetic differences in growth, biomass allocation, or photosynthetic parameters between native and introduced genotypes contribute to observed differences in habitat distribution and abundance. Results from previous tests of this hypothesis have been equivocal; greater size and reproductive effort of some species in their introduced than native range is genetically determined (Blossey and Nötzold 1995, Blossey and Kamil 1996, Siemann and Rogers 2001, Leger and Rice 2003), but is not for other species (Willis et al. 1999, Willis et al. 2000). I controlled for the effect environmental differences might have on these variables by conducting a greenhouse common garden experiment with *Clidemia hirta* grown from seed collected in four populations each from both Costa Rica and Hawaii. Plants were grown under high or low light for six months.

In Chapter 4, I ask whether one environmental characteristic, insect herbivore and fungal pathogen pest loads, affects the habitat distribution, growth, and survival of *C. hirta* in its native and introduced ranges. Specifically, I test the enemy release hypothesis, which posits that herbivores and pathogens (natural enemies) limit growth or survival of plants in native areas, that natural enemies have less impact in the introduced than in the native range, and that the release from natural enemy regulation in areas of introduction accounts in part for observed changes in plant abundance and habitat distribution. Almost all examinations of the enemy release hypothesis have been limited to observational studies comparing pest loads on plants in native and introduced ranges (Wolfe 2002, Mitchell and Power 2003) or have compared the effects of natural enemies on co-occurring native and exotic congeners (Schierenbeck et al. 1994, Blaney and Kotanen 2001). I tested experimentally the enemy release hypothesis by planting *C. hirta* into understory and open habitats in Costa Rica and Hawaii and applied pesticides to examine the effects of fungal pathogen and insect herbivore exclusion on survival and growth.

Chapter 5 departs from tests of invasion hypotheses to evaluate the potential for biological control agents to control *Clidemia hirta* in Hawaiian rainforests. I use stage-structured (Lefkovitch) matrix projection models parameterized with field data collected over three years in two lowland Hawaiian forests as well as the results from Chapter 4 to project the likely effects
on the population growth rate of specific changes in vital rates (growth, survival, and fecundity of different life history stages). I recommend a biological control strategy that the analyses suggest would cause the greatest declines in the asymptotic population growth rate of C. hirta in Hawaii. This demographic approach promises to help design effective and parsimonious biological control programs (Shea and Kelly 1998, McEvoy and Coombs 1999, Parker 2000).

Chapter 6 provides a general conclusion, practical applications of the research, and future directions for the study of exotic pest plants. Overall, this dissertation provides a novel comparison of various aspects of the ecology and genetics of a woody tropical invader in its native and introduced ranges. Few studies have undertaken to examine a non-native, invasive species in both its native and introduced ranges, despite the better understanding of invasions that may come of such an approach (Sakai et al. 2001).

LITERATURE CITED


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Division of Plant Industry. 1992. List of plant species designated as noxious weeds. Hawaii Department of Agriculture, Honolulu, Hawaii, USA.


Chapter 2
A Comparison of Genetic Diversity in Populations from Native and Introduced Ranges

INTRODUCTION

For exotic plants, the amount and distribution of genetic variation are determined by founder population genetic diversity, number of founders, life history characteristics, and post-introduction processes (e.g., founder effects, selection, and genetic drift). Theory predicts that plants in their introduced range will have diminished within-population genetic variation and increased among-population genetic differentiation relative to their native range (Brown and Marshall 1981, Barrett and Richardson 1986, Husband and Barrett 1991) because founder effects and genetic drift tend to reduce heterozygosity and lead to interpopulation differentiation when introduced population sizes are small. However, colonizations that stem from multiple introductions or involve large founding populations may not exhibit large reductions in genetic variation (Novak and Mack 1993, Wang et al. 1995, Amsellem et al. 2000). In addition, separate introductions from multiple parts of the native range may result in intermingling of genotypes that increase sampled genetic diversity (intraspecific hybridization; sensu Ellstrand and Schierenbeck 2000).

Life history characteristics, such as the mating system, also affect levels of within- and among-population genetic diversity of introduced plants. Many exotic weeds have uniparental reproduction (selfing or apomixis). Whereas outcrossing sexually reproducing species generally maintain higher within-population genetic variation and have less divergence among populations and subpopulations, asexual or selfing species generally have lower overall genetic variation, higher homozygosity, and increased genetic structure, resulting from genetic drift and low levels of gene flow (Hamrick and Godt 1989, 1996). Uniparental reproduction reduces the number of recombinations and thus the frequency of observed heterozygotes in a population, but levels of expected heterozygosity may be similar to those in outcrossing populations.

Most attempts to determine predictive characteristics of exotic invasive species have met with little success (Mack et al. 2000). Generalizations about levels of genetic diversity for
introduced plants may also not be possible given the diversity of mating systems, life forms, dispersal syndromes, and introduction histories that they encompass. As there are multiple ways to be rare (Karron 1987, Gitzendanner and Soltis 2000), there seem to be many ways to be introduced. However, more insights on genetic variation and its relative importance in determining invasiveness will come from comparative ecological and genetic studies between plant species in their native and introduced ranges.

Our objective was to estimate the genetic diversity of the tropical shrub *Clidemia hirta* in Hawaii, where this plant is an exotic pest, and to place this diversity in the context of its native range. We did not attempt to identify source populations of *C. hirta* on Hawaii as this would have entailed extensive sampling throughout Central and South America. Instead, we compared populations across the Hawaiian archipelago to populations across Costa Rica, where *C. hirta* occurs naturally. Our interest in the genetic structure of *C. hirta* in Costa Rica and Hawaii is part of a larger project in which we are examining how environmental and genetic factors may contribute to the greater abundance and habitat breadth of this species in its introduced than in its native range (Chapters 3 and 4).

In this paper, we address the following questions: (1) How much genetic diversity is held within and among introduced Hawaiian populations of *C. hirta*? (2) What does the distribution of genotypes and genetic diversity across the Hawaiian archipelago indicate about its introduction history and subsequent spread? and (3) How do the genetic diversity and structure of *C. hirta* populations in introduced Hawaiian populations compare to native populations sampled in Costa Rica? We further compare levels of genetic diversity in *C. hirta* related to other weeds, native Hawaiian species, and widespread tropical woody species.

**MATERIALS AND METHODS**

**Study Organism**

*Clidemia hirta* (L.) D. Don (Melastomataceae) is native to the lowlands of Central and South America and the Caribbean Islands where it occurs in mesic to wet environments to 1500 m elevation. It was introduced and is now naturalized throughout the tropics including the Hawaiian archipelago, American Samoa, Fiji, Mauritius, Seychelles, Southeast Asia (Peninsular Malaysia and Borneo), Sri Lanka, and Tanzania (Lever 1937, Wester and Wood 1977, Gerlach 1993, Sheil 1994, Strahm 1999, Singhakumara et al. 2000, Peters 2001). *Clidemia hirta* was first
reported on the island of Oahu in the Hawaiian archipelago in 1941 (Anonymous 1954) and spread in the 1970s and 1980s to Kauai, Maui, Molokai, Lanai, and Hawaii (Smith 1992). Unlike ornamental or crop plants that have been introduced worldwide, *C. hirta* was likely an accidental introduction (Simmonds 1933, Wester 1992). The precise origin of introduced *C. hirta* is not known. Fruits are pulpy, dark-blue berries produced year-round and contain from 200 – 900 seeds, each about 0.5 mm in diameter (Chapter 5). Seeds are animal-dispersed, primarily by birds. The mating system of *C. hirta* in its native range includes both sexual reproduction through bee pollination and asexual reproduction through agamospermy (a form of apomixis; Renner 1989). The mating system of *C. hirta* in its introduced range has not been examined. The proportion of seeds produced through apomixis versus sexual reproduction is not known for either the native or introduced range. Although a vigorous resprouter following damage, *C. hirta* does not propagate vegetatively.

**Sample Collections**

We collected *Clidemia hirta* fruit in 20 populations across four of the main Hawaiian Islands (Kauai, Oahu, Maui, and Hawaii) and 20 populations in five geographic regions of Costa Rica (EARTH, La Selva, Quepos, Los Chiles, and San Carlos) (Figure 2.1). Populations were separated geographically by at least 2 km. We collected three fruits from each of 11 – 13 plants per population and placed them in labeled bags. Fruit were collected in Hawaiian populations located along roadsides, trailsides, and forest understory from 0 – 800 m a.s.l. and in Costa Rican populations located along pastures, roadsides, and young plantations of *Bactris gasipaes* from 0 – 300 m a.s.l. (Table 2.1). Maternal plants were chosen haphazardly within populations. Seeds were separated from the fruit pulp, dried at room temperature, and stored in Eppendorf tubes labeled with a number unique to the individual fruit and maternal plant.

Ten to 20 seeds per fruit were surface sown on Fafard 3B mix (Conrad Fafard, Awawam, MA) in trays divided into individual cells for each fruit. Trays were placed on a mistbench at the University of Georgia greenhouse until seedlings were established (about 1.5 mo after sowing). At this time, they were moved off the mistbench, and seedlings were hand-watered. In this way, we ensured that seeds did not move between cells. One seedling per cell (fruit) was randomly
Figure 2.1 Collection sites of the 40 *Clidemia hirta* populations used in this study that were located in (a) Hawaii and (b) Costa Rica. Shaded areas around populations in Costa Rica delimit the five regions chosen *a priori* based on geographic proximity. Numbers correspond to populations listed in Table 2.1.
Table 2.1 Locations of 40 *Clidemia hirta* sample sites in (a) Hawaii and (b) Costa Rica. The population numbers correspond to those in Figure 2.1. $N_b$ is the number of samples collected in the bulk data set and $N_i$ is the number collected in the individuals data set.

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>$N_b$ ($N_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Hawaii</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kauai</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Na Pali Trail</td>
<td>22°10’</td>
<td>159°35’</td>
<td>29 (12)</td>
</tr>
<tr>
<td>2</td>
<td>Limahuli Botanical Garden</td>
<td>22°13’</td>
<td>159°34’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>3</td>
<td>Hanalei</td>
<td>22°09’</td>
<td>159°27’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>4</td>
<td>Sleeping Giant</td>
<td>22°05’</td>
<td>159°26’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>Oahu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Hauula</td>
<td>21°38’</td>
<td>157°59’</td>
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</tr>
<tr>
<td>6</td>
<td>Kahana Valley</td>
<td>21°31’</td>
<td>157°57’</td>
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</tr>
<tr>
<td>7</td>
<td>Maunawili</td>
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<td>157°50’</td>
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</tr>
<tr>
<td>8</td>
<td>Kuliouou</td>
<td>21°19’</td>
<td>157°49’</td>
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</tr>
<tr>
<td>9</td>
<td>Manoa</td>
<td>21°22’</td>
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</tr>
<tr>
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<td>Kahili St.</td>
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<td>157°58’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>11</td>
<td>Aiea</td>
<td>21°26’</td>
<td>158°02’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>Maui</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Road to Hana 1</td>
<td>20°55’</td>
<td>156°14’</td>
<td>30 (12)</td>
</tr>
<tr>
<td>13</td>
<td>Road to Hana 2</td>
<td>20°54’</td>
<td>156°13’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>14</td>
<td>Road to Hana 3</td>
<td>20°53’</td>
<td>156°12’</td>
<td>31 (12)</td>
</tr>
<tr>
<td>Hawaii</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Laupahoehoe Nature Reserve</td>
<td>19°55’</td>
<td>155°21’</td>
<td>32 (12)</td>
</tr>
<tr>
<td>16</td>
<td>Road B, Waiakea Forest Reserve</td>
<td>19°41’</td>
<td>155°08’</td>
<td>31 (12)</td>
</tr>
<tr>
<td>17</td>
<td>Waiakea Forest Reserve</td>
<td>19°39’</td>
<td>155°11’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>18</td>
<td>Lava Tree State Park</td>
<td>19°27’</td>
<td>154°59’</td>
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</tr>
<tr>
<td>19</td>
<td>Leilani Estates</td>
<td>19°24’</td>
<td>155°02’</td>
<td>28 (11)</td>
</tr>
<tr>
<td>20</td>
<td>South Kona</td>
<td>19°22’</td>
<td>155°50’</td>
<td>35 (12)</td>
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</table>
(Table 2.1 continued)

<table>
<thead>
<tr>
<th>Population Location</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>$N_b (N_i)$</th>
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</thead>
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<td>(b) Costa Rica</td>
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<tr>
<td><strong>EARTH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 CATIE</td>
<td>9°53’</td>
<td>83°39’</td>
<td>34 (12)</td>
</tr>
<tr>
<td>2 Carlos</td>
<td>10°06’</td>
<td>83°30’</td>
<td>34 (12)</td>
</tr>
<tr>
<td>3 EARTH University</td>
<td>10°09’</td>
<td>83°33’</td>
<td>32 (12)</td>
</tr>
<tr>
<td><strong>La Selva</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Nazareth</td>
<td>10°16’</td>
<td>83°54’</td>
<td>39 (13)</td>
</tr>
<tr>
<td>5 Teal House</td>
<td>10°19’</td>
<td>83°55’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>6 Ticari</td>
<td>10°22’</td>
<td>83°55’</td>
<td>33 (11)</td>
</tr>
<tr>
<td>7 El Tigre</td>
<td>10°24’</td>
<td>83°59’</td>
<td>34 (12)</td>
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<tr>
<td><strong>San Carlos</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Chicken</td>
<td>10°22’</td>
<td>84°10’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>9 Pino</td>
<td>10°20’</td>
<td>84°12’</td>
<td>36 (11)</td>
</tr>
<tr>
<td>10 Venecia</td>
<td>10°15’</td>
<td>84°18’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>11 Muelle</td>
<td>10°24’</td>
<td>84°28’</td>
<td>31 (11)</td>
</tr>
<tr>
<td><strong>Los Chiles</strong></td>
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<td></td>
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</tr>
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<td>12 Poco Sol</td>
<td>10°40’</td>
<td>84°32’</td>
<td>36 (12)</td>
</tr>
<tr>
<td>13 Stream</td>
<td>11°00’</td>
<td>84°40’</td>
<td>34 (12)</td>
</tr>
<tr>
<td>14 Los Chiles</td>
<td>10°58’</td>
<td>84°42’</td>
<td>33 (12)</td>
</tr>
<tr>
<td>15 Caño Negro</td>
<td>10°52’</td>
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<tr>
<td><strong>Quepos</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16 Naranjito</td>
<td>9°27’</td>
<td>84°04’</td>
<td>34 (12)</td>
</tr>
<tr>
<td>17 Villa Nueva</td>
<td>9°30’</td>
<td>84°03’</td>
<td>38 (13)</td>
</tr>
<tr>
<td>18 Londres</td>
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<td>36 (12)</td>
</tr>
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<td>19 Platanillo</td>
<td>9°22’</td>
<td>83°46’</td>
<td>35 (12)</td>
</tr>
<tr>
<td>20 Molle Jones</td>
<td>9°16’</td>
<td>83°30’</td>
<td>31 (12)</td>
</tr>
</tbody>
</table>
selected 4 – 6 mo after sowing, when the plants were of sufficient size for enzyme extraction. Seedlings ranged in height from 1 – 12 cm at the time of extraction.

**Enzyme Extraction and Electrophoresis**

Enzymes were extracted from fresh leaves, stems, and roots of small seedlings or from new leaves of larger seedlings. Plant material was ground to a wet paste with a mortar and pestle using a polyvinylpyrrolidone buffer (Wendel and Parks 1982) to stabilize the enzymes. The extract was absorbed onto Whatman filter paper wicks, which were stored in microtiter trays at -70 to -80°C until analysis.

Wicks were loaded into 10% starch gels, and electrophoresis was conducted at 4°C. We resolved 16 loci in Hawaii and 17 loci in Costa Rica from eight enzyme systems. We assayed the following enzymes (abbreviation, number of loci): malic enzyme (ME, 1), leucine aminopeptidase (LAP, 3), fluorescent esterase (FE, 3 in Hawaii, 4 in Costa Rica), triosephosphate isomerase (TPI, 3), colormetric esterase (CE, 1), peroxidase (PER, 1), diaphorase (DIA, 2), and aspartate aminotransferase (AAT, 2). FE-2 was a fixed heterozygote for Costa Rican plants and for all analyses we treated it as two monomorphic loci (FE-2 and FE-3). We treated FE-2 as absent for Hawaiian plants. We used four gel and buffer systems: Poulik (CE and PER) from Mitton et al. (1979); and Soltis 7 (DIA and AAT), Soltis 10 (FE and TPI), and a modified Soltis 8 (ME and LAP) from Soltis et al. (1983). We stained enzymes following procedures in Soltis et al. (1983) and Cheliak and Pitel (1984). The age of seedlings was a factor determining banding patterns in glutamate dehydrogenase (GDH); therefore, this enzyme was not included in our analysis.

**Genetic Diversity Analyses**

Analyses were conducted on two sets of the data. The “bulk” collection data set used the genotypic data from three fruits per maternal plant that produced at least one seedling (N = 21 - 39 per population). This data set mimics bulk collections, in which multiple seeds may be collected from the same plant (e.g., Novak and Mack 1993, Chase et al. 1995, Chamberlain 1998). The “individuals” data set used the genotypic data from one randomly chosen fruit per plant (N = 11 - 13 per population). Analyses refer to the bulk data set unless specified otherwise. We described genetic variability within *C. hirta* at the population and species levels (Berg and Hamrick 1997) in terms of percentage of polymorphic loci (%P), mean number of alleles per
locus ($A$) and per polymorphic locus ($AP$), mean effective number of alleles ($A_e$), the observed heterozygosity ($H_o$), and the expected heterozygosity ($H_e = 1 - \sum p_i^2$ where $p_i$ is the frequency of the $i$th allele; averaged over all loci). Species-level values were calculated based on the sum of all the polymorphic loci or alleles pooled over all of the Hawaiian and Costa Rican populations (the “true” species level for this study). Species-level values were also calculated separately for each area (species level within Hawaii or Costa Rica). We used these species-level parameters to compare genetic diversity in $C. hirta$ to other species of exotic invasive plants, native Hawaiian plants, and widespread tropical woody plants. Population-level parameters were calculated to statistically compare within-population levels of genetic diversity between Hawaii and Costa Rica and among regions or islands (in Costa Rica or Hawaii, respectively).

Population-level fixation indices ($F = 1 - H_o/H_e$) were calculated for each polymorphic locus and significant deviations from Hardy-Weinberg expectations ($F = 0$) were identified by $\chi^2$ analysis (Li and Horovitz 1953). The proportional deviation from Hardy-Weinberg expectations (HWE) for a group of populations, $F_{IS}$, was calculated for each polymorphic locus and averaged across loci. A mean $F_{IS} > 0$ indicates an excess of homozygotes and generally is interpreted as a measure of inbreeding in the absence of other sources of heterozygote deficit (Berg and Hamrick 1997). Founder effects (one form of bottleneck) may also contribute to heterozygote deficit.

We examined among-population variation three ways. First, $\chi^2$ analysis was used to test allele frequency heterogeneity among populations at each locus (Workman and Niswander 1970). Second, we calculated Nei’s (1973, 1977) genetic diversity statistics to examine genetic structure. Total genetic diversity at each polymorphic locus, $H_T$, was partitioned into within-population genetic diversity, $H_S$, and among-population genetic diversity, $D_{ST} (H_T - H_S)$. We calculated the proportion of genetic variation explained by among-population variation, $G_{ST}$, as $D_{ST}/H_T$. A mean $G_{ST}$ was calculated over all polymorphic loci at different levels: between the two areas (Costa Rica and Hawaii; $G_{STA}$), among islands in Hawaii ($G_{STI}$), among regions in Costa Rica ($G_{STR}$), and among all populations within Hawaii ($G_{STH}$) and within Costa Rica ($G_{STC}$). Third, Nei’s genetic identity ($I$) and distance ($D = -\ln(I)$) were calculated for all possible pairwise comparisons among the 40 populations (Nei 1972).

Relationships among populations were also examined graphically for the bulk data set using non-metric multidimensional scaling (NMDS) for three comparisons: 1) all sampled
populations, 2) only Hawaiian populations, and 3) and only Costa Rican populations.

Ordinations were based on the matrix of Nei’s genetic distances among each pair of populations. NMDS is an iterative ordination technique based on ranked distances of $n$ entities on $k$ axes that seeks to minimize distortions caused by reductions in dimensionality (Minchin 1987). The SAS Version 8 routine PROC NMS was used with a minimum acceptable stress set at 0.001 and maximum iterations set at 200 (SAS Institute 2000). Solutions with two and three axes were examined, but solutions with two axes adequately reduced the stress and are presented for easier interpretation. We examined the ordinations for each area to determine whether populations clustered in relation to the hierarchical arrangement of populations among regions of Costa Rica or among islands in the Hawaiian archipelago.

We tested for significant genetic isolation by geographic distance within Costa Rica and Hawaii using IBD (Bohonak 2002). The level of significance was assessed by a Mantel test for matrix correlation between the log of Slatkin’s (1993) similarity measure $\hat{M}$ and log geographic distance between each population pair.

Historical levels of gene flow ($Nm$) were estimated using Wright’s (1951) equation $F_{ST} = 1/(1 + 4Nm)$, where $N$ is the effective population size of the recipient population, $m$ is the rate of gene flow, and $F_{ST}$ is equivalent to $G_{ST}$. This method has been criticized on the grounds that $F_{ST}$ can not be used to accurately estimate $Nm$ (Whitlock and McCauley 1999); however, we were unable to use the method of Slatkin (1985) to estimate gene flow because no private alleles, i.e. alleles found in a single population, were found in Hawaii.

**RESULTS**

**Species Level**

A total of 1356 *C. hirta* individuals from Costa Rican populations (690) and Hawaiian populations (652) were analyzed in the bulk data set (Table 2.1). At the species level (across Hawaii and Costa Rica), 43.8% of the 16 loci (excluding FE-2) were polymorphic (LAP-3, FE-1, FE-3, FE-4, TPI-2, AAT-1, and AAT-2). The average number of alleles was 1.69 alleles per locus and 2.57 per polymorphic locus, while the effective number of alleles was 1.56 (Table 2.2). Total expected heterozygosity for the species was 0.225. The results were similar using the
individuals data set, for which we analyzed 238 plants each from Costa Rican and Hawaiian seed sources (Table 2.2).

At the true species level, the overall mean genetic diversity for polymorphic loci \((H_T)\) was 0.514 and was partitioned largely between Costa Rica and Hawaii \((G_{STA} = 0.72)\). Thus 72% of the total variation in allele frequencies for polymorphic loci occurred between Costa Rica and Hawaii and 28% occurred within the two areas.

**Within-Population Variation**

Within areas, Costa Rican populations were only polymorphic for FE-1 and AAT-2, whereas Hawaiian populations were variable for FE-3 and AAT-2. In addition, Costa Rican populations had what appeared to be a fixed heterozygotic allele combination for FE-2, which is considered as two monomorphic loci here. Four loci (AAT-1, LAP-3, FE-3, and TPI-2) were monomorphic for different alleles in the two areas. Costa Rican and Hawaiian populations shared at least one allele for the polymorphic loci FE-1 and FE-3 but not for AAT-2. Thus, 11 alleles were found in both Costa Rican and Hawaiian plants, while eight alleles were unique to Costa Rican plants and seven alleles were unique to Hawaiian plants. Species-level measures of within-population genetic variation were similar between *C. hirta* from Costa Rica \((%P = 12.5, A = 1.19, AP = 2.50, A_e = 1.14, H_e = 0.064)\) and Hawaii \((%P = 12.5, A = 1.19, AP = 2.50, A_e = 1.17, H_e = 0.068)\).

Costa Rican populations generally had lower within-population genetic diversity than Hawaiian populations for both the bulk and individuals data sets (Table 2.2). In the bulk data set, Hawaiian populations had significantly greater \%\(P_{pop}\) (Mann-Whitney \(U = 210, P < 0.001\)), \(H_e (U = 223, P < 0.001)\), alleles per polymorphic locus \((U = 230, P < 0.001)\), mean number of alleles per locus \((U = 210, P < 0.001)\), and effective number of alleles \((U = 218, P < 0.001)\) than Costa Rican populations. Observed heterozygosity did not differ significantly between the two areas \((U = 413, P = 0.48)\).

There was little indication that islands in Hawaii or regions in Costa Rica differed in levels of genetic diversity. In the Hawaiian archipelago, the percentage of polymorphic loci and number of alleles per polymorphic locus were identical for populations on the four islands. Mean observed and expected heterozygosity differed slightly among islands \((H_o: \text{Kruskal-Wallis} \ H = 8.21, df = 3, P = 0.04; H_e: \text{Kruskal-Wallis} \ H = 7.8, df = 3, P = 0.05)\). Mean \(H_o\) was lower for Hawaii (0.042) and Maui (0.042) than for Kauai (0.059) and Oahu (0.056), while mean \(H_e\) was
Table 2.2 Within-population genetic diversity measures for (a) Hawaiian and (b) Costa Rican populations of *Clidemia hirta* in the bulk and individuals data sets. Population-level means for both areas and the species-level statistics also are presented.

<table>
<thead>
<tr>
<th>Population</th>
<th>%P (SD)</th>
<th>AP (SD)</th>
<th>$A_e$ (SD)</th>
<th>$H_e$ (SD)</th>
<th>$H_o$ (SD)</th>
<th>Individuals</th>
<th>$A_e$ (SD)</th>
<th>$H_e$ (SD)</th>
<th>$H_o$ (SD)</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
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<td></td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Kauai</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.5</td>
<td>2.5</td>
<td>1.16</td>
<td>0.058 (0.033)</td>
<td>0.067 (0.048)</td>
<td>1.14</td>
<td>0.042 (0.050)</td>
<td>0.055 (0.045)</td>
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</tr>
<tr>
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<td>2.5</td>
<td>1.18</td>
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<td>0.072 (0.050)</td>
<td>1.17</td>
<td>0.068 (0.046)</td>
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</tr>
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<td>2.5</td>
<td>1.17</td>
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<td>0.069 (0.048)</td>
<td>1.17</td>
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<td>4</td>
<td>12.5</td>
<td>2.5</td>
<td>1.16</td>
<td>0.061 (0.029)</td>
<td>0.069 (0.047)</td>
<td>1.16</td>
<td>0.063 (0.044)</td>
<td>0.069 (0.047)</td>
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<tr>
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<td>12.5</td>
<td>2.5</td>
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<td>0.068 (0.047)</td>
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<td>2.5</td>
<td>1.17</td>
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<td>0.069 (0.048)</td>
<td>1.14</td>
<td>0.052 (0.050)</td>
<td>0.065 (0.045)</td>
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<td>2.5</td>
<td>1.13</td>
<td>0.061 (0.032)</td>
<td>0.061 (0.043)</td>
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<td>2.5</td>
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<td>0.065 (0.044)</td>
<td>1.07</td>
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<td>2.5</td>
<td>1.10</td>
<td>0.055 (0.031)</td>
<td>0.055 (0.038)</td>
<td>1.16</td>
<td>0.063 (0.051)</td>
<td>0.063 (0.048)</td>
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</tr>
<tr>
<td>10</td>
<td>12.5</td>
<td>2.5</td>
<td>1.12</td>
<td>0.038 (0.028)</td>
<td>0.059 (0.044)</td>
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<td>0.052 (0.051)</td>
<td>0.059 (0.043)</td>
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<td>11</td>
<td>12.5</td>
<td>2.5</td>
<td>1.15</td>
<td>0.077 (0.028)</td>
<td>0.067 (0.048)</td>
<td>1.17</td>
<td>0.073 (0.050)</td>
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<tr>
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<td>12.5</td>
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<td>0.048 (0.030)</td>
<td>0.057 (0.049)</td>
<td>1.15</td>
<td>0.032 (0.040)</td>
<td>0.055 (0.051)</td>
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</tr>
<tr>
<td>14</td>
<td>12.5</td>
<td>2.5</td>
<td>1.14</td>
<td>0.039 (0.030)</td>
<td>0.064 (0.046)</td>
<td>1.15</td>
<td>0.032 (0.045)</td>
<td>0.063 (0.048)</td>
<td></td>
</tr>
<tr>
<td>Hawaii</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>12.5</td>
<td>2.5</td>
<td>1.12</td>
<td>0.037 (0.023)</td>
<td>0.058 (0.041)</td>
<td>1.14</td>
<td>0.037 (0.042)</td>
<td>0.059 (0.044)</td>
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<tr>
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<td>1.12</td>
<td>0.039 (0.031)</td>
<td>0.056 (0.045)</td>
<td>1.12</td>
<td>0.032 (0.048)</td>
<td>0.054 (0.045)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>12.5</td>
<td>2.5</td>
<td>1.17</td>
<td>0.034 (0.027)</td>
<td>0.064 (0.050)</td>
<td>1.15</td>
<td>0.037 (0.049)</td>
<td>0.061 (0.048)</td>
<td></td>
</tr>
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<td>18</td>
<td>12.5</td>
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<td>1.13</td>
<td>0.033 (0.028)</td>
<td>0.062 (0.044)</td>
<td>1.14</td>
<td>0.074 (0.047)</td>
<td>0.062 (0.047)</td>
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</tr>
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<td>1.15</td>
<td>0.056 (0.033)</td>
<td>0.065 (0.047)</td>
<td>1.11</td>
<td>0.037 (0.048)</td>
<td>0.051 (0.040)</td>
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</tr>
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<td>20</td>
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<td>2.5</td>
<td>1.17</td>
<td>0.050 (0.029)</td>
<td>0.069 (0.049)</td>
<td>1.17</td>
<td>0.047 (0.048)</td>
<td>0.067 (0.048)</td>
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(Table 2.2 continued)

<table>
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<tr>
<th>Population</th>
<th>%P (SD)</th>
<th>AP (SD)</th>
<th>Bulk</th>
<th>Individuals</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>A_e (SD)</td>
<td>H_o (SD)</td>
</tr>
<tr>
<td>(b) Costa Rica</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EARTH</td>
<td>11.76</td>
<td>2.0</td>
<td>1.10</td>
<td>0.080 (0.028)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.08</td>
<td>0.052 (0.030)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.08</td>
<td>0.048 (0.029)</td>
</tr>
<tr>
<td>La Selva</td>
<td>11.76</td>
<td>2.0</td>
<td>1.08</td>
<td>0.044 (0.027)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.05</td>
<td>0.026 (0.025)</td>
</tr>
<tr>
<td></td>
<td>5.88</td>
<td>2.0</td>
<td>1.01</td>
<td>0.007 (0.014)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.09</td>
<td>0.052 (0.026)</td>
</tr>
<tr>
<td>San Carlos</td>
<td>11.76</td>
<td>2.0</td>
<td>1.09</td>
<td>0.049 (0.028)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.08</td>
<td>0.034 (0.026)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.11</td>
<td>0.069 (0.026)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.06</td>
<td>0.029 (0.025)</td>
</tr>
<tr>
<td>Los Chiles</td>
<td>11.76</td>
<td>2.0</td>
<td>1.03</td>
<td>0.020 (0.022)</td>
</tr>
<tr>
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<td>11.76</td>
<td>2.0</td>
<td>1.06</td>
<td>0.021 (0.022)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.11</td>
<td>0.061 (0.013)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.10</td>
<td>0.051 (0.031)</td>
</tr>
<tr>
<td>Quepos</td>
<td>11.76</td>
<td>2.5</td>
<td>1.09</td>
<td>0.042 (0.029)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.11</td>
<td>0.076 (0.019)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.5</td>
<td>1.12</td>
<td>0.078 (0.021)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.10</td>
<td>0.079 (0.020)</td>
</tr>
<tr>
<td></td>
<td>11.76</td>
<td>2.0</td>
<td>1.11</td>
<td>0.076 (0.021)</td>
</tr>
<tr>
<td>Hawaii mean</td>
<td>12.50 (1.85)</td>
<td>2.50 (0.00)</td>
<td>1.14 (0.02)</td>
<td>0.050 (0.007)</td>
</tr>
<tr>
<td>Costa Rica mean</td>
<td>11.47 (1.73)</td>
<td>2.05 (0.15)</td>
<td>1.08 (0.03)</td>
<td>0.050 (0.005)</td>
</tr>
<tr>
<td>Species values</td>
<td>43.75</td>
<td>2.57</td>
<td>1.56</td>
<td>-</td>
</tr>
</tbody>
</table>

*a %P = percentage polymorphic loci, AP = mean number of alleles per polymorphic locus, A_e = effective number of alleles per locus, H_o = observed heterozygosity, H_e = expected heterozygosity"
lower for Maui (0.059) and Oahu (0.059) than for Hawaii (0.062) and Kauai (0.069). No significant differences were found among islands for $H_o$ or $H_e$ using the individuals data set. Nor were differences found among the five regions in Costa Rica for any of the within-population diversity measures using either data set.

Many of the Hawaiian and Costa Rican populations showed significant deviations from Hardy-Weinberg expectations. In each area, we would expect 5%, or 2 of the 40 tests, to deviate from HWE by chance. In Hawaii, 14 of 40 fixation indices ($F$) calculated for each polymorphic locus deviated significantly from HWE (Table 2.3). Of the significant indices, all were positive for AAT-2, while four were positive and one was negative for FE-2. In Costa Rica, 20 of the 40

<table>
<thead>
<tr>
<th>Hawaiian Population</th>
<th>Island</th>
<th>Locus</th>
<th>FE-2</th>
<th>AAT-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Kauai</td>
<td></td>
<td></td>
<td>0.000</td>
<td>0.251</td>
</tr>
<tr>
<td>2 Kauai</td>
<td></td>
<td></td>
<td>-0.135</td>
<td>0.271</td>
</tr>
<tr>
<td>3 Kauai</td>
<td></td>
<td></td>
<td>-0.100</td>
<td>0.538 *</td>
</tr>
<tr>
<td>4 Kauai</td>
<td></td>
<td></td>
<td>-0.220</td>
<td>0.413 *</td>
</tr>
<tr>
<td>5 Oahu</td>
<td></td>
<td></td>
<td>-0.176</td>
<td>0.505 *</td>
</tr>
<tr>
<td>6 Oahu</td>
<td></td>
<td></td>
<td>0.043</td>
<td>0.492 *</td>
</tr>
<tr>
<td>7 Oahu</td>
<td></td>
<td></td>
<td>-0.173</td>
<td>0.138</td>
</tr>
<tr>
<td>8 Oahu</td>
<td></td>
<td></td>
<td>0.156</td>
<td>-0.117</td>
</tr>
<tr>
<td>9 Oahu</td>
<td></td>
<td></td>
<td>-0.020</td>
<td>0.276 *</td>
</tr>
<tr>
<td>10 Oahu</td>
<td></td>
<td></td>
<td>0.368</td>
<td>0.197</td>
</tr>
<tr>
<td>11 Oahu</td>
<td></td>
<td></td>
<td>-0.397 *</td>
<td>0.104</td>
</tr>
<tr>
<td>12 Maui</td>
<td></td>
<td></td>
<td>0.514 *</td>
<td>0.274</td>
</tr>
<tr>
<td>13 Maui</td>
<td></td>
<td></td>
<td>-0.056</td>
<td>0.196</td>
</tr>
<tr>
<td>14 Maui</td>
<td></td>
<td></td>
<td>0.418 *</td>
<td>0.394 *</td>
</tr>
<tr>
<td>15 Hawaii</td>
<td></td>
<td></td>
<td>0.905 *</td>
<td>0.115</td>
</tr>
<tr>
<td>16 Hawaii</td>
<td></td>
<td></td>
<td>0.325</td>
<td>0.313</td>
</tr>
<tr>
<td>17 Hawaii</td>
<td></td>
<td></td>
<td>0.361</td>
<td>0.458 *</td>
</tr>
<tr>
<td>18 Hawaii</td>
<td></td>
<td></td>
<td>0.462 *</td>
<td>0.500 *</td>
</tr>
<tr>
<td>19 Hawaii</td>
<td></td>
<td></td>
<td>-0.063</td>
<td>0.329</td>
</tr>
<tr>
<td>20 Hawaii</td>
<td></td>
<td></td>
<td>0.146</td>
<td>0.365 *</td>
</tr>
</tbody>
</table>

* $P < 0.05$
fixation indices showed significant differences from HWE (Table 2.4). All Costa Rican populations that showed significant deviations had an excess of heterozygotes for FE-1 and an excess of homozygotes for AAT-2. Most populations, regardless of region, showed this pattern, even if the indices were not significantly different from 0. Hawaiian populations had an overall tendency for a deficit of heterozygotes ($F_{IS} = 0.191$) and Costa Rican populations had a slight excess of heterozygotes ($F_{IS} = -0.144$) (Table 2.5). The overall species-level fixation index ($0.077 \pm 0.126$) was intermediate between the fixation indices for the two areas.

### Table 2.4 Fixation indices ($F$) for FE-1 and AAT-2 in Costa Rican populations of *Clidemia hirta*.

<table>
<thead>
<tr>
<th>Costa Rican Population</th>
<th>Region</th>
<th>Locus</th>
<th>FE-1</th>
<th>AAT-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 EARTH</td>
<td></td>
<td></td>
<td>-0.512 *</td>
<td>-0.393 *</td>
</tr>
<tr>
<td>2 EARTH</td>
<td></td>
<td></td>
<td>-0.260</td>
<td>-0.059</td>
</tr>
<tr>
<td>3 EARTH</td>
<td></td>
<td></td>
<td>-0.386 *</td>
<td>0.344</td>
</tr>
<tr>
<td>4 La Selva</td>
<td></td>
<td></td>
<td>-0.242</td>
<td>0.296</td>
</tr>
<tr>
<td>5 La Selva</td>
<td></td>
<td></td>
<td>-0.098</td>
<td>0.381 *</td>
</tr>
<tr>
<td>6 La Selva</td>
<td></td>
<td></td>
<td>0.000</td>
<td>-0.048</td>
</tr>
<tr>
<td>7 La Selva</td>
<td></td>
<td></td>
<td>-0.500 *</td>
<td>0.442 *</td>
</tr>
<tr>
<td>8 San Carlos</td>
<td></td>
<td></td>
<td>-0.353 *</td>
<td>0.425 *</td>
</tr>
<tr>
<td>9 San Carlos</td>
<td></td>
<td></td>
<td>0.310</td>
<td>0.162</td>
</tr>
<tr>
<td>10 San Carlos</td>
<td></td>
<td></td>
<td>-0.067 *</td>
<td>0.177</td>
</tr>
<tr>
<td>11 San Carlos</td>
<td></td>
<td></td>
<td>-0.017</td>
<td>0.171</td>
</tr>
<tr>
<td>12 Los Chiles</td>
<td></td>
<td></td>
<td>-0.095</td>
<td>0.468 *</td>
</tr>
<tr>
<td>13 Los Chiles</td>
<td></td>
<td></td>
<td>0.362 *</td>
<td>0.417 *</td>
</tr>
<tr>
<td>14 Los Chiles</td>
<td></td>
<td></td>
<td>-0.969 *</td>
<td>0.873 *</td>
</tr>
<tr>
<td>15 Los Chiles</td>
<td></td>
<td></td>
<td>-0.109</td>
<td>0.037</td>
</tr>
<tr>
<td>16 Quepos</td>
<td></td>
<td></td>
<td>0.225</td>
<td>0.112</td>
</tr>
<tr>
<td>17 Quepos</td>
<td></td>
<td></td>
<td>-0.974 *</td>
<td>0.367 *</td>
</tr>
<tr>
<td>18 Quepos</td>
<td></td>
<td></td>
<td>-0.821 *</td>
<td>0.274</td>
</tr>
<tr>
<td>19 Quepos</td>
<td></td>
<td></td>
<td>-0.971 *</td>
<td>0.097</td>
</tr>
<tr>
<td>20 Quepos</td>
<td></td>
<td></td>
<td>-0.968 *</td>
<td>0.393 *</td>
</tr>
</tbody>
</table>

* $P < 0.05$
Among-Population Variation

The mean total genetic diversity \( (H_T) \) was similar for Hawaiian (0.571) and Costa Rican populations (0.526) and most of this diversity was held within populations (low \( G_{ST} \)) in the two areas (Table 2.5). More genetic diversity was partitioned among populations (greater \( G_{ST} \)) in Costa Rica \( (G_{STC} = 0.235) \) than in Hawaii \( (G_{STH} = 0.074) \). Differentiation among regions within Costa Rica \( (G_{STC}) \) accounted for 5.9% of the among-population differentiation, but only 1.5% of the genetic diversity was held among islands in Hawaii \( (G_{STH}) \). Chi-square analyses to test allele frequency heterogeneity among populations showed significant \( (P < 0.001) \) differences overall between the Hawaiian and Costa Rican populations, as well as within Hawaii and Costa Rica for each of their two polymorphic loci in each area: FE-3 and AAT-2 in Hawaii, and FE-1 and AAT-2 in Costa Rica.

Nei’s genetic identities and the NMDS ordinations based on Nei’s genetic distances among pairs of populations also showed that allele frequencies differed greatly between Costa Rica

<table>
<thead>
<tr>
<th>Area</th>
<th>Locus</th>
<th>( H_T )</th>
<th>( H_S )</th>
<th>( G_{ST} )</th>
<th>( F_{IS} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii</td>
<td>FE-2</td>
<td>0.500</td>
<td>0.456</td>
<td>0.088</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>AAT-2</td>
<td>0.642</td>
<td>0.604</td>
<td>0.060</td>
<td>0.319</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.571</td>
<td>0.530</td>
<td>0.074</td>
<td>0.191</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.100</td>
<td>0.104</td>
<td>0.020</td>
<td>0.180</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>FE-1</td>
<td>0.575</td>
<td>0.355</td>
<td>0.383</td>
<td>-0.527</td>
</tr>
<tr>
<td></td>
<td>AAT-2</td>
<td>0.476</td>
<td>0.435</td>
<td>0.087</td>
<td>0.240</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.526</td>
<td>0.395</td>
<td>0.235</td>
<td>-0.144</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.070</td>
<td>0.057</td>
<td>0.210</td>
<td>0.542</td>
</tr>
</tbody>
</table>

Table 2.5 Among-population genetic diversity statistics for polymorphic loci of *Clidemia hirta* in (a) Hawaii and (b) Costa Rica, using the bulk data set. Results for the individuals data set were similar and are not shown here.
Figure 2.2 Non-metric multidimensional scaling ordination of Nei’s (1972) genetic distances for (a) Hawaiian and Costa Rican populations (stress = 0.01), (b) only Hawaiian populations (stress = 0.08), and (c) only Costa Rican populations (stress = 0.08) of the tropical shrub *Clidemia hirta*. Each point represents one population. Different symbols in (b) represent different islands: Kauai (open squares), Oahu (gray triangles), Maui (black diamonds), and Hawaii (black circles). Different symbols in (c) represent different regions chosen *a priori*: EARTH (black squares), La Selva (open circles), San Carlos (open inverted triangles), Los Chiles (black triangles), and Quepos (gray diamonds).
Rican and Hawaiian populations but differed little within areas. The mean genetic identity of Costa Rican and Hawaiian populations was only 0.64 and they separated markedly along Dimension 1 of the combined-area ordination (Figure 2.2a). Hawaiian populations clustered more tightly together along Dimension 2, reflecting the higher similarity among Hawaiian populations showed no geographic differentiation in allele frequencies by island (Figure 2.2b).

Within Costa Rica, there was some geographic differentiation (Figure 2.2c). Four of the five populations on the western side of the Cordillera Central in the Quepos region were differentiated from the rest of the Costa Rican populations by a unique allele for FE-1. Some seedlings from the fifth Quepos population had the unique allele while others had alleles found elsewhere in Costa Rica.

In the isolation by distance analysis, no significant correlation was found between log geographic and log genetic similarity (M) either in Costa Rica or Hawaii (P > 0.05, Reduced Major Axis regression $R^2 = 0.03$ and 0.006, respectively). As expected, estimated gene flow between Costa Rican and Hawaiian populations using Wright’s method was low ($Nm = 0.10$). Estimated levels of historic gene flow among populations were lower in Costa Rica ($Nm = 0.81$) than in Hawaii ($Nm = 3.14$).

**DISCUSSION**

**Genetic Variation in Costa Rica and Hawaii**

Lower intrapopulation genetic diversity and greater interpopulation differentiation of populations in introduced versus native ranges is predicted from theory (Brown and Marshall 1981), yet various comparisons have failed to find substantial reductions in intrapopulation genetic diversity in areas of introduction (Novak and Mack 1993, Wang et al. 1995, Neuffer and Hurka 1999, Squirrell et al. 2001, Bartlett et al. 2002). In fact, we found greater mean intrapopulation diversity in introduced populations of *C. hirta*; within-population levels of allozyme variation for the native Costa Rican populations ($P_{pop} = 11.5\%, A_e = 1.08, H_e = 0.045$) were lower than for introduced Hawaiian populations ($P_{pop} = 12.5\%, A_e = 1.14, H_e = 0.063$). We also did not find support for the prediction of greater differentiation in introduced populations than in native populations. Instead, less of the total genetic diversity was partitioned
Clidemia hirta populations in Hawaii also had relatively low levels of within-population genetic diversity ($\%P_{\text{pop}} = 12.5\%$, $H_e = 0.063$) compared to many other exotic species such as Epipactis helleborine (Squirrell et al. 2001), Carpobrotus edulis (Gallagher et al. 1997), Pueraria lobata (Pappert et al. 2000), Lonicera japonica (Schierenbeck et al. 1995), Carpobrotus edulis (Gallagher et al. 1997), and Lathyrus latifolius (Godt and Hamrick 1991). These species had an average $\%P_{\text{pop}} > 40\%$ and $H_e > 0.10$. Levels of genetic variation of C. hirta in Hawaii were comparable to other species such as Sorghum halepense (Warwick et al. 1984), Abutilon theophrasti (Warwick and Black 1986), Setaria faber (Warwick et al. 1987), Bromus tectorum (Novak et al. 1991), and Bryonia alba (Novak and Mack 1995). These species had low levels of genetic diversity (average $\%P_{\text{pop}} < 15\%$ and $H_e < 0.02$), although some studies did not report the expected heterozygosity.

Multiple introductions likely are responsible for the high genetic diversity of some species in their introduced range (Novak and Mack 1993, Neuffer and Hurka 1999). Neuffer and Hurka (1999) found that multiple introductions of Capsella bursa-pastoris (Brassicaceae) from different areas of its native range offset any reductions in genetic diversity that may have occurred during founder events. Multiple introductions were also cited as the reason for two-fold greater $\%P$ and $H_e$ of Bromus tectorum (Poaceae) in introduced populations in the northwestern United States compared to native populations in Eurasia (Novak and Mack 1993). Fewer introductions to the eastern United States may explain why genetic variation within B. tectorum populations in the East was similar, and not greater, than those in the native range (Bartlett et al. 2002). Multiple introductions were suggested to be the “rule and not the exception” for many introduced plants (Novak and Mack 1993, Novak et al. 1993), and may be the case for C. hirta on Hawaii.

The higher levels of genetic variation in Hawaiian populations than Costa Rican populations could result from multiple introductions, potentially from different areas of the native range. Levels of genetic variation, although greater than that found in Costa Rica, were still low compared to many other plant species. It seems unlikely that intraspecific hybridization of native genotypes from geographically distant parts of the native range has occurred. Instead, the source populations of C. hirta on Hawaii may have relatively higher levels of genetic
variation than the populations sampled in Costa Rica (see discussion of source population under Evidence Points to a Caribbean or South American Origin).

Population fixation indices showed that there was a deficit of heterozygotes in Hawaii ($F_{IS} = 0.191$) and a slight excess of heterozygotes in Costa Rica ($F_{IS} = -0.144$). Such differences in the direction of deviations from Hardy-Weinberg equilibrium could result from a predominance of uniparental reproduction in Hawaii but outcrossing in Costa Rica or from the effects of population bottleneck in Hawaii. Analyses of progeny arrays would be needed to ascertain whether outcrossing rates are in fact higher in the native populations. In Costa Rica, some populations had large differences in the proportional deviation from Hardy-Weinberg expectations between the two polymorphic loci. These patterns may arise if populations were founded by particular genotypes heterozygotic for FE-1 and homozygous for AAT-2 and then reproduction was predominantly asexual. The number of heterozygotes and homozygotes for each locus would then reflect the make-up of the original genotypes.

Although low levels of genetic diversity may hinder our abilities to detect differentiation among the Hawaiian Islands, genetic drift does not appear to be shaping the distribution of genetic variation of *C. hirta* in Hawaii. The high levels of historic gene flow ($Nm$) among *C. hirta* populations in Hawaii would suggest that these newly founded populations have not yet reached equilibrium between genetic drift and gene flow. Additionally, current gene flow through pollen or seed movement among the islands may prevent differentiation caused by genetic drift. Indeed, some gene flow must occur among islands for the widespread and ecologically variable dominant canopy tree, *Metrosideros polymorpha* (Myrtaceae). Populations of this species on the islands of Hawaii and Kauai had a genetic distance of only 0.053 (Treseder and Vitousek 2001), which is comparable to the distance among populations of *M. polymorpha* found only on Maui (Aradhya et al. 1993). Human-mediated dispersal of small-seeded exotic species such as *C. hirta* among islands probably also is substantial. Even if gene movement among islands is infrequent, genetic divergence may not have occurred yet because *C. hirta* was introduced to Hawaii relatively recently. It has been on the island of Oahu circa 1940 and spread as recently as the 1970s and 1980s to Kauai, Maui, and Hawaii (Smith 1992). The high genetic identity of Hawaiian populations ($I = 0.99$) and lack of island-unique alleles suggests that populations may have been founded by a relatively large number of individuals, there may have
been multiple founding events between islands, or subsequent gene flow following introduction has been substantial.

Species-level genetic variation of Clidemia hirta in Hawaii (%P = 12.5, A = 1.17, He = 0.068) was lower than many native Hawaiian angiosperms and particularly low compared to M. polymorpha. Endemic species of Brighamia (Campnulaceae), Wilkesia (Asteraceae), and Dubautia (Asteraceae) had more alleles per locus (A = 1.3 – 1.4), a higher percentage of polymorphic loci (%P = 18.2 – 27.3), and similar expected heterozygosity (He = 0.063 – 0.076) (Witter and Carr 1988, Gemmill et al. 1998) as C. hirta. Metrosideros polymorpha, on the other hand, was exceptionally diverse with 2.9 alleles per locus and an expected heterozygosity of 0.36 (Aradhya et al. 1991).

**Clidemia hirta in Its Native Range**

Native, Costa Rican populations of C. hirta harbored little within-population allozyme variation (%P = 12.5, A = 1.14, He = 0.064) compared to that reported for other widespread tropical woody species with mixed mating systems (i.e., neither outcrossing nor uniparental reproduction predominates) and animal-dispersed seeds (%P = 32 – 50%, A =1.13 –1.21, He = 0.109 – 0.149; Hamrick and Godt 1989). The proportion of genetic diversity partitioned among native populations of C. hirta (GST = 0.235) was somewhat higher than that reported for many outcrossing, animal-dispersed species (GST = 0.197; Hamrick and Godt 1996).

The only geographic isolation found in Costa Rica was between the eastern and western sides of the Cordillera Central. Phylogeographic differences between these regions have been documented in other woody taxa studied in Costa Rica (Chase et al. 1995, Gillies et al. 1997). The high among-population variation in Clidemia hirta may result partly from reduced long distance pollen flow because apomixis occurs, potentially frequently, in natural C. hirta populations (Renner 1989).

**Evidence Points to a Caribbean or South American Origin**

Genetic similarity between Costa Rican and Hawaiian C. hirta populations (Nei’s I = 0.64) was low compared to intraspecific genetic similarity among other species sampled in their native and introduced ranges, but comparable to that found across the native ranges of some tropical woody species. Most introduced species studied in both ranges were herbaceous plants, which could account for the high genetic similarity. For example, the grasses Bromus tectorum
and *Setaria viridis* had high genetic identity values (0.93 and 1.0, respectively) even between native Eurasian and introduced North American populations (Novak and Mack 1993, Wang et al. 1995). In contrast, large intraspecific differences in genetic similarity between Central and South American populations were observed in two species of *Stylosanthes* (Fabaceae), which were only 67% and 72% similar across populations sampled from Mexico to southern Brazil (Sawkins et al. 2001). Central American and Caribbean populations of *Pterocarpus officinalis* (Fabaceae) were only about 68% similar (Rivera-Ocasio et al. 2002). The widespread tree species *Calliandra calothyrsus* (Fabaceae) had a genetic identity (Nei’s *I*) of only 0.42 between populations in Mexico and Panama (Chamberlain 1998). In contrast, Central American (Panama and Costa Rica) populations of *P. officinalis* and *C. calothyrsus* were less genetically distant.

The markedly low genetic similarity between the Costa Rican and Hawaiian populations strongly suggests that Costa Rica was not the source of *C. hirta* introduced to Hawaii. We tentatively suggest that a South American or Caribbean origin seems more likely than a Central American origin for two reasons. First, previous examples show that genetic distances often are substantial between populations of tropical woody species sampled over large geographic distances or between islands and mainland areas. Thus, genetic similarity in allele frequencies of 64% may be reasonable between Central and South American or Caribbean populations of *Clidemia hirta*. Second, some anecdotal evidence based on leaf morphology supports the hypothesis of a South American origin. Simmonds (1933) found that the leaf morphology of introduced *C. hirta* on Fiji was more similar to individuals in British Guiana (Guyana) than those in Panama or Trinidad (an island off the coast of Venezuela). Simmonds (1937) postulated that seeds of *C. hirta* were introduced to Fiji as contaminants in nursery stock of coffee plants. This vector also could have been the mode of introduction to Hawaii. Whether the Hawaiian introduction of *C. hirta* stems directly from native populations or whether material was introduced from other parts of the introduced range (e.g., Fiji or Java where it was reported occurring as early as the late 1880s) cannot be determined by this study. A phylogeographic study that examined genetic similarity of individuals across a broader part of the native and introduced ranges would resolve this issue.

Alternatively, large differences between Costa Rican and Hawaiian populations may result if our comparisons were based on different varieties of *Clidemia hirta*. Three varieties are recognized in Wurdack (1980): *hirta*, *elegans*, and *tiliaefolia*. The *elegans* and *tiliaefolia*
varieties are restricted to South America, whereas *C. hirta* var. *hirta* is found in both Central and South America (Wurdack 1980). We find the sampling of different varieties scenario unlikely because both Hawaiian and Costa Rican specimens are identified as *Clidemia hirta* var. *hirta* (F. Almeda, personal communication), despite some morphological differences (Chapter 3). In Costa Rica, we encountered only *C. hirta* var. *hirta*.

**Effect of Genetic Variation on Invasiveness**

Ultimately, a goal of studies of introduced species’ genetics should be to assess the selective advantage of genetic variability in the species’ native and introduced ranges. It might be expected that greater diversity would be introduced for plants with high genetic diversity in the native range. High genetic variation may be advantageous in colonizing species by allowing populations to radiate into environmental conditions different than those experienced in the native range, but a partially asexually reproducing “general purpose” genotype might also be favored (Baker 1974). From compilations of allozyme literature by Hamrick et al. (1979, 1992), Schierenbeck et al. (1995) found that native populations of woody angiosperms that are invasive in their introduced range had high levels of genetic variation (mean species level $H_e = 0.258$; range = 0.085 – 0.489). However, we found little evidence for substantial variation in part of the native range of *C. hirta*. It would be informative to sample *C. hirta* more extensively within its native range to determine variability in the source populations. Nevertheless, substantial genetic variation within the native range likely is not a prerequisite for producing successful invaders. *Clidemia hirta* also has few allozyme-detectable genotypes in Hawaii. From this we conclude that high levels of genetic variation are not necessary for successful proliferation in the introduced range. In fact, Amsellem (2000) found lower levels of genetic diversity in the tropical shrub *Rubus alceifolius* in highly invasive populations on La Réunion, Mauritius, Mayotte, and Queensland than in relatively non-invasive populations on Madagascar. Although it may be impossible to generalize genetic diversity estimates across introduced species, we found that *C. hirta* exhibits all the characteristics of an asexually reproducing weedy species with relatively high dispersal ability that has opportunistically colonized the Hawaiian Islands.


Chapter 3
A Test of the Genetic Shift Hypothesis: Biomass Allocation, Growth, and Photosynthesis in Native and Introduced Genotypes

INTRODUCTION

The high environmental damage and economic costs of non-native, invasive plants have prompted research to identify morphological, growth, and photosynthetic traits associated with invasiveness (e.g., Roy 1990, Rejmánek and Richardson 1996, Williamson and Fitter 1996, Reichard and Hamilton 1997). These and other studies generally have found that non-native, invasive species have greater relative growth rates (RGR), specific leaf areas (SLA; leaf area per unit leaf mass), leaf area ratios (LAR; total leaf area per unit plant mass), and maximal photosynthetic rates ($A_{\text{max}}$) as well as lower respiratory costs than native species that occur in the same area or non-invasive congeners (Pattison et al. 1998, Baruch and Goldstein 1999, Durand and Goldstein 2001, Grotkopp et al. 2002, McDowell 2002). In addition, phenotypic plasticity of non-native species grown in different light environments has been found to be greater than for native species (Pattison et al. 1998, Schweitzer and Larson 1999).

This variation in traits among species is also demonstrated at the intraspecific level; conspecifics often differ in key ways between their native and introduced ranges. Plants may become more abundant (Fowler et al. 1996, Williamson and Fitter 1996), have faster growth rates (Blossey and Nötztold 1995, Fowler et al. 1996), grow to greater stature (Pritchard 1960, Blossey and Nötztold 1995), or have higher seed production (Noble 1989, Paynter et al. 1996) where they have been introduced than where they are native. In addition, some species invade habitats, such as forest understory, in which they do not occur in their native range (Chapter 1).

Two non-mutually exclusive hypotheses have been proposed to account for these changes: the enemy release hypothesis and the genetic shift hypothesis. The enemy release hypothesis states that increased abundance, growth rates, and habitat distribution between native
and introduced ranges result from lower herbivore or fungal pest loads in areas of introduction (Chapter 4; Keane and Crawley 2002). The genetic shift hypothesis states that native and introduced genotypes differ genetically in ways that lead to differences in biomass allocation, growth, or photosynthesis between their native and introduced ranges (Pritchard 1960, Blossey and Nötzold 1995). Genetic divergence could result from founder effects, post-introduction natural selection (Blossey and Nötzold 1995), inter- or intraspecific hybridization (Ellstrand and Schierenbeck 2000), or increases in ploidy (Pritchard 1960). The two hypotheses are not mutually exclusive because reduced natural enemy attack in areas of introduction could lead to stronger selection for introduced genotypes that allocate fewer resources to defense and more toward increased “vigor” (Blossey and Nötzold 1995). Such competitively superior genotypes might therefore have increased size (height or biomass), faster growth rates, or greater allocation to reproduction than native genotypes. Support for the genetic shift hypothesis has been equivocal; introduced genotypes of some species showed increased vigor compared to native genotypes when grown in common gardens (Blossey and Nötzold 1995, Blossey and Kamil 1996, Willis et al. 1999, Siemann and Rogers 2001, Leger and Rice 2003), while others did not (Willis et al. 2000).

The environmental attributes of the areas being invaded also affect which life history characteristics may differ between native and introduced genotypes. In mesic to wet tropical forests, light is generally considered the most limiting and heterogeneous resource to plant growth and survival (Chazdon 1988, Chazdon et al. 1996). Plant species differ in their response both to gross scale differences in light between gaps and understory (Denslow et al. 1990, Veneklaas and Poorter 1998) and to fine scale differences in the understory (Montgomery and Chazdon 2002). In the understory, morphological and physiological characteristics that maximize photosynthetic area, minimize carbon costs, and maximize recovery from abiotic or biotic damage are important (Pattison et al. 1998, Baruch and Goldstein 1999, McDowell 2002). Low light compensation points also are considered beneficial in the shade (Boardman 1977) because below this point there is insufficient light to maintain a positive carbon balance. Higher LAR and SLA demonstrate an increase in allocation to whole plant carbon gain and should thus be maximized in low light; however, high-SLA leaves may be more vulnerable to herbivory (Grime et al. 1996) because they are generally less tough than low-SLA leaves (Reich et al. 1991, Witkowski and Lamont 1991, Moles and Westoby 2000). In the absence of natural
enemies, trade-offs between high-light growth and low-light survival demonstrated for various woody taxa in their native ranges (Kitajima 1994, Walters and Reich 1999, 2000) may be relaxed. Thus, high survivorship may be coupled with fast growth rates, resulting from greater allocation to light interception (SLA and LAR) and less allocation to storage (e.g., roots). In open areas (gaps, forest margins, pastures), maximization of photosynthetic area may be the most important trait to maintain high growth rates (Kitajima 1994).

We tested the genetic shift hypothesis by growing native and introduced genotypes of the tropical shrub *Clidemia hirta* var. *hirta* (L.) D. Don. (Melastomataceae) under either high or low light in a common greenhouse environment (hereafter called “common garden”). *Clidemia hirta* occurs primarily in high light environments and is absent from forest understory in its native range, which includes tropical Central and South America and some Caribbean Islands (Wester and Wood 1977). Paradoxically, the species is an aggressive, disruptive invader of open areas as well as forest understory where it has been introduced to various islands in the Pacific and Indian Oceans and continental areas of Asia and eastern Africa (Wester and Wood 1977, Smith 1992, Gerlach 1993).

The genetic shift hypothesis predicts that *Clidemia hirta* genotypes from the introduced range will have increased vigor, shade tolerance, and phenotypic plasticity than genotypes from the native range when grown in a common garden. Evidence of increased vigor for *Clidemia hirta* in both high and low light environments would include greater final biomass, height, RGR, $A_{\text{max}}$, and reproductive effort. Evidence of greater shade tolerance of plants grown in low light would be seen in traits that maximize carbon gain and minimize carbon costs, such as higher SLA, LAR, LMR, and $A_{\text{max}}$ as well as lower dark respiration rates and light compensation points. In the absence of natural enemies, genotypes that allocate more to growth and reproduction and less to defense or storage might be at a selective advantage (Blossey and Nötzold 1995). In particular, specific leaf area would be expected to be greater for introduced genotypes because this trait has a strong positive relationship with RGR for many woody taxa, especially in low light conditions (Lusk et al. 1997, Huante and Rincon 1998, Wright and Westoby 1999, Shipley 2002). SLA was also found to be the primary parameter responsible for differences in RGR between invasive and non-invasive pines grown in a common garden (Grotkopp et al. 2002). Evidence for greater genetically determined phenotypic plasticity was examined here by measuring the difference in several morphological and physiological variables.
of plants measured on plants grown in high and low treatments. Thus, here we are testing whether plasticity (variation among the high and low light treatment) is part of a suite of traits that differ between *C. hirta* sampled in native and introduced ranges.

**METHODS**

**Study Species**

*Clidemia hirta* is a densely branching woody shrub that grows to a maximum height of 2-3 m and occurs in mesic to wet areas from sea level to about 1500 m a.s.l. in both its native and introduced ranges (Wester and Wood 1977). Although the species resprouts vigorously when damaged and may root along fallen stems, it does not exhibit clonal growth. The fruits of *C. hirta* are pulpy, dark-blue berries that are produced year-round. Fruit may contain between 200 and 900 seeds, each about 0.5 mm in diameter.

Seeds used in this experiment were from multiple individuals of four Costa Rican (native) and four Hawaiian (introduced) populations (Table 3.1). These populations were randomly chosen from 20 populations in each area for which we had seed collections (Chapter 2). Source populations used in the experiment occurred between 0 and 300 m in elevation. Ripe

<table>
<thead>
<tr>
<th>Source population</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Costa Rica</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nazareth</td>
<td>10°16´</td>
<td>83°54´</td>
<td>Young <em>Bactris gasipaes</em> plantation</td>
</tr>
<tr>
<td>Ticari</td>
<td>10°22´</td>
<td>83°55´</td>
<td>Semi-shaded garden</td>
</tr>
<tr>
<td>Los Chiles</td>
<td>10°58´</td>
<td>84°42´</td>
<td>Roadside</td>
</tr>
<tr>
<td>Platanillo</td>
<td>9°22´</td>
<td>83°46´</td>
<td>Pasture</td>
</tr>
<tr>
<td><strong>Hawaii</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hanalei (Kauai)</td>
<td>22°09´</td>
<td>159°27´</td>
<td>Trailside</td>
</tr>
<tr>
<td>Sleeping Giant (Kauai)</td>
<td>22°05´</td>
<td>159°26´</td>
<td>Roadside</td>
</tr>
<tr>
<td>Waiakea Forest Reserve (Hawaii)</td>
<td>19°41´</td>
<td>155°08´</td>
<td>Shaded side of dirt road</td>
</tr>
<tr>
<td>South Kona (Hawaii)</td>
<td>19°22´</td>
<td>155°50´</td>
<td>Shaded side of dirt road</td>
</tr>
</tbody>
</table>
fruits were collected from populations in Hawaii in January and February 2000 and in Costa Rica in March 2000. Seeds were separated from the fruit pulp, dried at room temperature, and stored in Epindorf tubes at room temperature.

**Common Garden Light Treatments**

Six replicate sections of greenhouse benches (1.6 x 0.9 m) were assigned to low or high light treatments. Plants in the low light treatment were grown under one of three frames (1.4 m tall) covered with neutral density shade cloth (50% light interception) that hung down on all sides to the bench. Our low light treatment was designed to represent intermediate light levels found in small gaps or along trails in tropical forests (Valladares et al. 1997). Plants in the high light treatment were exposed to ambient light levels in the greenhouse. The high light treatment represented light levels received in large gaps. The three shade frames (low light) and three open sections (high light) were alternated in position over two benches in such a way as to reduce shading of the open sections by the shade frames. We increased the number of layers of shade cloth twice over the course of the experiment (February – August) to offset the increase in day length that occurred and to decrease light levels slowly. One layer was in place from 12 February to 6 April, two layers from 7 April to 14 June, and three layers from 14 June – 11 August 2002 (Table 3.2). We measured incident radiation in the open bench sections and shade houses (under 1 – 3 layers of shade cloth) as well as immediately outside the greenhouse to calculate percent diffuse transmittance (%T) of the treatments relative to full sunlight. These measurements were made early in the morning and late in the afternoon with quantum sensors (LI-190SA; LI-COR Inc., Lincoln, NE) mounted on a LAI-2000 canopy analyzer operated in the two-instrument mode. Mean daily photosynthetic photon flux density (PPFD) in the high and low light treatments (Table 3.2) was calculated using %T and the total PPFD measured daily during our experiment in a large clearing nearby at the University of Georgia Watkinsville-Plant Sciences Farm (G. Hoogenboom, *unpublished data*). On a daily basis, plants in our high light treatment received 37 – 50% of full sunlight received in large clearings in lowland areas of Costa Rica (27.9 mol m\(^{-2}\) d\(^{-1}\); Oberbauer et al. 1989). The mean daily PPFD for the low light treatment (Table 3.2; 5 – 16.5% full sunlight) is in the upper range of light levels found in forest understory in Panama (Valladares et al. 2000) and is higher than the maximum found in forest understory in Costa Rica (Chazdon and Fetcher 1984, Montgomery and Chazdon 2002). The light levels in
Seeds were germinated on the surface of Fafard 3B soil mix (Conrad Fafard, Inc, Agawam, MA, USA) under a misting system in a temperature-controlled greenhouse at the University of Georgia in Athens, GA, USA in mid July 2001. There was no noticeable difference in timing or percentage germination in the Hawaiian or Costa Rican genotypes. Single seedlings (26 – 40 per population) were transferred to 3.8-L pots filled with a mixture of pine bark, vermiculite, and limestone in early December 2001. We waited until this time to be assured that seedlings were established and hardy enough to be transplanted to individual pots. At the time of transplanting, seedlings had two to three pairs of true leaves. Seedlings were grown under ambient light until mid-February 2002. At this time, there were no significant differences in the total stem length (TSL) or number of leaves of plants from Hawaii or Costa Rica (data shown for TSL in Figure 3.1).

Four plants per population were randomly assigned to each block (3) x light treatment (2). Initially, there were 32 pots in each block (4 plants x 4 populations x 2 source areas), but several plants were damaged by liquid fertilizer application and were discarded. At least two plants per population in each block were undamaged (no empty cells in the experimental design).

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Layers of 50% shade cloth</th>
<th>Total daily PPFD (mol m⁻² day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Feb - 3 Apr</td>
<td>0</td>
<td>10.3 ± 3.4</td>
</tr>
<tr>
<td>4 Apr - 2 June</td>
<td>0</td>
<td>12.3 ± 4.2</td>
</tr>
<tr>
<td>3 June - 10 Aug</td>
<td>0</td>
<td>13.9 ± 3.1</td>
</tr>
<tr>
<td>Low light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Feb - 3 Apr</td>
<td>1</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td>4 Apr - 2 June</td>
<td>2</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>3 June - 10 Aug</td>
<td>3</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Table 3.2 Mean daily PPFD of the high and low light treatments (N = 3 each) during the course of the experiment. The number of layers of shade cloth used for the low light treatment was increased twice during the experiment.
Fertilizer was subsequently applied with encapsulated, slow release Osmocote 15-9-12 and Peter’s 20-10-20 Peat-lite special several times over the course of the experiment. Plants were watered as needed. Plants were relocated within their designated block every 14 – 21 days to reduce possible positioning effects.

**Biomass Allocation and Growth Analyses**

We measured the biomass allocation and growth of plants between February and August 2002 using non-destructive (TSL and basal diameter) and destructive measurements (whole plant harvests in June and August). Initial dry above- and below-ground biomass of experimental seedlings was estimated from TSL and basal diameter using allometric equations ($R^2 = 0.82$ and 0.74, respectively) developed from harvested plants (mid-January) that had been transplanted to pots in December but were not used in the experiment. Half of the experimental plants were harvested in June to provide mass-based measures for photosynthetic parameters conducted at the same time (see below) and to provide more space for the remaining plants. We repotted the remaining plants, which were becoming too large for the 3.8-L pots, into 9.5-L pots on 4 June. These plants were harvested between 7 and 12 August 2002. Biomass allocation results from the final (August) harvest only are presented, but results were similar between harvests. We used the final harvest because we presumed that any differences between the Costa Rican and Hawaiian genotypes should have become more apparent over time.

At the final harvest, the leaf area and dry mass of the two most recently fully expanded leaves were measured to calculate SLA. Leaf area was measured on fresh leaves with a LI-COR 3100 leaf area meter (LI-COR, Inc., Lincoln, NE, USA). Plants were divided into roots, leaves, stems (branches and petioles), and reproductive parts (peduncles, buds, flowers, fruit) and dried at 60°C for 48 h. Four variables were calculated from these measurements: root mass ratio (RMR; root mass per whole plant mass), stem mass ratio (SMR; stem mass per whole plant mass), leaf mass ratio (LMR; leaf mass per whole plant mass), and leaf area ratio (LAR; total leaf area per whole plant mass). At the final harvest, all plants in the high light treatment were reproductive, therefore the reproductive mass ratio (ReMR; reproductive mass per whole plant mass) was also calculated. Relative biomass growth rates ($RGR_b$) were calculated as: $RGR_b = [\ln(\text{final plant mass at harvest}) – \ln(\text{initial plant mass})]/\text{time in treatment (days)}$ (Evans 1972). Relative stem length growth rates were also calculated to compare plants non-destructively among census dates: $RGR_{st} = [\ln(\text{final total stem length at harvest}) – \ln(\text{initial total stem length})]/\text{time in treatment (days)}$ (Evans 1972).
length)/time in treatment (days). The initial plant mass was estimated from non-experimental plants harvested in January, while the initial total stem length was measured for each plant.

**Gas Exchange Measurements**

Responses of net CO₂ assimilation (\(A\)) to PPFD were measured on one randomly chosen plant from each population and block (\(N = 48\)) between 25 May and 3 June 2002. Measurements were made on the most recently fully expanded leaf between 08:00 and 14:00 hours on clear days (to ensure that leaves were induced) in a laboratory close to the greenhouse with a LI-COR 6400 gas exchange system (LI-COR, Inc., Lincoln, NE, USA). In the leaf chamber, each leaf was acclimated to 400 µmol m⁻² s⁻¹ PPFD for 1 – 3 min and then to 1000 µmol m⁻² s⁻¹ for 1 – 3 min prior to gas exchange measurements. Light response curves were started after acclimation by decreasing PPFD stepwise in the following order: 1500, 1000, 500, 250, 100, 50, and 10 µmol m⁻² s⁻¹. Dark respiration was measured three to five times at 0 PPFD at 2 min intervals. The mean of the final three dark respiration measurements was used as the gas exchange rate at 0 PPFD for the light response curve. During gas-exchange measurements, cuvette air was maintained at 22 – 25°C, 74 – 88% relative humidity, and 360 µmol mol⁻¹ sample CO₂ partial pressure. If a leaf displayed a conductance less than 0.08 mol m⁻² s⁻¹, another leaf or plant was chosen. The portion of each leaf measured for photosynthesis (area = 6 cm²) was cut out and dried for mass determination. Dark respiration (\(R_d\)), apparent quantum efficiency (AQE), maximum photosynthetic rates (\(A_{\text{max}}\)), light compensation points (LCPT), and light saturation points were calculated from the light response curve data using the program Photosyn Assistant (Dundee Scientific, Dundee, Scotland), which employs the standard method of Prioul and Chartier (1977).

**Phenotypic Plasticity Index**

An index of phenotypic plasticity (Valladares et al. 2000) was calculated for each population for morphological variables measured at the August harvest and for photosynthetic variables measured at the June harvest. Only variables that differed significantly between light treatments were used. The index ranges from zero to one and was calculated per population as the positive difference between the mean value of each variable for the high light and low light treatments divided by the greater mean value (high or low light; Valladares et al. 2000, Balaguer
et al. 2001). For each variable, a mean plasticity index was calculated for each source area (Hawaii or Costa Rica) by averaging over the four populations per area.

Data Analysis

Analysis of variance (ANOVA) was used to compare the effects of light and source area on morphological and photosynthetic variables. The design of the experiment was a split-split plot with a completely randomized design at the within split-plot level (block). The main effect was light (high or low). The experimental unit for the effect of light was the block ($N = 3$ per treatment). Light level and seed source area (Hawaii or Costa Rica) were treated as fixed effects, whereas the source population, block, and all interactions containing these effects were treated as random effects in PROC MIXED of SAS Version 8 (SAS Institute 2000). When significant area x light interactions were detected, we tested for differences between Hawaiian and Costa Rican genotypes under each light level. The residuals for all tests were normally distributed and no data transformations were necessary. No empty cells were present even though some plants died initially, and therefore our design was balanced.

We examined whether Hawaiian and Costa Rican genotypes separated in multivariate space and determined which variables contributed most to this separation by conducting principal components analysis (PCA; PROC PRINCOMP in SAS). We used the mean response of each population in each of the two light treatments for 14 growth, morphological, and photosynthetic variables. Each PCA was performed on a correlation matrix because the 14 variables had different units of measurement.

RESULTS

Biomass Allocation and Growth

Both Hawaiian and Costa Rican genotypes responded predictably to light treatments; *Clidemia hirta* grown in high light had significantly greater RGR_b and RGR_a, more leaves, greater total leaf area, and lower SLA and LAR than plants grown in low light (Table 3.3; Figure 3.1). Plants in high light also allocated proportionately less biomass to leaves and stems and more to roots than those grown in low light (Table 3.3).
Table 3.3 Growth and biomass allocation variables for Costa Rican and Hawaiian genotypes grown in two light treatments. Means ± 1 SE are listed for each light treatment and source area. F-values and significance levels of ANOVAs testing for main effects of source area, light treatment, and their one-way interaction are listed for each variable. The denominator df for the effects of area and light are 6 and 4, respectively. The df for the interaction term are indicated for each variable. When the interaction term was significant, F-values and levels of significance are included for the effect of area within each light treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low light</th>
<th>High light</th>
<th>F-values</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Costa Rica</td>
<td>Hawaii</td>
<td>Costa Rica</td>
<td>Hawaii</td>
</tr>
<tr>
<td>RGRst (mm cm⁻¹ day⁻¹)</td>
<td>20.1 ± 0.6</td>
<td>19.1 ± 0.6</td>
<td>27.6 ± 0.5</td>
<td>27.2 ± 0.6</td>
</tr>
<tr>
<td>RGRb (mg g⁻¹ day⁻¹)</td>
<td>20.9 ± 0.7</td>
<td>19.7 ± 0.5</td>
<td>28.6 ± 0.5</td>
<td>28.4 ± 0.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>61.4 ± 1.4</td>
<td>59.7 ± 2.0</td>
<td>68.3 ± 0.9</td>
<td>69.5 ± 0.9</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>49.1 ± 2.7</td>
<td>49.6 ± 4.1</td>
<td>239.1 ± 10.9</td>
<td>295.4 ± 14.0</td>
</tr>
<tr>
<td>Total leaf area (cm²)</td>
<td>2274 ± 163</td>
<td>2243 ± 163</td>
<td>7906 ± 247</td>
<td>8015 ± 220</td>
</tr>
<tr>
<td>SLA (cm² g⁻¹)</td>
<td>265.1 ± 8.5</td>
<td>249.3 ± 8.4</td>
<td>135.6 ± 4.7</td>
<td>122.2 ± 5.1</td>
</tr>
<tr>
<td>LAR (cm² g⁻¹)</td>
<td>130.0 ± 2.9</td>
<td>114.8 ± 4.0</td>
<td>67.1 ± 1.8</td>
<td>62.8 ± 1.4</td>
</tr>
<tr>
<td>LMR (g g⁻¹)</td>
<td>0.505 ± 0.012</td>
<td>0.478 ± 0.009</td>
<td>0.434 ± 0.006</td>
<td>0.422 ± 0.006</td>
</tr>
<tr>
<td>RMR (g g⁻¹)</td>
<td>0.147 ± 0.005</td>
<td>0.190 ± 0.009</td>
<td>0.217 ± 0.006</td>
<td>0.273 ± 0.008</td>
</tr>
<tr>
<td>SMR (g g⁻¹)</td>
<td>0.347 ± 0.009</td>
<td>0.333 ± 0.003</td>
<td>0.329 ± 0.007</td>
<td>0.266 ± 0.006</td>
</tr>
<tr>
<td>ReMR (g g⁻¹)</td>
<td>-</td>
<td>-</td>
<td>0.020 ± 0.003</td>
<td>0.039 ± 0.004</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001
Relative stem growth rates (RGRst) did not differ significantly between the genotypes from the native (Costa Rican) and introduced (Hawaiian) ranges at any time interval during the course of the experiment in either light environment (Figure 3.1). No significant differences between Costa Rican and Hawaiian genotypes were found for relative biomass growth rates (RGRb) or maximum plant height (Table 3.3).

Although there were no differences in growth rates, Costa Rican and Hawaiian *C. hirta* genotypes differed significantly in some biomass allocation patterns. Compared to Hawaiian genotypes, Costa Rican genotypes allocated proportionately more biomass to stems and branches (SMR) in the high light treatment, more to leaf area per unit whole-plant biomass (LAR) in the low light treatment, and less to roots (RMR) in both light treatments (Table 3.3). Biomass

Figure 3.1 Total stem length over the course of the experiment for plants grown from Hawaiian and Costa Rican seed sources in two light treatments. No significant differences were found between Hawaiian and Costa Rican genotypes for any time interval.
allocation to leaves (LMR) did not differ significantly between the genotypes from the native and introduced ranges in either light treatment. Plants from the two source areas also did not differ significantly in number of leaves, total leaf area, or SLA (Table 3.3).

In the high light treatment, plants from both source areas became reproductive, producing flowers and immature fruit, but not mature fruit. Mean ReMR for Hawaiian genotypes was almost two-fold greater than for Costa Rican genotypes (Table 3.3).

Other differences between the Costa Rican and Hawaiian genotypes were observed, but not measured. Growth form of plants differed with the Hawaiian genotypes exhibiting a more upright compact form than the Costa Rican genotypes (Figure 3.2). Hawaiian genotypes also had fewer and shorter trichomes on the leaves and stems and smaller flowers.

Figure 3.2 Clidemia hirta individuals grown in a common garden in high light for 10 months from Hawaiian (left) or Costa Rican (right) seed sources. Note that the Hawaiian plant has a more upright and compact form than the Costa Rican plant.
Photosynthetic Gas Exchange

Costa Rican and Hawaiian genotypes responded to increasing PPFD with increases in net CO₂ assimilation per unit area until light saturation (Figure 3.3). As expected, plants grown in the high light treatment had greater maximal photosynthetic rates calculated on an area basis ($A_{\text{max}_a}$), LCPTs, and light saturation points as well as lower maximal photosynthetic rates calculated on a mass basis ($A_{\text{max}_m}$) and dark respiration rates calculated on an area basis ($R_d$) than plants grown in the low light treatment (Table 3.4). Apparent quantum efficiency was the only photosynthetic variable that did not show any significant difference between light treatments.

Neither $A_{\text{max}_a}$ nor $A_{\text{max}_m}$ differed between Costa Rican and Hawaiian genotypes in

![Figure 3.3 Net CO₂ assimilation as a function of photosynthetic photon flux density (PPFD) in Costa Rican and Hawaiian genotypes of *Clidemia hirta* grown in high or low light treatments. Error bars represent ± 1 SE of the mean.](image-url)
Table 3.4 Photosynthesis variables for Costa Rican and Hawaiian genotypes grown in two light treatments. Means ± 1 SE are listed for each light treatment and source area. F-values and significance levels of ANOVAs testing for main effects of source area, light treatment, and their one-way interaction are listed for each variable. The df for the effect of area and light are 1,6 and 1,4, respectively. The df for the interaction term are 1, 34. When the interaction term was significant, F-values and levels of significance are included for the effect of area within light treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low light</th>
<th>High light</th>
<th>F-values</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Costa Rica</td>
<td>Hawaii</td>
<td>Costa Rica</td>
<td>Hawaii</td>
</tr>
<tr>
<td>$A_{\text{max}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>11.35 ± 0.43</td>
<td>11.48 ± 0.72</td>
<td>13.94 ± 0.78</td>
<td>15.25 ± 0.77</td>
</tr>
<tr>
<td>$A_{\text{max}}$ (nmol CO$_2$ g$^{-1}$ s$^{-1}$)</td>
<td>238.9 ± 9.8</td>
<td>251.8 ± 19.2</td>
<td>157.9 ± 10.4</td>
<td>170.0 ± 12.6</td>
</tr>
<tr>
<td>AQE (mol CO$_2$ mol photons$^{-1}$)</td>
<td>0.0826 ± 0.0022</td>
<td>0.0801 ± 0.0028</td>
<td>0.0807 ± 0.0017</td>
<td>0.0788 ± 0.0022</td>
</tr>
<tr>
<td>$R_d$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>0.494 ± 0.017</td>
<td>0.517 ± 0.027</td>
<td>0.782 ± 0.043</td>
<td>1.041 ± 0.073</td>
</tr>
<tr>
<td>LCPT (µmol m$^{-2}$ s$^{-1}$)</td>
<td>6.04 ± 0.28</td>
<td>6.51 ± 0.38</td>
<td>9.73 ± 0.50</td>
<td>13.00 ± 1.22</td>
</tr>
<tr>
<td>Light saturation point (µmol m$^{-2}$ s$^{-1}$)</td>
<td>145.0 ± 7.4</td>
<td>150.0 ± 8.7</td>
<td>183.3 ± 10.3</td>
<td>206.9 ± 9.7</td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$
either light treatment (Table 3.4). At PPFD levels ≥ 250 µmol, net CO₂ assimilation did not differ significantly between genotypes from the two source areas in either light treatment (Figure 3.3). In the high light treatment, however, Hawaiian genotypes had 33% higher $R_d$ and 34% higher LCPT than Costa Rican genotypes (Table 3.4). In the low light treatment, there were no significant differences in any of the photosynthetic gas exchange characteristics between the native and introduced genotypes. For plants in both light treatments, there was a positive linear relationship between $A_{\text{max}}$ and $R_d$ ($F_{1,47} = 25.7, P < 0.001, R^2 = 0.36$), but at a given dark respiration rate, photosynthetic rates did not differ for Costa Rican and Hawaiian genotypes (i.e., no difference in slopes; ANCOVA: $F_{1,47} = 1.39, P = 0.24$).

**Phenotypic Plasticity**

The plasticity index of only one of 12 variables differed significantly between Hawaiian and Costa Rican genotypes (Table 3.5). Hawaiian genotypes had significantly greater variation among the high and low light treatments for stem mass ratio than Costa Rican genotypes, which

### Table 3.5 Indices of phenotypic plasticity and ANOVA $F$-values testing for differences between *Clidemia hirta* collected from Costa Rican (native range) and Hawaiian (introduced range) populations for 12 variables that differed significantly between light treatments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Costa Rica</th>
<th>Hawaii</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>0.48</td>
<td>0.50</td>
<td>0.1</td>
</tr>
<tr>
<td>LAR</td>
<td>0.48</td>
<td>0.45</td>
<td>1.1</td>
</tr>
<tr>
<td>LMR</td>
<td>0.13</td>
<td>0.11</td>
<td>0.3</td>
</tr>
<tr>
<td>SMR</td>
<td>0.06</td>
<td>0.20</td>
<td>19.0 **</td>
</tr>
<tr>
<td>RMR</td>
<td>0.31</td>
<td>0.30</td>
<td>0.1</td>
</tr>
<tr>
<td>No. lvs</td>
<td>0.79</td>
<td>0.83</td>
<td>5.0</td>
</tr>
<tr>
<td>Height</td>
<td>0.27</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td>RGR$_b$</td>
<td>0.37</td>
<td>0.49</td>
<td>5.6</td>
</tr>
<tr>
<td>$R_d$</td>
<td>0.18</td>
<td>0.26</td>
<td>0.6</td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>0.38</td>
<td>0.49</td>
<td>2.0</td>
</tr>
<tr>
<td>LCPT</td>
<td>0.20</td>
<td>0.31</td>
<td>1.7</td>
</tr>
<tr>
<td>Light saturation point</td>
<td>0.10</td>
<td>0.15</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**$P < 0.01$**
displayed almost no variation between light treatments for this variable (plasticity index = 0.06). Populations from both areas had low plasticity for leaf mass ratio and light saturation point and high plasticity for number of leaves and SLA (Table 3.5).

**Principal Components Analysis**

The PCAs of 14 morphological and physiological characters provided a clear separation of Costa Rican and Hawaiian genotypes for the high light treatment (Figure 3.4a) and a moderate separation for the low light treatment (Figure 3.4b). For both analyses, the separation was mainly along the first axis and the first two axes explained most of the variation (68% in high light and 64% in low light). In the PCA for the high light treatment, RMR and SMR were the two variables most strongly correlated with the first axis (Table 3.6). This result supported the univariate analyses showing that Hawaiian genotypes had greater RMR and lower SMR than

<table>
<thead>
<tr>
<th>Variable</th>
<th>High light Axis 1</th>
<th>High light Axis 2</th>
<th>Low light Axis 1</th>
<th>Low light Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>-0.63</td>
<td>0.51</td>
<td>0.68</td>
<td>0.07</td>
</tr>
<tr>
<td>LAR</td>
<td>-0.60</td>
<td><strong>0.71</strong></td>
<td>0.77</td>
<td>0.35</td>
</tr>
<tr>
<td>LMR</td>
<td>-0.52</td>
<td>0.60</td>
<td>0.72</td>
<td>0.60</td>
</tr>
<tr>
<td>SMR</td>
<td><strong>-0.94</strong></td>
<td>-0.28</td>
<td>0.05</td>
<td>-0.43</td>
</tr>
<tr>
<td>RMR</td>
<td><strong>0.98</strong></td>
<td>-0.03</td>
<td><strong>-0.81</strong></td>
<td>-0.35</td>
</tr>
<tr>
<td>ReMR</td>
<td>0.81</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. lvs</td>
<td>0.91</td>
<td>0.17</td>
<td>0.75</td>
<td>-0.38</td>
</tr>
<tr>
<td>Height</td>
<td>-0.02</td>
<td>-0.49</td>
<td><strong>0.83</strong></td>
<td>-0.19</td>
</tr>
<tr>
<td>RGR&lt;sub&gt;b&lt;/sub&gt;</td>
<td>-0.32</td>
<td>0.16</td>
<td>0.62</td>
<td>0.64</td>
</tr>
<tr>
<td>R&lt;sub&gt;d&lt;/sub&gt;</td>
<td>-0.92</td>
<td>0.14</td>
<td>0.04</td>
<td><strong>0.84</strong></td>
</tr>
<tr>
<td>AQE</td>
<td>-0.35</td>
<td>-0.23</td>
<td>-0.67</td>
<td>0.60</td>
</tr>
<tr>
<td>A&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.46</td>
<td><strong>0.73</strong></td>
<td>-0.72</td>
<td>0.32</td>
</tr>
<tr>
<td>LCPT</td>
<td>0.89</td>
<td>-0.12</td>
<td>0.47</td>
<td><strong>-0.85</strong></td>
</tr>
<tr>
<td>Light saturation point</td>
<td>0.62</td>
<td><strong>0.72</strong></td>
<td>-0.23</td>
<td>-0.12</td>
</tr>
</tbody>
</table>
Figure 3.4 Principal components ordination of four Costa Rican and four Hawaiian populations based on morphological and photosynthetic variables listed in Table 3.6. Analyses were performed separately on population means for 14 variables for plants grown in (a) high light and (b) low light treatments. The letter “H” indicates Hawaiian population means and “C” indicates Costa Rican population means.
Costa Rican genotypes (Table 3.3). Height and RMR were influential characters for plants grown in low light (Table 3.6), and distinguished the Costa Rican from Hawaiian populations with the exception of the Hawaiian Road B population, which had an unusually low RMR. In general in low light, Costa Rican genotypes tended to be taller and allocated less biomass to roots relative to whole plant biomass than Hawaiian genotypes (Figure 3.4b). However, source area was not a significant effect in univariate analyses of plant height (Table 3.3).

DISCUSSION

Genetic changes among conspecifics in their native and introduced ranges have been hypothesized to account for differences in some life history traits observed between native and introduced plant genotypes (Blossey and Nötzold 1995). From this genetic shift hypothesis, we had predicted that Hawaiian genotypes of *Clidemia hirta* would grow faster, accumulate more biomass, and have greater SLA, LAR, and SMR but lower RMR than Costa Rican genotypes because such patterns are found for fast-growing compared to slow-growing woody species (e.g., Cornelissen et al. 1996, Reich et al. 1997, Grotkopp et al. 2002). However, our study provided almost no evidence that Hawaiian genotypes of *C. hirta* differed genetically from Costa Rican genotypes in ways that might account for observed differences in habitat distributions and abundances in its introduced and native ranges. In fact, we found most significant genetic differences were in the opposite direction predicted (Table 3.7).

In the high light treatment, Costa Rican and Hawaiian genotypes differed in some aspects of biomass allocation, but the primary characteristics that we expected to be greater in Hawaiian genotypes in high light, specifically higher RGR, final height, SLA, and maximum photosynthetic rates, did not differ significantly from those of Costa Rican genotypes. The ways they differed in biomass allocation were contrary to our predictions that introduced genotypes would allocate more to stems and leaves and less to roots (one type of storage organ). Instead, the native Costa Rican genotypes allocated proportionately more biomass to stems and less to roots than the introduced Hawaiian genotypes. The PCA of mean population values for 14 morphological or physiological variables clearly separated the Costa Rican and Hawaiian genotypes grown in the high light treatment, primarily on the basis of SMR and RMR. Several physiological variables, such as $R_d$, LCPT, and light saturation point, did differ significantly between source areas and were higher for Hawaiian than Costa Rican genotypes grown in high
Table 3.7 Predicted responses of introduced genotypes compared to native genotypes and observed support (“yes”) or no support (“no”) for these predictions for eight species grown in common garden experiments. Only this study examined plants grown in low light.

<table>
<thead>
<tr>
<th>Predicted response</th>
<th>Clidemia hirta&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lythrum salicaria&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sapium sebiferum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Carduus nutans&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Digitalis purpurea&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Echium vulgare&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Senecio jacobea&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Eschscholzia californica&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Any light environment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greater height or basal area</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greater total biomass</td>
<td>no</td>
<td>yes</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Greater shoot mass</td>
<td>no</td>
<td>no</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Greater reproductive effort&lt;sup&gt;f&lt;/sup&gt;</td>
<td>yes</td>
<td>-</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Less root mass</td>
<td>no</td>
<td>no</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Higher photosynthetic rates</td>
<td>no</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(b) Low light environment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower respiratory costs</td>
<td>no</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greater SLA</td>
<td>no</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greater LAR</td>
<td>no</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>This study
<br><sup>b</sup>Blossey and Nötzold (1995); Blossey and Kamil (1996); Willis and Blossey (1999)
<br><sup>c</sup>Siemann and Rogers (2001)
<br><sup>d</sup>Willis et al. (2000)
<br><sup>e</sup>Leger and Rice (2003) - only under reduced competition
<br><sup>f</sup>All reproductive parts biomass for (a), % trees producing seed for (c), inflorescence mass for (d), and number of seeds for (e)
light. The difference in LCPT has little practical meaning for carbon budgets because light
levels would have been above this point in the high light environment most days (and thus they
would have had positive carbon budgets). The higher area-based dark respiration rates of
Hawaiian genotypes could result from selection on correlated traits such as leaf nitrogen, SLA,
or $A_{\text{max}}$ (Reich et al. 1998). We did not examine leaf nitrogen, but found no difference in $A_{\text{max}}$
or SLA between Hawaiian and Costa Rican genotypes. In addition, the slopes for the
relationship between $A_{\text{max}}$ and $R_d$ did not differ significantly between the native and introduced
genotypes indicating that they have similar respiratory costs at increasing rates of
photosynthesis. In contrast, studies comparing natives and non-native invasive species generally
have found higher photosynthetic rates per unit dark respiration for non-native species (Pattison
et al. 1998, McDowell 2002).

The only difference found in the direction predicted in the high light treatment was that
Hawaiian genotypes allocated a greater proportion of biomass to reproductive parts than the
Costa Rican genotypes. This could result from a shorter juvenile period relative to Costa Rican
genotypes or greater overall allocation to reproduction. Invasive Pinus species have an earlier
onset of reproduction than non-native congeners (Rejmánek and Richardson 1996). We did not
track the onset of flowering, and the plants had not yet produced mature fruit, thus we cannot
determine the timing of the onset of reproduction in this experiment. Nonetheless, the greater
allocation to reproduction of the introduced genotypes is the only difference supported by our
study in the high light treatment that was predicted to occur if genetic differences contribute to
greater abundance of C. hirta in Hawaii than Costa Rica.

In the low light treatment, the separation between Costa Rican and Hawaiian genotypes
for variables considered in this study was less definitive than in the high light treatment. Most
populations from each area clustered together in the PCA, but there was some mixing of
populations from the two source areas. The primary differences were that Costa Rican
genotypes allocated significantly more to leaf area and less to roots relative to whole plant
biomass than Hawaiian plants in the shade. In these morphological variables, the Hawaiian
genotypes were somewhat more characteristic of plants that allocate more to recovery from
damage than the Costa Rican genotypes. A well-established root system is thought to enhance
the recovery from loss of tissue from above-ground herbivores and pathogens (Kitajima 1994).
Damage to plants by above-ground herbivores and pathogens is lower for C. hirta in Hawaii than
in Costa Rica (Chapter 4), thus we had predicted that Hawaiian genotypes would allocate relatively more to growth and less to storage (roots), but this was not the case. High allocation to roots also may result from selection in nutrient- or water-limited environments in Hawaii. Other variables that affect light harvesting ability or photosynthetic efficiency, such as SLA, LCPTs, and $R_d$, did not differ between Costa Rican and Hawaiian genotypes in the low light treatment, suggesting that the genotypes from the introduced range were no more efficient in light capture or photosynthetic utilization than the genotypes from the native range in low light.

Up to this point, mean values of native and invasive genotypes of *C. hirta* in the two light treatments have been considered and found to differ in only a few ways. We also found little evidence that Hawaiian genotypes were more plastic; only the shoot mass ratio plasticity index was significantly higher for the Hawaiian vs. Costa Rican genotypes. We had predicted genotypes from the introduced range might be more plastic than those from the native range because *C. hirta* occupies a wider range of habitats (open areas as well as forest understory) in Hawaii than in Costa Rica and because greater morphological plasticity has been found for some non-native, invasive species in some comparisons with non-invasive, native species (Pattison et al. 1998, Schweitzer and Larson 1999). Greater plasticity in stem length might allow *C. hirta* in Hawaii to respond to changes in light environments more effectively.

The source area for *Clidemia hirta* in Hawaii is not known, thus we make no assumptions that the little evidence for genetic differences between our representative native populations collected in Costa Rica and introduced populations collected in Hawaii indicates post-introduction evolutionary change. Individuals in the native source population(s) may have allocation patterns more similar to the Hawaiian than Costa Rican plants and thus the differences observed, such as allocation to roots vs. stems, may simply result from differences among populations within the native range. Indeed, the differences in growth form (e.g., Figure 3.2) and biomass allocation observed in this study support results from a study of allozyme-detectable genetic variation that found a high level of genetic dissimilarity between *C. hirta* genotypes in Costa Rica and Hawaii (Chapter 2). The origin of *C. hirta* on Hawaii likely is not Costa Rica (Chapter 2).

The results of other common garden experiments comparing conspecifics grown from seed from native and introduced ranges have provided mixed support for the genetic shift hypothesis that genetic differences exist that confer greater success for non-native, invasive
species (Table 3.7). Support for the hypothesis was found for one tree species, *Sapium sebiferum* (Siemann and Rogers 2001), and one herbaceous species, *Lythrum salicaria* (Blossey and Nötzold 1995, Blossey and Kamil 1996), but not for four biennial species (Willis et al. 2000) (Table 3.7). Siemann and Rogers (2001) found that native, Taiwanese genotypes of *S. sebiferum* (Chinese tallow) that were grown for 14 years in the United States had less basal area and a lower likelihood of being reproductive (fewer trees were producing seeds) than introduced, North American genotypes, particularly those stemming from later introductions. In addition, native genotypes of the wetland invader, *Lythrum salicaria* (purple loosestrife), were consistently shorter and acquired less biomass than introduced genotypes (Blossey and Nötzold 1995, Blossey and Kamil 1996, Willis and Blossey 1999). Our results were more similar to those found by Willis et al. (2000) who found that aboveground and inflorescence biomass did not differ between native and introduced genotypes of four non-native, invasive biennial species (*Carduus nutans, Digitalis purpurea, Echium vulgare*, and *Senecio jacobaea*). The relative importance of genetic responses may differ among plant taxa, the environmental conditions in areas of introduction, and the time since introduction (Pritchard 1960, Willis and Blossey 1999); hence, there may be discrepancies in results from different common garden studies. As Willis et al. (2000) remarked, however, a genetically determined increase in plant size does not appear to be a widespread phenomenon among non-native, invasive plants.

The results of our study suggest that some genetic differences in morphological variables are present between the Costa Rican and Hawaiian genotypes sampled (e.g., growth form and differential allocation to reproduction, stems and roots), but there was no compelling pattern that suggests that Hawaiian genotypes are more vigorous (grow to greater size) than Costa Rican genotypes. The higher allocation to reproduction of Hawaiian genotypes could contribute to their greater abundance there than in Costa Rica. We would like to investigate this aspect further as we only indirectly measured fecundity (reproductive mass, not number of seeds produced). Nonetheless, mechanisms other than post-introduction shifts in allocation likely account for the differences in habitat distribution of *C. hirta* in Costa Rica and Hawaii. Indeed, we found support for the enemy release hypothesis (Chapter 4; Keane and Crawley 2002). Herbivores and fungal pathogens significantly decreased survival of *C. hirta* in forest understory in Costa Rica but were absent from forest understory in Hawaii where *C. hirta* survival was almost 100% (Chapter 4). Enemy release may ultimately lead to genetic shifts in resource allocation away
from defense and toward greater growth and reproduction, as found for *Lythrum salicaria* (Blossey and Nötzdol 1995, Blossey and Kamil 1996) and *Sapium sebiferum* (Siemann and Rogers 2001). For now the expanded habitat distribution and vigor of *C. hirta* in its introduced range seems to result from an ecological response to enemy release rather than a genetic shift in resource acquisition, allocation, or phenotypic plasticity.

**LITERATURE CITED**


Chapter 4

A Test of the Enemy Release Hypothesis: The Importance of Habitat

INTRODUCTION

Insect herbivores and fungal pathogens can limit plant abundance through depression of plant growth, survival, or reproduction. Numerous studies of temperate and tropical plants have found that herbivores affect individual plants by decreasing lifetime fitness (Louda 1982a, b, Doak 1992, Louda and Potvin 1995, Louda and Rodman 1996, Root 1996) and growth (Marquis 1984, Aide and Zimmerman 1990, Marquis 1992, Schierenbeck et al. 1994), whereas fungal pathogens can cause substantial leaf damage (Coley and Barone 1996) and are implicated causal mechanisms of numerous tree seedling fatalities (Augspurger 1984a, b, Stanosz 1994). However, some experimental manipulations of folivorous herbivore densities or levels of leaf damage do not show negative effects on plant fitness traits (Whitham et al. 1991). In fact, some studies have shown either no or positive effects of herbivores on plant growth (Owen and Wiegert 1976, Simberloff et al. 1978, McNaughton 1983, Anten and Ackerly 2001). Whether or not natural enemies affect plant demography likely depends on the type and extent of damage, life history traits of the plant species, and availability of resources (Whitham et al. 1991).

The demographic consequences of insect herbivory and fungal pathogen attack may be conditional on the habitat in which the plant is growing (Whitham et al. 1991). Plants growing in adjacent habitats often experience different levels of herbivore damage (Huffaker and Kennett 1959, Harper 1969, Louda et al. 1987, Louda and Rodman 1996). Depending on the plant species, natural enemies may be more abundant or damaging in high-light (e.g., Lincoln and Mooney 1984, Harrison 1987, Louda and Rodman 1996) or low-light habitats (Maiorana 1981, MacGarvin et al. 1986, Denslow et al. 1990, Folgarait et al. 1995). Substantial loss of leaf area to herbivores and pathogens may decrease individual growth rates and cause substantial mortality particularly in low light conditions where low carbon fixation rates can be further reduced by loss of leaf area and photosynthate. Chronic herbivore attack could effectively exclude plants from such habitats.
If natural enemies exhibit a strong selective pressure on plant abundance and habitat distribution, then species may increase dramatically in abundance and expand their habitat distribution in the absence of natural enemies. One such scenario occurs when species are introduced accidentally or intentionally to areas outside their native range. In this case, specialist natural enemies may be absent or scarce if herbivores are rare in general (as hypothesized for islands; Carlquist 1974), if specialist enemies are not introduced at the time of plant introduction, and if host switching to the new introduction does not occur (Keane and Crawley 2002). Generalist herbivores and pathogens present in the introduced range may prefer native to non-native species and thus have limited impact on the non-natives (Keane and Crawley 2002). In fact, several inventories of herbivores or pathogens have found fewer species and individuals attacking plants where they are introduced compared to where they are native (e.g., Bossard and Rejmánek 1994, Szentesi 1999, Fenner and Lee 2001, Wolfe 2002, Mitchell and Power 2003), but few studies have examined the consequences of lowered levels of herbivory or pathogen attack.

Biological invasions thus present an experimental system to address the effects of herbivores and pathogens on plant demography and habitat distribution. The release of plant populations from control by natural enemies in areas of introduction has been hypothesized to contribute to observed increases in plant abundance (Elton 1958, Crawley 1987, Fowler et al. 1996) and invasions of habitats in which species do not occur in their native range. This is called the enemy release hypothesis (Keane and Crawley 2002). Classical biological control is predicated on the underlying assumptions that herbivores and pathogens limit population growth in native areas and that introduction of these enemies will therefore limit population growth in introduced areas. However, these assumptions have not been directly tested (Callaway et al. 1999).

We used the tropical woody shrub *Clidemia hirta* (L.) D. Don (Melastomataceae) as a model organism to examine the role of natural enemies in areas of its native and introduced ranges. *Clidemia hirta* is native to lowlands of Central and South America and Caribbean Islands where it colonizes naturally and anthropogenically disturbed, relatively open areas such as pastures, riversides, roadsides, and tree plantations. In its native range, it apparently does not occur in old-growth forests (Cook 1929, Wester and Wood 1977). *Clidemia hirta* is naturalized now throughout the tropics including several islands in the Pacific and Indian Oceans, Peninsular
Malaysia, the Indian subcontinent, and eastern Africa (Tanzania) (Wester and Wood 1977, Rejmánek 1996, Strahm 1999). The introduction of *C. hirta* around the world likely was accidental (Simmonds 1933, Wester 1992). In its introduced range, *C. hirta* is abundant and invades open areas as well as gaps and understory of old-growth forest (Smith 1992, Rejmánek 1996, Strahm 1999).

In this study, we conducted a natural enemy exclusion experiment in replicated field sites in *C. hirta*’s native and introduced ranges to test whether there were differences in leaf damage between the two areas and whether these differences affect growth and survival. We evaluated the effect of aboveground leaf-chewing insects, sucking insects, and fungal pathogens on *C. hirta* in one part of its native range (Costa Rica) and one part of its introduced range (Hawaii). Based on predictions of the enemy release hypothesis, we expected to find high levels of herbivory on plants exposed to insect herbivores and fungal pathogens in the native area, but little to no damage in the introduced area. Given the lack of *C. hirta* in forest understory in Costa Rica but abundance in many light environments in Hawaii, we predicted enemy exclusion to have significant effects on plant growth and survival in understory sites in native but not introduced areas, to have greater positive effects on growth and survival in understory than open habitats in the native range, and to have little or no effect in either habitat in the introduced range.

**METHODS**

**Study Species**

*Clidemia hirta* is a densely branching woody shrub that grows to a height of 2 – 3 m and occurs in mesic to wet areas from sea level to about 1500 m in both native and introduced ranges (Wester and Wood 1977). *Clidemia hirta* was first reported within the Hawaiian archipelago on Oahu in 1941 (Anonymous 1954) and spread in the early 1970s and 1980s to the islands of Kauai, Maui, Molokai, Lanai, and Hawaii (Smith 1992). It has been declared a noxious weed by the Hawaii Department of Agriculture, and since 1953 seven biological control agents have been introduced in an attempt to limit its spread (P. Conant, personal communication; Nakahara et al. 1992). At the time of our study, only *Liothrips urichi* Karny (Phlaeothripidae, a thrips which attacks terminal leaves and internodes), *Lius poseidon* Napp (Buprestidae, a leaf-mining beetle), and *Colletotrichum gloeosporioides* f.s. *clidemiae* (Melanconiaceae, a fungal pathogen of leaves)
were established on the island of Hawaii, where this experiment was conducted. *Liothrips urichi* was first introduced in Hawaii in the 1950s and has reduced *C. hirta* populations in open habitats, but apparently has not affected populations in forest understory because the thrips lay eggs preferentially in the open (Reimer and Beardsley 1989). *Lius poseidon*, although established, is not widespread on the island of Hawaii, whereas *Colletotrichum gloeosporioides f.s. clidemiae* purportedly is established on all five Islands (P. Conant, personal communication). Several non-native generalist insects introduced accidentally or to control other plants also feed on *C. hirta* in Hawaii. There are no reports of vertebrate herbivores of *C. hirta* in either its native or introduced range.

**Study Sites**

At each site in the native and introduced areas, we planted *C. hirta* in paired open and forest understory habitats. Most habitat pairs were within 100 m of each other, and all were within 400 m. Experimental sites in Costa Rica were located in the northeastern Caribbean lowlands at the La Selva Biological Station, El Bejuco Biological Station and adjoining pasture, and the Escuela de Agricultura de la Región Tropical Húmeda (EARTH) (Figure 4.1a). *Clidemia hirta* occurred naturally at all three sites: wild populations were noted adjacent to experimental plots at El Bejuco and La Selva, and within 1 km of the EARTH site. Understory sites at La Selva and EARTH were located in plantations of native and introduced trees. The plantations were not managed actively and understories of native plants were well developed at both sites. The understory site at El Bejuco was in secondary forest of at least 20 y. Open sites were abandoned pastures dominated by ferns at La Selva, and grass at El Bejuco and EARTH. Mean annual rainfall and temperature at La Selva are 4000 mm and 26°C (Sanford et al. 1994). Soils are ultimately of volcanic origin and are a mixture of ultisols and inceptisols.

Experimental sites in Hawaii were located on the windward side of the island of Hawaii at the Waiakea Forest Reserve, University of Hawaii at Manoa (UHM) Agricultural Station, and Malama Ki Forest Reserve and Agricultural Experiment Station (Figure 4.1b). Mean annual rainfall and temperature in Hilo are 3300 mm and 23°C. Soils at all Hawaiian sites are inceptisols. All understory sites were in lowland wet forest with native *Metrosideros polymorpha* Gaud. in the canopy. *Clidemia hirta* occurred at all three sites but was most common at Waiakea. Open environments at Waiakea and UHM were located under in a power line.
Figure 4.1 Location of the three field sites in (a) Costa Rica and (b) Hawaii.
right-of-way dominated by the pantropical fern, *Dicranopteris linearis* (Burm.f.) Underw., and non-native trees, shrubs, and grasses. The Malama Ki open site was periodically mown and dominated by non-native grasses.

**Experimental Plants**

In Costa Rica, cuttings were taken from plants of four *C. hirta* populations in tree plantations, roadsides, and pastures. Collected material was trimmed to three nodes and a single small leaf or half of a large leaf and dipped in Daconil fungicide (active ingredient: chlorothalonil; ISK Biotech Corp., FL, USA). The first node was dipped in rooting hormone (Dip’N Grow 1.5 SL, Astoria Pacific, Inc. Clackamas, OR) and the cutting placed in sand on a mist-bench under neutral-density shade cloth and clear plastic (15% incident radiation). After two weeks, rooted cuttings were transplanted to individual bags filled with alluvial soil and grown for two weeks. Plants were 3.5 – 35.5 cm in total stem length when they were planted bare root at the three sites in May 1999.

In Hawaii, small seedlings were collected from three populations along roadsides and in tree plantations on the eastern side of the island of Hawaii. We used seedlings rather than cuttings because cuttings proved difficult to root in Hawaii. Plants were kept in a solution of Vita Start (Lily Miller, Clackamas, OR) for two days to reduce transplant shock. Seedlings were planted in individual bags filled with Promix BX, which is a mixture of sphagnum moss, Vermiculite, and Perlite (Premier Brands, Inc., Stamford, CT). The plants were kept on a mist-bench within a greenhouse for two weeks. Seedlings were 6.5 – 33.0 cm when outplanted in August 1999 with Promix included. By the end of the experiment, roots had grown beyond the potting mixture.

**Exclusion of Natural Enemies**

We randomly assigned 624 plants each in Costa Rica and Hawaii to sites, habitat within each site (understory or open), and treatments within light level (control, fungicide, insecticide, or dual application). Twenty-six seedlings or cuttings were planted as samples of each of the four treatments within each habitat (104 plants per habitat per site). There were no initial differences in total stem length among treatments, habitat, or sites within each area (data not shown). Plants were positioned 1.5 to 2 m apart. In the open, above-ground vegetation was cleared prior to planting and periodically mowed or clipped around experimental plants.
throughout the experiment to control for the effect of above-ground competition and to ensure that plants received similar light levels. Vegetation in the understory was not manipulated except that fallen leaves and branches were removed if they were touching experimental plants.

Pesticides were used to exclude fungal pathogens and insect herbivores. Specific formulations of the pesticides differed in Costa Rica and Hawaii, but the same concentration of active ingredient was used in each area. Fungal pathogens were excluded by spraying plants with a 1.25% solution of the systemic fungicide benomyl [Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] (Costa Rica: Benomil 50 WP, Helm AG, Hamburg, Germany; Hawaii: Benlate 50 WP, DuPont, Wilmington, DE, USA). Our insecticide treatment contained a mixture of the systemic chloronicotinyl insecticide imidacloroprid (1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) to control sucking insects such as thrips and the synthetic, contact pyrethroid insecticide cyfluthrin (cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethycyclopropane carboxylate) to control chewing insects such as lepidopteran larvae. Percent active ingredient after dilution was 17.4% for the imidacloroprid and 0.00375% for cyfluthrin. In Costa Rica these two chemicals were formulated as Confidor 70 WG and Baythroid 2.5 EC (Bayer AG, Leverkusen, Germany), respectively. In Hawaii they were Provado 1.6 F and Tempo 20 WP (Bayer, Kansas City, MO, USA). We attempted to exclude both insect herbivores and fungal pathogens (hereafter called “dual application”) by applying a mixture of the fungicide and insecticides. Control plants were sprayed with water.

Individual plants were sprayed with one of the four treatments using a hand sprayer approximately every 2 wk. Stems and leaves were sprayed to the drip point so the total volume of solution applied was proportional to the size of the plant. Pesticide application was initiated 2 wk after planting in Costa Rica and 1 mo after planting in Hawaii. In December 1999 in Costa Rica, heavy rains occurred daily, and pesticides were not applied during that month. Care was taken to avoid spraying the ground because the fungicide has been shown to inhibit functioning of arbuscular mycorrhiza when used as a soil drench (Pedersen and Sylvia 1997). Translocation of benomyl from leaves to the active site of mycorrhizal infection in the roots has been found to be minimal (Larsen et al. 1996, Pedersen and Sylvia 1997).

Some pesticides are phytotoxic under certain conditions, while others may stimulate growth through the addition of nutrients such as nitrogen (e.g., Brown et al. 1987, Paul et al. 1989, Root 1996). We assume the pesticides did not directly affect C. hirta growth in Costa Rica.
or Hawaii. This assumption was supported for the effect of Benlate fungicide, whose active ingredient benomyl contains four molecules of nitrogen, in a test using Hawaiian seedlings in a greenhouse study. We found that relative growth rates (total stem length) of *C. hirta* seedlings did not differ if Benlate or water only were sprayed to the drip point every 2 wk for 8 mo ($F_{1,17} = 0.75, P = 0.40$). The cyfluthrin and imidacloprid insecticides, with one and five nitrogen molecules respectively, also likely had little effect on plant growth or survival as the amount of nitrogen applied was small and there were no differences in growth rates between plants sprayed with these insecticides and control plants (see results under Results: Effects of Natural Enemy Exclusion on Survival and Growth). In addition, previous studies have shown that Costa Rican seedlings of related taxa are not limited by nitrogen availability (Vitousek and Denslow 1986, Denslow et al. 1998).

**Measurements**

Total stem length was measured on all plants at the time of initial planting. Mortality during the first 2 mo was attributed to transplant shock and those plants were excluded from all analyses. Costa Rican plants were measured and harvested after 450 d. Hawaiian plants in the open sites were harvested approximately 300 d after planting because some individuals had become reproductive and removal of reproductive plants was a condition of our research permits. We continued the experiment with understory plants in Hawaii for 410 d because these plants did not become reproductive. Growth rates and survival patterns in understory plants in Hawaii were similar at 300 and 410 d (data not shown); therefore harvest data only are presented.

At harvest, total stem length was measured on a randomly selected subset of plants in the open ($N = 5 – 8$ plants per treatment per site) and all plants in the understory. Relative growth rate (RGR) was calculated as $(\ln(L_1) - \ln(L_0))/t$, in which $L_0$ and $L_1$ were the total cumulative stem lengths in cm at time of planting and harvest, respectively, and $t$ is the number of days between measurements (Evans 1972). RGR reflects the net increase due to growth or decrease in total stem length due to loss to herbivores, pathogens, or damage from falling debris.

Herbivory was assessed as proportion of leaf area missing measured at harvest. Following Coley and Barone (1996), we use the term “herbivory” to include damage due to both insects and pathogens. We did not follow individual leaves and therefore cannot estimate leaf loss. Estimates of leaf damage are therefore conservative (Lowman 1984, Filip et al. 1995) and reflect only the activity of chewing insects and fungal lesions. At harvest, leaf area and leaf area
missing were determined using a LI-COR 3100 leaf area meter (LI-COR Inc., Lincoln, NE). Proportion of leaf area missing was calculated from the difference between actual leaf area and total leaf area when holes were covered with opaque tape. Necrotic tissue was also treated as leaf area missing and was removed prior to measuring the actual leaf area. For open habitats, total leaf area and leaf area missing were measured on 20 randomly chosen leaves for 5 - 8 plants per treatment. For understory habitats, leaf area was determined for all plants alive at harvest at El Bejuco and La Selva, and 12 plants per treatment at EARTH and at the three Hawaiian sites. We also examined plants for insects such as thrips, stemborers, gallmakers, and leafrollers that caused damage not quantified by leaf area missing.

The objective of the experiment was to examine relative effects of reduction in pest load in Costa Rica and Hawaii, not to account for differences in growth rates between the two areas. Several factors that may affect growth rates likely differed among sites and areas. The source of plants (cutting or seedling), soil fertility, climate, and forest structure differed between the two areas. We therefore limit our analyses to differences among treatments within areas. Nevertheless, we think the habitats were relatively similar between the sites in Costa Rica and Hawaii for two reasons. First, we evaluated understory light environments by measuring photon flux density (PFD) above > 30 plants at each site with quantum sensors (LI-190SA; LI-COR Inc., Lincoln, NE) mounted on a LAI-2000 canopy analyzer operated in the two-instrument mode. Above-canopy PFD was estimated from measurements taken in a nearby clearing. Mean percent diffuse transmittance (PFD understory/PFD clearing x 100) did not differ significantly between Hawaiian (2.6%) and Costa Rican sites (2.7%; $F_{1,4} = 0.02, P = 0.89$). (Percent transmittance was natural log-transformed for analysis to normalize residuals.) Thus, we expect that the difference between understory and open light levels did not differ significantly between the two areas. Second, we found indirect evidence that habitats were similar in that cutting and seedling responses to habitat differences were comparable. Relative growth rates of plants that survived to the final harvest were similar for both open and understory habitats between Costa Rica and Hawaii (understory: $F_{1,244} = 0.02, P = 0.89$; open: $F_{1,171} = 0.53, P = 0.47$). This may also indicate that the choice of cuttings or seedling did not affect the outcome of the experiment.

Data Analysis

All statistical analyses were conducted in SAS Version 8 (SAS Institute 2000). We examined the effects of habitat and natural enemies on survival, growth, and leaf area missing
separately within each area because of the methodological differences between each area. The design was a split-plot with a completely randomized design at the within plot (habitat) level. ANOVA tests were performed using PROC MIXED with site and site x habitat treated as random effects and pesticide treatment as a fixed effect. Percent survival per treatment per habitat per site was calculated and analyzed as a continuous variable with \( N = 3 \) sites per habitat within each area. Differences among pesticide treatments were analyzed as a full factorial of application or no application of fungicide or insecticide. For example, we examined the effect of insecticide by comparing treatments in which insecticide was applied (insecticide and dual (insecticide + fungicide)) vs. not applied (fungicide and control). Fungicide was examined similarly. We looked for synergistic effects with the interaction term of fungicide x insecticide. Proportion of plants with galls per pesticide treatment was analyzed with PROC GENMOD using a binomial distribution and logit link function; variation among sites was not examined in that analysis. Percent leaf area missing was square-root transformed to normalize residuals.

RESULTS

Effects of Natural Enemy Exclusion on Survival and Growth

In Costa Rica, survival of plants differed markedly between open and understory habitats and among treatments. Percent survival to harvest was significantly lower in the understory (44.7%) than in the open in Costa Rica (91.3%; Table 4.1). Application of insecticide and fungicide significantly affected survivorship of \( C. \) hirta depending on the habitat (Table 4.1). Both insecticide and fungicide showed a significant, positive effect on survivorship in the understory but not open (Figure 4.2a, b). Neither the second- or third-order interaction term of insecticide x fungicide or habitat x insecticide x fungicide was significant for survivorship in either habitat, suggesting that the effect of one type of pesticide did not differ depending on whether the other pesticide was added or not (Table 4.1). The effects of each pesticide treatment, therefore, were additive in the understory in Costa Rica. In relation to control plants in the understory in Costa Rica, \( C. \) hirta survival increased by 12% if sprayed with insecticide, 19% with fungicide, and 41% with dual application. Thus, both insect herbivores and fungal pathogens restricted \( C. \) hirta survival in one area of its native range, but only in understory habitats.
Fungal pathogens also had an effect on relative growth rates of plants that survived to harvest in Costa Rica (Figure 4.3a, c). Fungicide application had an overall positive effect on RGR, but insecticide application had no effect on RGR in either habitat (Table 4.1).

In Hawaii, *C. hirta* survival in both open and understory sites was high (Figure 4.2b). We found no difference in percent survival between open (98.7%) and understory sites (99.2%; Table 4.1). Pesticide application had no effect on survival or RGR of plants in either habitat (Figure 4.2b; Figure 4.3b, d).

### Natural Enemy Damage

We found that natural enemy damage to leaves differed between areas of *C. hirta*’s native range, Costa Rica, and introduced range, Hawaii. At harvest percent of leaf area missing on
control plants that lived to harvest was four to six times greater in Costa Rica than in Hawaii in the understory (4.6% vs. 1.1%) and open habitats (5.1% vs. 0.8%; Figure 4.4). Leaf damage levels did not differ between understory and open-grown plants in Costa Rica or Hawaii (Table 4.1).

Herbivore damage in Costa Rica was attributed to gall-makers, stemborers, leafrolling moth larvae, and leaf-sucking curculionid weevils. Other leaf chewing damage was evident, but the insects responsible could not be determined. The galls were thought to be formed by cecidomyiid fly larvae (P. Hanson, personal communication). At least one species of leafroller was found at all sites and was identified as a microlepidoptera (Compsolechia sp., Gelechiidae; D. Wagner and R. Hodges, personal communication). The type of herbivory differed dramatically but not consistently between paired open and understory habitats. For example, most plants in the EARTH understory had galls, whereas only 2% had galls in the open. In contrast, at el Bejuco none had galls in the understory, but most plants had galls in the open.

Despite frequent application of two types of insecticides, neither leaf chewers nor other herbivores were completely excluded in Costa Rica. By harvest, considerable leaf area was missing even on plants sprayed with insecticide (Figure 4.4a). Mean percent leaf area missing

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**Figure 4.2 Survival of Clidemia hirta plants to harvest in understory and open habitats in (a) Costa Rica and (b) Hawaii in four natural enemy exclusion treatments. Survivorship was higher in Costa Rican understory sites when plants were sprayed either with fungicide ($F_{1,6} = 17.1$, $P = 0.01$) or insecticide ($F_{1,6} = 8.7$, $P = 0.03$). No effect of either type of pesticide was found in the open or in either habitat of Hawaii. LSMeans + 1 S. E. are shown.**
Figure 4.3 Relative total stem growth rates of *Clidemia hirta* in understory and open habitats in Costa Rica and Hawaii in four natural enemy exclusion treatments. Fungicide application significantly increased growth in Costa Rica. LSMeans ± 1 S. E. are shown.

did not differ significantly among plants in the insecticide vs. non-insecticide treatments in Costa Rica in the open or understory (Table 4.1). Leaf rollers also appeared undeterred by insecticide application. Among plants in open habitats with rolled leaves, there was a significantly higher proportion of leaves rolled at harvest among plants sprayed with insecticide than among those not sprayed with insecticide ($F_{1,89} = 9.26$, $P < 0.01$). We were unable to estimate the damage caused by the galls and stemborers, but we observed that both promoted branch death.

Probability of gall presence was significantly lower for open-grown plants that received insecticide ($\chi^2 = 4.6$, $P = 0.03$) but not for plants in the understory ($\chi^2 = 1.2$, $P = 0.28$).
What little herbivory was found on plants in Hawaii could be attributed to introduced insects. In open habitats, *Liothrips urichi*, an introduced biological control agent, was found on 10 out of 312 plants and caused some damage. A non-native generalist moth, the Mexican leafroller *Amorbia emigratella* Busck, caused substantial damage, but only on a few plants at one site. We found no insects or insect damage on plants in Hawaiian understory sites. Instead, falling debris likely caused most of the 1% of leaf area missing on control plants. However, in Hawaii, insecticide application significantly decreased the amount of leaf area missing in both habitats (Figure 4.4b; Table 4.1). Fungicide had no effect on proportion of leaf area missing in either habitat.

**DISCUSSION**

**Evidence for the Enemy Release Hypothesis**

We found that that pest loads are greater in native than introduced areas of *C. hirta*’s range and that these natural enemies may limit the abundance and habitat distribution of *C. hirta* in its native range. Regardless of habitat, natural enemies that attacked *C. hirta* caused greater leaf area loss in Costa Rica than Hawaii, with missing leaf area four to six times greater in Costa
 Rica than in Hawaii. Galls and stem borers caused further, and potentially more detrimental, damage on plants in the native Costa Rican sites. In contrast, plants in Hawaii sustained very little damage. The fitness effects of natural enemies in the native range were apparent as well because application of insecticide or fungicide significantly increased survivorship in the understory in Costa Rica and fungicide application increased relative growth rates. In contrast, exclusion had no effect on survivorship or growth in Hawaii, where regardless of treatment, almost 100% of plants survived. These results are consistent with the enemy release hypothesis.

We also found strong experimental support for the habitat-specific prediction that natural enemies have greater effects on survival in understory than open habitats in the native range and have no effect in either habitat in the introduced range. In the native sites, enemy exclusion increased survivorship in the understory but not in the open, whereas no difference in survivorship was found in either light environment in the introduced range. The lack of an effect of the fungicide on growth or survival of plants in Hawaii suggests that the introduced biological control fungal pathogen, *Colletotrichum gleosporiodes* f.s. *clidemiae*, does not have an effect on *C. hirta* in Hawaii. Benomyl, the active ingredient in the fungicide treatment, has been shown to be effective against the fungal pathogen *Colletotrichum gloeosporioides* on other plants (Childers 1992, Elmer et al. 2001).

Our results suggest that enemy release may contribute to the success of *C. hirta* in Hawaii, but does not preclude the effects of other factors on its spread. Suitable climate and adequate resource availability are certainly prerequisites for invasion. Higher resource availability (Denslow 2003), dispersal or recruitment limitation (Tilman 1997, Hubbell 2001), or the appearance of more vigorous genotypes (Blossey and Nötzold 1995, Blossey and Kamil 1996) in introduced than native areas may also account for differences in abundance and habitat distribution.

Although use of biological control agents to control invasive plants has demonstrated that a degree of population control often can be obtained by herbivores in the introduced range of plants (Huffaker and Kennett 1959, McEvoy et al. 1991, Nötzold et al. 1998), our study shows that these species also can be controlled by their pests in their native range. Furthermore, differences in the impacts of pest exclusion in shaded and sunny habitats in *C. hirta*’s native range suggest that natural enemies are particularly effective at excluding *C. hirta* from low light environments. Interestingly, our study also suggests that introduction of pests from our study
areas in Costa Rica would not reduce survival of *C. hirta* in high light environments in Hawaii because we found no evidence that natural enemies affected survival in open sites. Fungal pathogens apparently decrease growth in both open and understory habitats. However, had we conducted this study only in open areas, we would have found no support for the hypothesis that natural enemies limit survival of this species in its native range. However, we did not examine the effects of competition in our study, and natural enemies could maintain low population sizes of *C. hirta* in open areas of Costa Rica by reducing its growth rate enough to be overtopped by competing vegetation. Below-ground herbivores and pathogens also may have negative effects on fitness (Blossey 1993, Maron 1998). In addition, insect herbivores and pathogens may be released from their own natural enemies, such as parasites and parasitoids, and have unforeseen effects when introduced outside their native ranges.

Several other studies have found evidence that natural enemies are either more abundant or have greater effects on demographic parameters on plants in their native than in their invasive ranges. Fenner and Lee (2001) examined flowerheads of 13 species of Asteraceae in Britain where they are native and in New Zealand where they have become naturalized and found that seed-eating insect larvae infected six times more flowerheads in Britain than New Zealand. Two studies of *Cytisus scoparius* (L.) Link (Fabaceae), one from the native range and one from the introduced range conducted almost 20 y later, found results similar to ours. Adults sprayed regularly with an insecticide in Europe, where *C. scoparius* is native, had higher survivorship and growth than unsprayed plants (Waloff and Richards 1977), suggesting that chronic insect herbivory may contribute to smaller population densities where the species is native. In California, where this species was introduced, Bossard and Rejmánek (1994) also sprayed plants with insecticide but detected no effect on growth. The number of phytophagous species found on plants in California was lower than in sites within the native range (Bossard and Rejmánek 1994). Interestingly, another study of this species in its native range detected no impact of invertebrates or pathogens on seedling survival, growth, or minimum age of reproduction (Paynter et al. 1998). Natural enemies therefore may affect only some life history stages, may vary in impact across the native and introduced ranges, or may have episodic rather than chronic impacts (e.g., Carson and Root 2000).

In our study system, the absence of native Melastomataceae makes it unlikely that specialist natural enemies native to Hawaii could limit *C. hirta* growth, survival, or habitat
distribution (i.e., there are few biotic barriers to naturalization; Mack 1996). Fenner and Lee (2001) hypothesized that the low incidence of colonization of non-native Asteraceae by insects in New Zealand could be attributed to the relatively recent introduction of the plants (100-200 y) or the lack of closely related native flora. *Clidemia hirta* is also a relatively recent introduction to the Hawaiian archipelago (70 y) and is even more recent on the island of Hawaii (30 y) where our study was conducted (Smith 1992). Host switching by generalist herbivores may not yet have occurred to deter establishment. On Silhouette, the primary island of the Seychelles where there are native Melastomataceae, an endemic generalist cricket, *Pelerinus rostratus*, was found feeding on *Clidemia hirta* within 10 y of its introduction (C. Awmack, unpublished data). It would be valuable to assess the impact of herbivores on *C. hirta* where it is invasive in the presence of native Melastomataceae (e.g., Malaysia, India, or Indonesia) to determine how the role of enemy release in invasion success differs across its introduced range. We expect herbivore and pathogen impacts to be higher where there are closely related species. In addition, areas with high plant species diversity also have a more diverse herbivore assemblage, increasing the probability of host-switching to an exotic species (Crawley 1983, Prieur-Richard et al. 2002).

To reduce the impacts of *C. hirta* in forested environments in its invasive range, biological control agents active in shaded humid conditions should be sought. However, surveys of natural enemies of *C. hirta* where it occurs naturally, i.e. open disturbed habitats such as roadsides and pastures in its native range, may not yield the biocontrol agents effective across the habitat range where it is invasive. Where exclusion by natural enemies has been most complete, the effective agents may be detected only by use of transplants and experimental pest exclusions. *Clidemia hirta* is not a special case in this regard. Habitat expansion is a phenomenon found for other woody tropical species in their introduced range (Chapter 1). The apparent success of the biological control agent *Liothrips urichi* in reducing *C. hirta* populations in open areas in Fiji and Hawaii (Reimer and Beardsley 1989) suggests that control of woody pioneers such as *C. hirta* in both open and understory habitats may require introduction of a suite of herbivores or pathogens, perhaps taken from different parts of the species’ native range and evaluated on outplantings in a variety of habitats.

**Natural Enemies as Determinants of Habitat Distribution**

There are at least two mechanisms by which herbivory or fungal damage may restrict the habitat distribution of *C. hirta* where it is native. First, spatial variation in extent of damage
could drive habitat distribution. Habitat and host plant selection by some herbivores is affected by variation in temperature, irradiation, and humidity among open and shady light environments (Sipura and Tahvanainen 2000). Preference for particular light environments has been found for insect herbivores of plants in their native range (Collinge and Louda 1988, Louda and Rodman 1996, Sipura and Tahvanainen 2000) as well as for particular biological control agents released against species in introduced areas (Huffaker and Kennett 1959, Harper 1969, Reimer and Beardsley 1989). Restriction of Hypericum perforatum L. to shaded areas in California where it is an exotic pest was attributed to significant consumption of leaves by a biological control agent in open but not shaded areas (Huffaker and Kennett 1959). Individual herbivore species may have light or habitat preferences, but the net effect of all the natural enemies of a given plant species is what may affect habitat distribution. In this study, we found no evidence for differences in habitat preference among the suite of natural enemies of C. hirta in Costa Rica. We found similar one-time estimates of leaf area missing between C. hirta in open and understory habitats, and the occurrence of galls and weevils did not differ systematically between habitats. These results differ from those of Denslow et al. (1990) who found that Miconia (Melastomataceae) and Piper (Piperaceae) cuttings transplanted into understory had higher percent leaf area missing than those planted into gaps in Costa Rica. In our study, the suite of herbivores (such as galls and weevils) varied, even among relatively nearby sites. Although the native range of C. hirta extends from Mexico to Bolivia, the insect and pathogen ranges may be more limited, and species and densities of herbivores and pathogens likely vary throughout C. hirta’s native range. Indeed, none of the moth, thrips, or leaf mining beetle species introduced as biological control agents in Hawaii and Fiji from other parts of the native range were found on experimental plants in Costa Rica. It is therefore unlikely that spatial variation in natural enemy habitat preferences drives the observed light-related habitat distribution of C. hirta throughout its native range.

Second, the consequences of herbivory and pathogen attack may differ in shaded and open habitats. In the understory, carbon assimilation rates may be too low to replace tissue or photosynthate lost to herbivores and pathogens. Natural enemies may not reduce growth or survival appreciably in open areas if plants growing in high light can compensate for high rates of leaf area loss (Whitham et al. 1991). In a greenhouse study, Anten and Ackerly (2001) found that photosynthetic rates of an understory palm grown at high light levels increased sufficiently
to compensate for the loss of leaf area when defoliated. In contrast, individuals grown in low light were unable to compensate for the loss in photosynthetic area. This seems to be the case with *C. hirta* as well.

Physiologically, *C. hirta* appears relatively shade-tolerant; it occurs in understory in Hawaii and elsewhere in its introduced range and had high survival and relative growth rates under low light levels in a greenhouse study in Hawaii (Baruch et al. 2000). In the presence of natural enemies, however, it is *effectively* shade intolerant. Genetic differences in shade tolerance between native and introduced genotypes do not seem to explain the changed habitat distribution of this invasive shrub between Costa Rica and Hawaii (Chapter 3). Thus, this study provides some of the first experimental evidence that natural enemy regulation can be an important factor in plant species’ distributions at both geographic and local scales. A characteristic of tropical forest invaders unacknowledged heretofore could be exclusion from forest shade in the native range.

**LITERATURE CITED**


Chapter 5
Potential Impacts of Biological Control on Population Dynamics in Hawaiian Rainforests: A Demographic Approach

INTRODUCTION

The high environmental and economic costs of non-native, invasive plant species are increasingly apparent (Parker et al. 1999, Pimentel et al. 2000), but the methods most effective for control of these species are often far from obvious (McEvoy and Coombs 1999). Biological control reduces the abundance of invasive plants by using natural enemies (herbivores, nematodes, and pathogens) to reduce seed production, stunt growth, or kill plants outright. In classical biological control non-native natural enemies are introduced to effect these changes. Biological control agents have been notably successful in controlling some plant species (e.g., *Senecio jacobea* in the northwestern United States (McEvoy et al. 1991), *Opuntia ficus-indica* in South Africa (Zimmermann and Moran 1982), and *Hypericum perforatum* in California (Huffaker and Kennett 1959), and these are seen as powerful tools for invasive species management (e.g., Huffaker and Kennett 1959, Huffaker et al. 1983, McEvoy et al. 1991).

However, others have raised concerns about the risk exotic biological control agents pose to non-target species (Howarth 1991, Simberloff and Stiling 1996a, Simberloff and Stiling 1996b, Louda et al. 1997, Thomas and Willis 1998), especially the introduction of many insects or pathogens against one target species (Thomas and Willis 1998). There is interest in methods to determine the minimum number of agents that will control a given target species and to estimate which among several potential agents is likely to cause the greatest decline in population growth rates of target species (McEvoy and Coombs 1999).

Stage-structured matrix projection models can be used to simulate the effects of potential control agents. These models use probabilities of survival, growth, and retrogression as well as the fecundity of each life history stage to estimate the asymptotic population growth rate, stable stage distribution, and stage-specific reproductive values under particular environmental
circumstances (Caswell 2001). Effects of differences in survival, growth, or reproduction in time or space can be modeled easily with these calculated variables. Perhaps the most important aspect of matrix model analysis with respect to invasive species management is elasticity analysis, which projects the relative contribution of each matrix element (survival, growth, or reproduction) to the population growth rate under current conditions (de Kroon et al. 1986). In theory, biological control agents that affect stages or transitions with the largest elasticities will cause the largest decline in the population growth rate (Shea and Kelly 1998, McEvoy and Coombs 1999, de Kroon et al. 2000). Even large changes in elasticity parameters provide robust predictions for the direction and magnitude of the change in the population growth rate (de Kroon et al. 2000). Matrix models also can be used to project the consequences of changes in particular matrix elements (vital rates) on asymptotic population growth rate in different environments or time periods (Shea and Kelly 1998, McEvoy and Coombs 1999, Parker 2000).

Matrix population models focus on asymptotic growth rates under constant conditions and generally do not incorporate changing vital rates such as result from density dependence (Bierzychudek 1999, Fox and Gurevitch 2000). However, most environments are dynamic, populations rarely approach stable stage distributions, and invasive species often occur at high densities. Thus, matrix models are heuristic tools to project, but not predict, long-term population dynamics (Caswell 2001) and to guide control strategies of invasive species (Parker 2000).

The goal of this study was to evaluate the potential of different types of biological control agents on the population dynamics of a non-native, invasive tropical shrub, *Clidemia hirta* (L.) D. Don (Melastomataceae). This species is native to Central and South America and the Caribbean Islands where it is a minor weed of disturbed areas in mesic to wet environments from sea level to 1,500 m elevation (Wester and Wood 1977). Though absent in forest understory in its native range, the species was introduced and has become an aggressive invader of forest understory on tropical islands, such as Hawaii, American Samoa, Fiji, Mauritius, Madagascar, Seychelles, Borneo, and Sri Lanka, and in continental areas, such as Peninsular Malaysia and Tanzania (Lever 1937, Wester and Wood 1977, Gerlach 1993, Sheil 1994, Strahm 1999, Singhakumara et al. 2000, Peters 2001). It is a densely branching, slightly woody shrub that grows to a maximum of 2 – 3 m in height in its introduced range. Although it does not grow clonally, it is a vigorous resprouter and may root along fallen stems. The fruits are pulpy, dark-
blue berries that contain hundreds of seeds, each about 0.5 mm in diameter. Flowering and fruiting may occur throughout the year. *Clidemia hirta* is listed on the Hawaii State Noxious Weed list (Division of Plant Industry 1992). Several biological control agents have been introduced to Fiji and Hawaii against *C. hirta* (Simmonds 1933, 1937, Nakahara et al. 1992, Smith 1992), but their impact on population dynamics in forest understory has received little attention. Insect herbivores and fungal pathogens cause high mortality of this species in forest understory in its native range, suggesting that biological control agents could provide effective control of this species in its introduced range (Chapter 4).

Using Lefkovitch stage-structured projection matrices, I asked three questions: (1) How does the asymptotic population growth rate vary among years and locations in Hawaii? (2) What is the relative importance of different life cycle transitions to the population growth rate at each site in each year? and (3) What are the potential effects of hypothetical biological control agents that affect different parts of the life cycle? To address these questions, I tagged and measured plants in four censuses of a *C. hirta* population representing recently invaded forest with a relatively open canopy (Laupahoehoe) and another population representing less-recently invaded forest with dense canopy (Waiakea).

**METHODS**

**Study Sites**

In 1998, I established permanent plots at Waiakea Forest Reserve and Laupahoehoe Forest Reserve on the windward side of the island of Hawaii. In both sites, forests are dominated by the native canopy tree *Metrosideros polymorpha* (Myrtaceae) but are heavily invaded by non-native shrubs and trees, such as *Psidium cattleianum* and *Melastoma candidum* at Waiakea and *P. cattleianum* and *Buddleia asiatica* at Laupahoehoe. *Clidemia hirta* likely has been present at Waiakea for at least 15 years, but it was rare at the Laupahoehoe site in 1988 (DOFAW Natural Areas Reserve survey, B. Stormont, personal communication). Mean annual rainfall is ca. 3500 mm at Waiakea (250 m a.s.l.) and Laupahoehoe (790 m a.s.l.) (Giambelluca et al. 1986). Relatively nutrient poor soils at Waiakea are derived from a recent (750 – 1500 y BP) Mauna Loa Volcano lava flow, whereas the relatively nutrient-rich soils at Laupahoehoe are derived
from a more highly weathered Mauna Kea flow (5,000 – 10,000 y BP) (Crews et al. 1995, Wolfe and Morris 1996).

At each site, I established a plot in an area heavily invaded by *C. hirta*. Approximately 300 plants were marked at each site; therefore, plots were 20 m x 100 m at Laupahoehoe and 10 x 20 m at Waiakea. Plants were censused in June or July of 1998, 1999, 2000, and 2001. Over the course of the four years of the study, I marked and followed 2906 plants at Laupahoehoe and 600 plants at Waiakea.

**Stage Classification**

Stages were assigned using life history characteristics and estimated plant biomass (Figure 5.1). Only plants ≥ 4.5 cm were tagged and followed. Independently rooted ramets for which connections to larger individuals were obvious were measured separately but were treated as part of the larger individual. Above-ground biomass for each individual was estimated from plant height and basal stem diameter with a regression equation developed from a sample of plants harvested outside of the two plots (*N* = 22 for Waiakea and 35 for Laupahoehoe). The plants were cut at the soil surface, dried, and weighed. The resulting equation was $Y = \exp(1.08 + 2.27\ln(\text{diameter})+0.706\ln(\text{height}))$, where $Y = \text{estimated biomass in g} (R^2 = 0.98, P < 0.001)$. Biomass ranges for each stage were seedling (< 10 g), small adult (10–50 g), medium adult (50–100 g), large adult (100–200 g), and extra-large adult (> 200 g). No seedlings larger than 10 g were found as new recruits in any year. I also assumed that no adults could regress to be a seedling because elements in that row of the matrix were reserved for fecundity estimates. There were only three instances when adults had shrunk this substantially. All adults were potentially reproductive (see below).

Fecundity was calculated as the mean number of seedlings per adult that recruited into the population during the growth year. Calculations follow those of Valverde and Silvertown (1998). First, I estimated the number of fruits a plant of biomass $b$ in year $t$ would produce before year $t + 1$ using equations specific to the site:

fruits/year (Laupahoehoe) = $-77.09 + 5.79b$ ($R^2 = 0.79, N = 95, P < 0.001$),

fruits/year (Waiakea) = $-8.868 + 0.977b$ ($R^2 = 0.71, N = 163, P < 0.001$).

These equations were developed from a subset of plants monitored monthly for ripe fruit in each population between June 1998 and June 1999. *Clidemia hirta* fruit in Hawaii generally contain between 300 and 500 seeds (mean ± SD: 412 ± 65; $N = 10$). Thus, I estimated the total number
of seeds produced per plant by multiplying the number of fruit per year by 412. Second, I summed the estimated number of seeds produced by all plants in each stage class and then divided by the estimated total number of seeds produced each year to calculate the proportional contribution of each plant in each adult stage to seed production in that year. Third, I multiplied this proportion by the total number of seedlings counted the following year to allocate to determine the number of recruits produced by each stage class. Finally, the mean fecundity per adult in each adult stage class was calculated by dividing the number of recruits produced by each stage class by the number of adults in each adult category at time \( t \).

**Stage Class Transitions**

The life history transitions of *C. hirta* plants are represented in its life-cycle graph (Figure 5.1) and matrix model structure (Table 5.1). The seed stage is not included in this life cycle because the transition probabilities of such small seeds are poorly known and were not examined in this study. Enforced dormancy in *C. hirta* does not occur, but seeds may live in the soil for more than one year (S. DeWalt, unpublished data). Thus, the transition from adult to seedling comprises multiple vital rates including germination, establishment, and growth to 4.5 cm in height.

I used Lefkovitch (stage-based) matrices (Lefkovitch 1965) to describe the demography of *C. hirta* populations. The projection matrix model has the form

\[
n_{(t+1)} = An_{(t)}
\]

where \( n_{(t)} \) and \( n_{(t+1)} \) are vectors of stage abundances at time \( t \) or \( t+1 \), and \( A \) is a matrix of elements, \( a_{ij} \), which represent transitions or contributions of individuals in stage \( j \) to stage \( i \) after one time step. Matrix entries are subdivided into fecundity (F, production of new seedlings), growth (G, transition to larger stages), and survival (S, persistence in the same stage or regression to smaller stages; Table 5.1). Plants occasionally shrank if they decreased in height or lost a ramet. Non-fecundity matrix elements above the main diagonal represent regression to smaller stage classes (Table 5.1). The dominant eigenvalue \( \lambda \) of \( A \) represents the asymptotic population growth rate. The left and right eigenvectors correspond to the stage-specific reproductive values and the stable stage distribution, respectively (Caswell 2001). The biological implications of the mathematical components of matrix models have been explored
Figure 5.1 Life-cycle graph of *Clidemia hirta*, showing all transitions observed in at least one of the two Hawaiian populations studied between 1998 and 2001. Circles represent plant stages, arrows represent transitions between stages, and letters correspond to the matrix entries in Table 5.1. Zero entries in Table 5.1 are not pictured in this graph.
elsewhere (Horvitz and Schemske 1995, de Kroon et al. 2000, Caswell 2001) and will not be described in great detail here.

Each population was censused four times, yielding a total of six transition matrices. For each matrix, I calculated the elasticity of every matrix element to $\lambda$. Elasticity analysis estimates the effect of a proportional change in vital rates on $\lambda$ and is used to assess the importance of different life stages and vital rates on population dynamics (Caswell 2001). I summarized the elasticity analyses in two ways, using the property that elasticities sum to unity. As in Horvitz and Schemske (1995) and Parker (2000), I calculated composite elasticities for the fate of each vegetative stage by summing elasticities for each column, excluding fecundity values. A composite elasticity for fecundity was also calculated. Second, I calculated the total elasticities of $\lambda$ to changes in elements involving fecundity (F), survival (stasis or retrogression, L), and growth (G) by summing appropriate matrix elements. These were plotted on a demography triangle as in Silvertown et al. (1993, 1996). MATLAB 6.0 (Mathworks 2001) was used to calculate eigenvalues, eigenvectors, and elasticities.

I estimated the standard error of $\lambda$ using series expansion (Caswell 2001). First-order approximation to the variance $V(\lambda)$ was calculated by summing the variances among the matrix elements. This method assumes small variances and no covariance among the elements (Caswell 2001), and thus serves only to approximate values of variance. The variance for each adult stage for fecundity was estimated from the variance each year in the number of new recruits attributed to each adult in each stage. The standard error ($\sigma$) was calculated as the square root of the variance and was used to approximate 95% confidence intervals ($\lambda \pm 2\sigma$).

Log-linear analyses were used to determine whether transition probabilities differed among populations or years. Initial state (stage), time period (1998-1999, 1999-2000, and 2000-2001, location (population), and fate (stage $\tau+1$; including “dead” as a stage) were the categorical variables in these analyses and are referred to as $S$, $T$, $L$, and $F$, respectively. The response variable was the observed frequency of tagged individuals per category. A constant of 0.5 was added to each cell (Fingleton 1984).

I examined several models to test whether time and/or location affected the fate of $C. hirta$ plants, given the initial state. The null model that fate is dependent on initial state but not on time or location is denoted as $FS, STL$. As in Caswell (2001), interaction terms, such as $STL$, imply that the all single-factor terms ($S$, $T$, $L$) and the lower-order interactions ($ST$, $SL$, $TL$) are
also present in the model. The significance of the effects of $T$ and $L$ were tested by examining differences in the goodness-of-fit statistic ($\Delta G^2$) between models that include the effects of $T$ or $L$ and the null model. The significance of an interaction between time and location on fate was examined by comparing the model $SLT$, $FST$, $FSL$ to the saturated model $FSTL$. I divided the $SLT$, $FSL$ model into a set of $FT$ tables, one for each location, to examine whether the fate of individuals differed among time periods for the Waiakea or Laupahoehoe populations. Additionally, I divided the $SLT$, $FST$ model into a set of $FL$ tables to examine the effect of location for each year. Finally, each $FST$ table was decomposed into a set of $FT$ tables, one for each initial state and location. $G^2$ statistics were calculated for each model using the GENMOD procedure with a Poisson distribution and log link in SAS Version 8 (SAS Institute 2000).

**Biological Control Simulations**

Since 1953, several biological control agents have been introduced in Hawaii to control *Clidemia hirta* (P. Conant, personal communication, Nakahara et al. 1992). Of those that became established, *Carposina bullata* Myrick (Carposinidae) feeds on flower buds and *Mompha trithalama* (Momphidae) feeds on flowers and berries (Nakahara et al. 1992). Leaf-feeding beetles and moths, terminal bud-attacking thrips, and a leaf spot fungus also have been introduced, but none have had significant effects on *C. hirta* survival in forest understory on the island of Hawaii (Chapter 4).

I conducted three analyses to determine what level of damage from such agents would be needed to cause a decline in *C. hirta* populations at Laupahoehoe and Waiakea. First, I investigated the potential impact of biological control agents that affect seed production or seedling establishment by reducing fecundity values for all adult stages by fixed proportions. Second, I evaluated the effect of a control agent that causes mortality only in seedlings by decreasing the survival rate of seedlings by fixed proportions. Third, I examined the impact of biological control agents that decrease survival rates of all vegetative plants by reducing all non-fecundity elements of the matrix by a fixed proportion. For all analyses, I manipulated values in the 2000-2001 matrices. The latter two analyses assumed that growth is unaffected by the control agents and therefore growth transitions were decreased only by the fixed proportion of plants killed.
RESULTS

Population Growth Rates

Both populations of *C. hirta* studied in Hawaii were growing. This fact is demonstrated by the increase in plot biomass (Figure 5.2a) and plant density (Figure 5.2b) in both populations, and asymptotic population growth rates ($\lambda$) much greater than one (except during the final year at Waiakea; Figure 5.3). The 95% confidence intervals for $\lambda$ did not include one for any year or location (Figure 5.3). Probabilities of survival were greater for larger than smaller plants in both populations (Table 5.2).

Initial and final densities and plot biomasses were lower for Laupahoehoe than Waiakea, but the population was growing more quickly (Figure 5.3). Confidence intervals for $\lambda$ for the two populations did not overlap in any time interval. The declining rate of accumulation of plants at Waiakea (Figure 5.2b) suggests that fewer plants were recruiting into the population. Indeed, the Waiakea population structure in 2001 shows a smaller proportion of seedlings than in previous years (Table 5.2). Individual plants at Laupahoehoe also were growing in size more quickly than at Waiakea. This is apparent in the non-zero probabilities of multi-stage growth transitions (e.g., from seedling to large adult in one year) where these stage transitions were zero at Waiakea.

The null hypothesis that initial state was sufficient for predicting fate of *C. hirta* plants was rejected (Table 5.3; SLT, SF model $P < 0.001$). The log-linear analysis of transition matrices for vegetative plants indicated that location and time also significantly affected the fate of plants (Table 5.3). In addition, fate and time were not independent for some initial states (Table 5.4). For Waiakea, the fate of small adults varied among years, while for Laupahoehoe, the fates of seedlings and medium adults varied (Table 5.4). There was also a significant time x location interaction ($T \times L$; Table 5.3) because the fate of plants differed significantly among years at Laupahoehoe ($G^2 = 101.1$, $df = 50$, $P < 0.001$) but not at Waiakea ($G^2 = 67.0$, $df = 50$, $P > 0.05$). Location had a significant effect on fate for all time intervals (1998-1999: $G^2 = 114.1$, 1999-2000: $G^2 = 84.5$, and 2000-2001: $G^2 = 228.5$; $df = 25$, $P < 0.001$ for all intervals). This analysis confirms that the Laupahoehoe population was growing more quickly than the Waiakea population each year and that fate of *C. hirta* in Hawaiian populations cannot be predicted from initial state alone. Spatial and temporal effects are important, particularly for the smaller plants
Figure 5.2 Increases in (a) estimated total biomass of *Clidemia hirta* and (b) density over the four years censused in the two Hawaiian populations.
Figure 5.3 Asymptotic population growth rates (+ 2 SE) for the Waiakea and Laupahoehoe populations for each time interval. Each population growth rate represents the dominant eigenvalue of a five-stage matrix model. The standard errors were estimated analytically by first-order approximation. The dashed horizontal line indicates a $\lambda$ of one, the value of a population that is neither growing nor declining.
Table 5.2 Population projection matrices and main demographic results of the matrix analysis for *C. hirta* populations in (a) Waiakea and (b) Laupahoehoe studied during 1998-1999, 1999-2000, and 2000-2001 time intervals. The first column shows λ for each population during each time interval.

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Seedling</th>
<th>Small adult</th>
<th>Medium adult</th>
<th>Large adult</th>
<th>Extra-Large adult</th>
<th>n +1 distribution</th>
<th>Stable stage distribution</th>
<th>Reproductive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Waiakea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998-1999</td>
<td><strong>0.804</strong></td>
<td><strong>0.560</strong></td>
<td><strong>1.982</strong></td>
<td><strong>4.295</strong></td>
<td><strong>6.101</strong></td>
<td>0.751</td>
<td>0.735</td>
<td>0.025</td>
</tr>
<tr>
<td>λ = 1.396</td>
<td>Small adult</td>
<td>0.140</td>
<td>0.653</td>
<td>0.176</td>
<td>0.000</td>
<td>0.000</td>
<td>0.162</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Medium adult</td>
<td>0.003</td>
<td>0.347</td>
<td>0.588</td>
<td>0.000</td>
<td>0.000</td>
<td>0.057</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>Large adult</td>
<td>0.000</td>
<td>0.000</td>
<td>0.235</td>
<td>0.667</td>
<td>0.000</td>
<td>0.020</td>
<td>0.022</td>
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<tr>
<td></td>
<td>Extra-Large adult</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.333</td>
<td><strong>1.000</strong></td>
<td>0.010</td>
<td>0.019</td>
</tr>
<tr>
<td>n</td>
<td>321</td>
<td>49</td>
<td>17</td>
<td>9</td>
<td>2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1999-2000</td>
<td><strong>0.749</strong></td>
<td><strong>0.178</strong></td>
<td><strong>0.633</strong></td>
<td><strong>1.364</strong></td>
<td><strong>2.473</strong></td>
<td>0.619</td>
<td>0.628</td>
<td>0.051</td>
</tr>
<tr>
<td>λ = 1.378</td>
<td>Small adult</td>
<td>0.219</td>
<td>0.450</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.216</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>Medium adult</td>
<td>0.005</td>
<td>0.400</td>
<td>0.286</td>
<td>0.000</td>
<td>0.000</td>
<td>0.078</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
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<td>0.000</td>
<td>0.150</td>
<td>0.714</td>
<td>0.500</td>
<td>0.000</td>
<td>0.068</td>
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</tr>
<tr>
<td></td>
<td>Extra-Large adult</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.500</td>
<td><strong>1.000</strong></td>
<td>0.018</td>
<td>0.095</td>
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<td>n</td>
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<td>80</td>
<td>28</td>
<td>10</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000-2001</td>
<td><strong>0.779</strong></td>
<td><strong>0.045</strong></td>
<td><strong>0.166</strong></td>
<td><strong>0.318</strong></td>
<td><strong>0.800</strong></td>
<td>0.526</td>
<td>0.412</td>
<td>0.073</td>
</tr>
<tr>
<td>λ = 1.119</td>
<td>Small adult</td>
<td>0.176</td>
<td>0.718</td>
<td>0.048</td>
<td>0.000</td>
<td>0.000</td>
<td>0.260</td>
<td>0.199</td>
</tr>
<tr>
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<td>Medium adult</td>
<td>0.003</td>
<td>0.256</td>
<td>0.643</td>
<td>0.081</td>
<td>0.100</td>
<td>0.111</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>Large adult</td>
<td>0.000</td>
<td>0.009</td>
<td>0.310</td>
<td>0.838</td>
<td>0.000</td>
<td>0.081</td>
<td>0.173</td>
</tr>
<tr>
<td></td>
<td>Extra-Large adult</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.081</td>
<td><strong>0.900</strong></td>
<td>0.022</td>
<td>0.064</td>
</tr>
<tr>
<td>n</td>
<td>335</td>
<td>117</td>
<td>42</td>
<td>37</td>
<td>10</td>
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<td></td>
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Table 5.2 continued

<table>
<thead>
<tr>
<th></th>
<th>Seedling</th>
<th>Small adult</th>
<th>Medium adult</th>
<th>Large adult</th>
<th>Extra-Large adult</th>
<th>n&lt;sub&gt;t+1&lt;/sub&gt; distribution</th>
<th>Stable stage distribution</th>
<th>Reproductive value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laupahoehoe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998-1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling</td>
<td>0.552</td>
<td>0.502</td>
<td>3.362</td>
<td>6.271</td>
<td>13.631</td>
<td>0.746</td>
<td>0.768</td>
<td>0.031</td>
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<tr>
<td>Small adult</td>
<td>0.238</td>
<td>0.162</td>
<td>0.043</td>
<td>0.000</td>
<td>0.000</td>
<td>0.096</td>
<td>0.096</td>
<td>0.116</td>
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<tr>
<td>Medium adult</td>
<td>0.049</td>
<td>0.405</td>
<td>0.087</td>
<td>0.000</td>
<td>0.000</td>
<td>0.045</td>
<td>0.045</td>
<td>0.207</td>
</tr>
<tr>
<td>Large adult</td>
<td>0.040</td>
<td>0.351</td>
<td>0.261</td>
<td>0.308</td>
<td>0.083</td>
<td>0.053</td>
<td>0.053</td>
<td>0.261</td>
</tr>
<tr>
<td>Extra-Large adult</td>
<td>0.000</td>
<td>0.081</td>
<td>0.609</td>
<td>0.692</td>
<td>0.917</td>
<td>0.059</td>
<td>0.059</td>
<td>0.385</td>
</tr>
<tr>
<td>n</td>
<td>223</td>
<td>37</td>
<td>23</td>
<td>13</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling</td>
<td>0.588</td>
<td>0.341</td>
<td>1.906</td>
<td>4.317</td>
<td>11.480</td>
<td>0.780</td>
<td>0.764</td>
<td>0.025</td>
</tr>
<tr>
<td>Small adult</td>
<td>0.185</td>
<td>0.300</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.089</td>
<td>0.101</td>
<td>0.114</td>
</tr>
<tr>
<td>Medium adult</td>
<td>0.022</td>
<td>0.400</td>
<td>0.250</td>
<td>0.030</td>
<td>0.000</td>
<td>0.036</td>
<td>0.036</td>
<td>0.179</td>
</tr>
<tr>
<td>Large adult</td>
<td>0.011</td>
<td>0.267</td>
<td>0.679</td>
<td>0.394</td>
<td>0.000</td>
<td>0.045</td>
<td>0.048</td>
<td>0.269</td>
</tr>
<tr>
<td>Extra-Large adult</td>
<td>0.000</td>
<td>0.017</td>
<td>0.071</td>
<td>0.576</td>
<td>1.000</td>
<td>0.050</td>
<td>0.046</td>
<td>0.413</td>
</tr>
<tr>
<td>n</td>
<td>464</td>
<td>60</td>
<td>28</td>
<td>33</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000-2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling</td>
<td>0.535</td>
<td>0.559</td>
<td>2.847</td>
<td>5.843</td>
<td>19.129</td>
<td>0.778</td>
<td>0.750</td>
<td>0.028</td>
</tr>
<tr>
<td>Small adult</td>
<td>0.293</td>
<td>0.183</td>
<td>0.024</td>
<td>0.000</td>
<td>0.000</td>
<td>0.106</td>
<td>0.114</td>
<td>0.102</td>
</tr>
<tr>
<td>Medium adult</td>
<td>0.068</td>
<td>0.327</td>
<td>0.214</td>
<td>0.038</td>
<td>0.017</td>
<td>0.040</td>
<td>0.047</td>
<td>0.159</td>
</tr>
<tr>
<td>Large adult</td>
<td>0.016</td>
<td>0.433</td>
<td>0.619</td>
<td>0.377</td>
<td>0.034</td>
<td>0.040</td>
<td>0.052</td>
<td>0.251</td>
</tr>
<tr>
<td>Extra-Large adult</td>
<td>0.000</td>
<td>0.048</td>
<td>0.143</td>
<td>0.585</td>
<td>0.932</td>
<td>0.036</td>
<td>0.036</td>
<td>0.460</td>
</tr>
<tr>
<td>n</td>
<td>914</td>
<td>104</td>
<td>42</td>
<td>53</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n<sub>t+1</sub> distribution denotes the observed proportions of plants in each stage in 1999, 2000, and 2001 for each population. n denotes the number of plants from which transition values were calculated. Diagonal matrix elements, representing stasis, are underlined and fecundity entries are in bold.
Table 5.3. Results of the log-linear analysis for the models built with the entire transition matrices from two *C. hirta* populations for the three time intervals. Frequency matrices that include dead as a fate and excluded fecundities were used for this analysis. The explanatory variables are $S =$ initial stage (seedling, small adult, medium adult, etc.), $L =$ location (Waiakea and Laupahoehoe), and $T =$ time interval (1998-1999, 1999-2000, and 2000-2001). The response variable is $F =$ fate (dead, seedling, small adult, etc.). The significance of each factor is analyzed by examining the reduction in the goodness-of-fit statistic $G^2$ when each factor is added to a model that excludes it ($\Delta G^2$).

<table>
<thead>
<tr>
<th>Model</th>
<th>Effect</th>
<th>df</th>
<th>$G^2$</th>
<th>$\Delta G^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$SLT, FS$</td>
<td></td>
<td>125</td>
<td>538.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>$SLT, FST$</td>
<td></td>
<td>75</td>
<td>427.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>$T$</td>
<td></td>
<td>50</td>
<td>111.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>$SLT, FSL$</td>
<td></td>
<td>100</td>
<td>168.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>$L$</td>
<td></td>
<td>25</td>
<td>370.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>$SLT, FST, FSL$</td>
<td>$T \times L$</td>
<td>50</td>
<td>68.8</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.4. Results of log-linear analyses of effects of time period on the fate of *C. hirta* plants per initial stage in the two studied Hawaiian populations. $P$-values < 0.05 represent significant deviations from the null hypothesis that the fate of plants was not affected by the census interval (model $FT$). The df for each test was 10.

<table>
<thead>
<tr>
<th>Initial stage</th>
<th>Waiakea</th>
<th></th>
<th>Laupahoehoe</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G^2$</td>
<td>$P$</td>
<td>$G^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>Seedling</td>
<td>9.76</td>
<td>NS</td>
<td>64.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Small adult</td>
<td>28.32</td>
<td>&lt;0.05</td>
<td>9.00</td>
<td>NS</td>
</tr>
<tr>
<td>Medium adult</td>
<td>16.97</td>
<td>NS</td>
<td>22.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Large adult</td>
<td>10.54</td>
<td>NS</td>
<td>1.54</td>
<td>NS</td>
</tr>
<tr>
<td>Extra-large adult</td>
<td>1.36</td>
<td>NS</td>
<td>3.84</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant

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(seedling through medium adult). However, none of the years during the study was more conducive or detrimental to growth in both populations of *C. hirta*.

There was also significant temporal variation in the calculated stable stage distributions among the three time intervals at Waiakea ($G^2 = 187.2, df = 8, P < 0.001$) but not at Laupahoehoe ($G^2 = 9.39, df = 8, P > 0.05$). The observed population structures differed significantly among the four censuses in both populations (Waiakea $G^2 = 117.46$, Laupahoehoe $G^2 = 23.85, df = 12, P < 0.001$ and $< 0.05$, respectively).

**Elasticity Analysis**

The relative importance of each of the observed life history transitions on the asymptotic population growth rate was relatively similar, and none were overwhelmingly important in the life cycle of *C. hirta*. The three transitions with the greatest proportional contribution to $\lambda$ for the three time intervals made up only 39-41% at Laupahoehoe and 42 – 47% at Waiakea. The three parameters with the greatest elasticity at Laupahoehoe (range = 0.104 to 0.160) were extra-large adult survival ($S_{55}$), extra-large adult fecundity ($F_{15}$), and growth of seedlings to small adults ($G_{21}$). At Waiakea, the three parameters with the greatest elasticity (range = 0.120 to 0.206) varied more among years than they did at Laupahoehoe. The set of three always contained seedling stasis ($S_{11}$), but the identity of the other two parameters varied among growth of seedlings to small adults ($G_{21}$), small adult stasis ($S_{22}$), large adult survival ($S_{55}$), and extra-large adult survival ($S_{66}$).

The composite elasticity structure was fairly consistent across the three time intervals at Laupahoehoe but differed among years at Waiakea (Figure 5.4). The seedling stage class had the largest total composite elasticity in both populations in all years except the final year studied at Waiakea when the large adult class was proportionally more important to $\lambda$. For all but that year at Waiakea, the second most important stage was small adults at Waiakea and fecundity at Laupahoehoe. Medium, large, and extra-large adults had greater elasticities at Waiakea than Laupahoehoe.

The relative importance of the life cycle components of survival (without growth), fecundity, and growth for *C. hirta* in the two populations in each year can be seen in Figure 5.5. Both populations are concentrated toward the center, right-hand part of the triangular diagram which plots the total elasticities for these three elements. Elasticity of $\lambda$ to changes in growth ($G$) were greater than for survival ($L$) at Laupahoehoe, which had higher $\lambda$-values, than at Waiakea.
Figure 5.4 Composite elasticities for fecundity and the fate of each stage in the two Hawaiian populations. Different symbols represent the three time intervals.
Figure 5.5  Demography triangle plot of summed elasticities for fecundity (F), growth (G), and survival (L) for each population during each of the three time periods. Note the direction of tick marks for each axis to determine the direction of the reference lines.
Figure 5.6 Asymptotic population growth rates as a function of (a) the percent of new recruits destroyed, (b) the percent of seedlings killed, and (c) the percent reduction in survival of all vegetative plants by hypothetical biological control agents. The matrix model for the interval 2000-2001 for each Hawaiian population was used for these scenarios. The dashed horizontal line indicates a $\lambda$ of one, representing a population that is neither growing nor declining.
Elasticity of \( \lambda \) to fecundity (F) was also greater at Laupahoehoe. The point for the Waiakea population in 2000-2001, which had the lowest \( \lambda \) value of all time x location combinations, was located closer to the corner for survival than the other points.

**Potential Effects of Biological Control**

The analyses of the potential effects of biological control agents showed that smaller changes in plant survival across all vegetative stages than in fecundity or seedling survival alone would reduce \( \lambda \) below one (Figure 5.6). For both Waiakea and Laupahoehoe, \( \lambda \) remained above one until almost 100% of the new recruits per adult (Figure 5.6a) or seedlings (Figure 5.6b) were destroyed. However, \( \lambda \) dropped below one when plant survival was reduced across all vegetative stages by 12% at Waiakea and 64% at Laupahoehoe (Figure 5.6c).

**DISCUSSION**

*Clidemia hirta* demography exhibited temporal and spatial variation, but differences were mainly in the magnitude of the high rates of population increase. The Waiakea population, with a higher initial density and plot biomass, grew more slowly than the Laupahoehoe population, but demonstrated less temporal variation in the fate of vegetative stages. Decline in both the number of new recruits and \( \lambda \) in the final year at Waiakea suggest a slowing invasion. The high population density likely affects establishment success through negative density dependence. At Laupahoehoe, the proportion of plants in the seedling stage was relatively constant across all years. The more open tree canopy and lower population densities of *C. hirta* at Laupahoehoe likely contributed to the higher population growth rate there. However, causes of variation among years in the asymptotic population growth rates and both stable stage and observed population structures are unknown. The lack of corresponding variation in the two populations among years suggests that the causes resulted from local environmental factors.

The central position of the two forest populations of *C. hirta* in the center of the demographic triangle is characteristic of shrubs (Silvertown et al. 1996) and woody plants of open habitats (Silvertown et al. 1993). The relatively high population growth rate of the Laupahoehoe population is reflected in its relatively high elasticity of \( \lambda \) to growth, whereas the more slowly growing Waiakea population showed high elasticity to survival. This is consistent
with previous studies, which found faster growing populations to have higher elasticity of $\lambda$ to changes in fecundity and growth than survival (Silvertown et al. 1993, Oostermeijer et al. 1996).

The elasticity matrices of $C. \ hirta$ populations were not dominated by any one parameter or stage. Even the three transitions with the greatest elasticity values made up at most 47% of the contribution to the population growth rate. With respect to development of effective biological control agents, this indicates that there is no single “Achilles’ heel” that might be exploited to reduce population growth below a sustaining rate. Similarly, Parker (2000) found that elasticity was evenly distributed throughout the life cycle of another non-native, invasive shrub, $Cytisus \ scoparius$, in expanding populations in the northwestern United States. In contrast, the elasticity structure was dominated by fewer transitions for $Carduus \ nutans$, an exotic thistle in New Zealand (Shea and Kelly 1998); for example, seed production and germination each had elasticities $> 0.30$ in one of the populations studied. Thus, biological control agents that targeted these transitions in $Carduus \ nutans$ might have a strong negative impact on the population growth rate (Shea and Kelly 1998). The low elasticity of $\lambda$ to changes in most transitions in $C. \ hirta$ (all were $< 0.20$) suggests that control might be difficult to implement for this species. Extermination of populations under current environmental conditions is likely to be effected only by reducing multiple vital rates.

A biological control agent targeting survival and growth transitions in the seedling stage, as opposed to adult fecundity or growth transitions, is likely to be most effective because the composite elasticity for the seedling stage was higher than for other stages in both Hawaiian populations in most years. Adult stage classes generally had low composite elasticities, requiring larger changes in adult vital rates to cause a substantial decline in the population growth rate. The composite elasticity of extra-large adults, found to dominate the elasticity structure in some populations of $Cytisus \ scoparius$ (Parker 2000), was low for $C. \ hirta$ in both populations during most time intervals.

Simulated reductions in recruitment (fecundity), as might occur with the introduction of seed-eating insects, seed pathogens, or damping-off fungi, showed that almost 100% of all recruits would have to be killed to cause either of the two Hawaiian populations to be exterminated. Such high levels of impact are unlikely. Other analyses have reached similar conclusions. For example, it would be necessary to destroy an estimated 97 – 99.9% of seeds in some $Cytisus \ scoparius$ populations to reduce $\lambda$ below one in the northwestern United States and
Australia (Sheppard et al. 2002). In contrast, an estimated 69% seed loss was predicted to cause \( \lambda \) of *Carduus nutans* in New Zealand to drop below one (Shea and Kelly 1998). For *Cytisus scoparius* and *Clidemia hirta*, biological control agents that reduce fecundity likely will not be the most cost-effective means of extermination.

Similarly, biological control agents affecting mortality of seedlings alone do not show much promise for control of Waiakea or Laupahoehoe populations. Although the seedling stage exhibited the highest composite elasticity, the simulation projected almost 100% of seedling mortality would be necessary to cause the population to go towards extinction.

Biological control agents that reduce survival of *C. hirta* across all vegetative stages are most likely to cause declines in the population growth rate. Likewise, McEvoy and Coombs (1999) found that a flea beetle would provide the most effective control of ragwort, *Senecio jacobea*, because it affected several transitions, with the largest effect on transitions for which \( \lambda \) had the greatest elasticity. Our projections suggest that continuous reductions of *C. hirta* survival by 12% at Waiakea and 64% at Laupahoehoe would cause the *C. hirta* populations to go locally extinct. Natural enemies (fungal pathogens and insect herbivores) caused such high levels of mortality for small *C. hirta* planted into forest understory in its native range in Costa Rica (Chapter 4). Survival of plants unprotected from natural enemies during a 14-month experiment was 62% lower than plants protected from natural enemies by insecticide and fungicide (Chapter 4). Not surprisingly, *C. hirta* does not occur in closed-canopy forest understory similar to Waiakea in its native range. Similar mortality rates in Hawaiian forests across vegetative stages are projected to lead to a sharply declining population \( (\lambda << 1) \) at Waiakea and a slightly declining population at Laupahoehoe. These comparisons suggest that the leaf-feeding lepidoptera and weevils, gall-forming cecidomyiid flies, fungal pathogens, and stem borers found on *C. hirta* planted in Costa Rican forests (Chapter 4) should be explored as potential biological control agents for *C. hirta*.

It has been hypothesized that biological control agents have greater effects on species that are genetically depauperate (Levin 1975, Burdon and Marshall 1981). Genetic variation within and among populations of *C. hirta* on four of the main Hawaiian Islands is higher than levels of variation in Costa Rica, part of the native range, but still low compared to many other non-native, invasive species (Chapter 2). Thus, biological control may prove effective against this species, despite the lack of an Achilles’ heel.
This study provides information that will assist efforts to design more effective and parsimonious biological control programs for the invasive shrub *Clidemia hirta* in Hawaii. The demographic data from the three years of this study provide a useful baseline with which to compare the actual population effects of future biological control introductions. Research is needed to find and screen control agents that kill *C. hirta* in shaded conditions. As suggested in Chapter 4, such agents only may be found by outplanting *C. hirta* into forest understory in its native range because the species does not occur naturally in such dense shade. Furthermore, post-introduction studies should be conducted to determine whether the prospective and projection analyses used here, and in other studies (Lonsdale et al. 1988, Shea and Kelly 1998, Parker 2000), provides reasonable forecasts of the effects of actual biological control agents.

**LITERATURE CITED**


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Mathworks. 2001. MATLAB 6.0 (Release 12), Natick, Massachusetts, USA.


Chapter 6
Conclusions, Applications, and Future Directions

CONCLUSIONS

The goal of this dissertation was to answer the question why *Clidemia hirta* is scarce with a narrow habitat range in its native range but becomes abundant in a broad habitat range in its introduced range. Several non-exclusive hypotheses have been proposed to account for such changes in the behavior of introduced species. I used the tropical shrub *Clidemia hirta* as a model exotic pest plant to test two of these hypotheses, to quantify the amount of genetic variation present in parts of the native and introduced ranges, and to model the potential effects of biocontrol agents in Hawaii, part of its introduced range.

The genetic diversity of non-native, invasive species in their areas of introduction reflects levels of genetic diversity held within native populations, species life history traits, and the introduction history. Genetic variation within Hawaiian populations of *C. hirta* was comparable to other unintentionally introduced species but low compared to deliberate introductions. Contrary to theory, the native Costa Rican populations had a significantly lower percentage of polymorphic loci, expected heterozygosity, and number of alleles per polymorphic locus than the introduced Hawaiian populations. Most genetic diversity was held within rather than among populations in the two areas ($G_{ST} = 0.074$ and 0.235 in Hawaii and Costa Rica, respectively), but there was no genetic differentiation among islands in Hawaii and little among different regions in Costa Rica. Costa Rican populations differed only between Caribbean and Pacific sides of the central mountain range. Low levels of genetic diversity in the native range, a low level of outcrossing, and introduction from only one part of the native range may contribute to the low levels of genetic diversity within Hawaiian *C. hirta* populations. Not enough of the native range was sampled to determine the source populations of Hawaiian *C. hirta*; however, the low genetic similarity between the two areas sampled (Nei’s genetic identity $I = 0.64$) strongly suggests that
the source of *C. hirta* in Hawaii is not Costa Rica. Levels of genetic diversity seem unrelated to invasive success for this species.

The first hypothesis of invasion tested was that plants in the introduced range are more vigorous and more shade tolerant than those from the native range because of a genetic shift in resource acquisition, allocation, or plasticity. The common garden study I used to test this hypothesis provided little evidence that Hawaiian genotypes of *C. hirta* differed genetically from Costa Rican genotypes in ways that would contribute to differences in habitat distribution or abundance observed between the two areas. In this experiment, six-month old seedlings were placed in high (10.3 – 13.9 mol m⁻² day⁻¹) or low (1.4 - 4.5 mol m⁻² day⁻¹) light treatments and grown for an additional six months. Some of the genetic differences that were apparent, such as greater allocation to stems and leaf area relative to whole plant biomass in Costa Rican genotypes and greater allocation to roots in Hawaiian genotypes, were contrary to predictions that genotypes from the introduced range would allocate more biomass to growth and less to storage than those from the native range. Hawaiian and Costa Rican genotypes displayed no significant differences in relative growth rates, maximal photosynthetic rates, or specific leaf areas in either light treatment. In the high light environment, however, Hawaiian genotypes allocated more biomass to reproductive parts than Costa Rican genotypes. Phenotypic plasticity for only one of twelve morphological and photosynthetic variables was greater for Hawaiian than Costa Rican genotypes, suggesting that Hawaiian plants are no more plastic than Costa Rican plants.

The natural enemy exclusion study showed support for the enemy release hypothesis, which is also proposed to account for changes in habitat distribution and abundance in *C. hirta* between its native and introduced range. The amount of leaf area missing or damaged was four and six times higher in the understory and open habitats, respectively, of Costa Rica than Hawaii. *Clidemia hirta* survival overall was lower in Costa Rica than Hawaii in both open and understory habitats. Percent survival was much lower in the understory in Costa Rica (45%) than Hawaii (99%), but when insects and fungal pathogens were excluded 66% of *C. hirta* seedlings in Costa Rica survived. Exclusion of natural enemies had no effect on survival in either habitat in Hawaii or in open sites in Costa Rica. Relative growth rates of plants that survived to the end of the experiment were higher for plants sprayed with fungicide in Costa Rica, but not Hawaii, suggesting that fungal pathogens of *C. hirta* affect growth in both habitats in the native range.
The results from the natural enemy exclusion study suggest that herbivores and fungal pathogens may limit survival only in particular habitats. For *Clidemia hirta*, its absence from forest understory in its native range likely results in part from the strong pressures of natural enemies. Its invasion into Hawaiian forests is apparently aided by a release from these herbivores and pathogens.

In answer to the overall question posed at the beginning, it seems that *C. hirta* is less abundant and more restricted to high light environments in its native range because of limiting environmental conditions, specifically herbivore and fungal pathogen pest loads. Genetic differences are apparent between Hawaiian and Costa Rican genotypes, however, these genetic differences do not appear to account for the greater abundance or shade tolerance of *C. hirta* in its introduced range compared to its native range.

**APPLICATIONS**

These results, coupled with stage structured matrix model projection models, may help in designing a parsimonious and tailored biological control program to reduce the growth of Hawaiian populations of *C. hirta*. Control measures clearly are needed, as the asymptotic population growth rates of two studied populations in Hawaii were much greater than one, demonstrating that both populations were projected to continue growing rapidly. The elements of the matrix models were parameterized with field data collected over three years from 2906 plants in a recently invaded forest with an open overstory (Laupahoehoe) and 600 plants in a less recently invaded forest with a dense canopy (Waiakea). No Achilles’ heel was indicated by the elasticity structure of the matrix models. Biological control agents that affect only seeds or seedlings likely would not be cost-effective measures to cause populations to go toward extinction; however, herbivores or pathogens that reduce survival of all vegetative life stages may prove effective at causing substantial declines in the population growth rate. Simulations showed that a 12% and 64% reduction in survival across all plants at Waiakea and Laupahoehoe, respectively, would cause a decline in each population’s growth rate. The natural enemy exclusion study demonstrated that natural enemies caused these high levels of mortality of *C. hirta* planted into forest understory in Costa Rica. Further research will be needed to identify the particular insects or fungal pathogens that could cause these levels of damage in Hawaii, but
from the Costa Rican study we know that biological control agents potentially could control *C. hirta* populations in Hawaiian lowland forests.

**FUTURE DIRECTIONS**

Numerous future directions for research on exotic pest species and *C. hirta* in particular are apparent to me after completing this dissertation. First, the generality of my results could be explored by conducting common garden and enemy exclusion experiments with the other seven tropical woody species listed in Table 1.1, which also are limited to open areas in their native range but invade closed tropical forests in their introduced range. *Miconia calvescens* is a prime candidate because this melastome tree also is native to Central and South America and is an exotic pest on Hawaii. Second, it would be valuable to assess the impact of herbivores and pathogens on *Clidemia hirta* and other exotic pest plants in areas where native members of the Melastomataceae are present. For example, a natural enemy exclusion study of *C. hirta* in Malaysia, India, or Indonesia, where native Melastomataceae occur, would help determine whether enemy release also may explain its invasion success even when the probability of host switching by native insects is greater. Third, the results of the study of genetic similarity, or dissimilarity in this case, between Costa Rican and Hawaiian *C. hirta* piques my interest about the origin of the individuals brought to Hawaii. I would like to conduct a phylogeographic analysis to determine the origin of Hawaiian *C. hirta* and to track its introduction history throughout Oceania, Southeast Asia, and Africa. In addition, I would like to evaluate the genetic similarity of *C. hirta* throughout its native range. Are Central and South American genotypes of *C. hirta* as different as Costa Rican and Hawaiian genotypes? Finally, I would be interested in testing the results of my simulations to actual biological control releases. Do the populations decline toward extinction if survivorship of all vegetative stages is reduced by 12% and 64% at Waiakea and Laupahoehoe as predicted by the modeling efforts? I have several years of background data on the population dynamics at Waiakea and Laupahoehoe. Biological control agents could be introduced to these two populations, survival and growth of the tagged plants could be measured, and the actual effect on the population growth rate could be compared to the matrix model projections. Clearly, many interesting questions remain to be addressed.
Vita

Saara Jennie DeWalt was born August 24, 1972, in Willimantic, Connecticut, to Billie and Kathleen DeWalt. She grew up in Lexington, Kentucky and attended Henry Clay High School. She attended Brown University where she developed a passion for plant ecology and traveling. During her junior year, she spent six months in Ecuador working and studying, primarily in the eastern Amazonian lowlands. During this time she decided to make a career out of studying plants wherever the mean daily temperature was over 20ºC. After graduating in 1994, she received a Fulbright Fellowship and spent thirteen months in lowland Bolivia studying the ethnobotany of a lowland indigenous group. Eight days after returning from Bolivia, she left to go to Central America where she made pilgrimages to two famous biological field stations, Barro Colorado Island and La Selva. She decided to stay in warm areas and attend graduate school in plant biology at Louisiana State University. Her dissertation research focused on the ecology and genetics of an invasive shrub in Costa Rica, where it is native, and Hawaii, where it is introduced. She also conducted research in Malaysia (Peninsular and Borneo), Panama, Peru, Brazil, and French Guiana during this time. She hopes to continue her nomadic-style ecology, at least in the short-term.