

GENETIC VARIATION OF INTRODUCED HAWAIIAN AND NATIVE COSTA RICAN POPULATIONS OF AN INVASIVE TROPICAL SHRUB, *CLIDEMIA HIRTA* (MELASTOMATACEAE)¹

SAARA J. DEWALT^{2,4} AND J. L. HAMRICK³

²Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803 USA; and ³Departments of Plant Biology and Genetics, University of Georgia, Athens, Georgia 30602 USA

Clidemia hirta is one of the most common woody invasive plants in mesic to wet forests in Hawaii, where it was introduced around 1940. The species is relatively uncommon by comparison in its native range of Central and South America and some Caribbean Islands. We examined genetic variation in allozymes of 20 *C. hirta* populations on four Hawaiian Islands to determine the introduction history. For comparison, we measured genetic variation in 20 native populations across Costa Rica. Mean levels of genetic variation in Hawaiian and Costa Rican populations were low compared to other woody or introduced plants (11.5–12.5% polymorphic loci, 2.05–2.50 alleles per polymorphic locus, and 0.045–0.063 expected heterozygosity). Most genetic diversity was held within rather than among populations in both areas ($G_{ST} = 0.120$ and 0.271 in Hawaii and Costa Rica, respectively). Hawaiian populations had a high degree of genetic similarity, and no genetic differentiation was found among the four Hawaiian Islands sampled. These patterns of genetic variation in Hawaii suggest that no intraspecific hybridization of genotypes from different parts of the native range has occurred and that introductions to the different islands came from the same or similar source populations. The low levels of genetic diversity in parts of both the native and introduced ranges suggest that genetic variation is unrelated to invasiveness in *C. hirta*.

Key words: allozymes; Costa Rica; genetic diversity; Hawaii; invasive species; tropical rain forest.

For non-native plants, the amount and distribution of genetic variation are determined by the genetic diversity in the founder populations, number and source of founders, life history characteristics, and post-introduction processes (e.g., selection and genetic drift). As a result of founder effects and nonequilibrium conditions, populations in the introduced range are expected to have less within-population genetic variation and more among-population genetic differentiation than those in the native range (Brown and Marshall, 1981; Barrett and Richardson, 1986; Husband and Barrett, 1991). Founder effects and genetic drift tend to reduce heterozygosity and lead to interpopulation differentiation when introduced population sizes are small. However, colonizations stemming from multiple introductions or involving large founding populations may not exhibit large reductions in genetic variation (Novak and Mack, 1993; Wang et al., 1995; Amsellem et al., 2000). Indeed, separate introductions from multiple parts of the native range may result in intermingling of genotypes that may increase genetic diversity within populations (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 non-native 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 multilocus genotypic combinations and 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 non-native invasive species have met with little success (Mack et al., 2000). Generalizations about levels of genetic diversity for such plants may also not be possible given the diversity of mating systems, life forms, dispersal syndromes, and introduction histories that they encompass (Gray, 1986). High genetic variation may be advantageous in colonizing species by providing the basis for populations to adapt to heterogeneous environments and potentially to radiate into environmental conditions different from those experienced in the native range (Sakai et al., 2001), but a general-purpose genotype that proliferates in multiple habitats through phenotypic plasticity might also be favored (Baker, 1965; Parker et al., 2003). Just as there are multiple ways for plants to be rare (Gitzendanner and Soltis, 2000), there seem to be many ways for plants to be invasive. More insights on genetic variation and its 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* (L.) D. Don (Melastomataceae)

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⁴ Present address: Department of Ecology & Evolutionary Biology, MS 170, Rice University, Houston, Texas 77005 USA (e-mail: sdewalt@rice.edu).

in Hawaii, where this plant is an introduced 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 because this would have entailed extensive sampling throughout Central and South America and the Caribbean Islands. Instead, we used Costa Rica as a representative area of the native range and compared populations across the Hawaiian archipelago to populations across Costa Rica. *Clidemia hirta* occurs primarily in high light environments and is absent from forest understory in its native range, but it is an aggressive, disruptive invader of both open areas and forest understory where it has been introduced (Wester and Wood, 1977; Smith, 1992; Gerlach, 1993). Previously, we found that fungal pathogens and insect herbivores, but not genetically determined patterns of biomass allocation, photosynthesis, and growth, may contribute to the greater abundance and habitat breadth of this species in Hawaii than in Costa Rica (DeWalt et al., 2004a, b).

In this paper, we address the following questions: (1) How much genetic diversity occurs 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 population genetic structure of *C. hirta* in introduced Hawaiian populations compare to native populations sampled in Costa Rica? We further compare levels of genetic diversity in *C. hirta* to that in other non-native invasive plants, native Hawaiian species, and widespread tropical woody species.

MATERIALS AND METHODS

Study organism—*Clidemia hirta* is native to Central and South America and the Caribbean Islands where it occurs in mesic to wet environments to 1500 m above sea level (a.s.l.) (Wester and Wood, 1977; Steyermark and Huber, 1978). 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 (Wester and Wood, 1977; Gerlach, 1993; Sheil, 1994; Strahm, 1999; Singhakumara et al., 2000). The species 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). *Clidemia hirta* was likely introduced accidentally to Hawaii (Simmonds, 1933; Wester, 1992), but the precise origin from the native range is not known.

Three varieties of *C. hirta* 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). Both Hawaiian and Costa Rican specimens were identified as *Clidemia hirta* var. *hirta* (F. Almeda, California Academy of Sciences, personal communication). Nevertheless, the Costa Rican and Hawaiian plants have some morphological differences that are apparent even when grown in a common garden (DeWalt et al., 2004a). Costa Rican plants have longer and denser trichomes, larger flowers, and a more open growth form than Hawaiian plants.

The mating system of *C. hirta* in its native range includes both sexual reproduction through bee pollination and asexual reproduction through agamospermy (Renner, 1989; Melo et al., 1999). The proportion of seeds produced through agamospermy vs. sexual reproduction is not known for either the native or the introduced range. We are unsure whether bee pollination occurs in Hawaii, but introduced honey bees (*Apis mellifera*) and native Colletidae bees could conduct buzz pollination of *C. hirta*. The fruits are pulpy, dark-blue berries produced year-round and contain from 200 to 900 seeds, each about 0.5 mm in diameter. Seeds are primarily bird-dispersed. Although

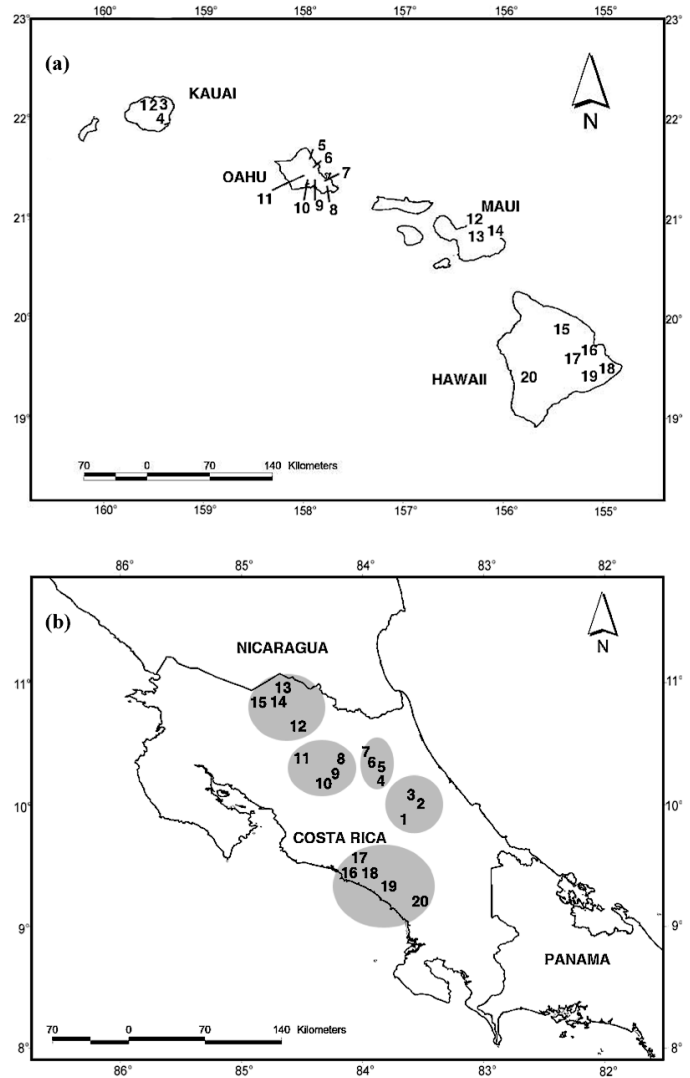


Fig. 1. Map of the populations of *Clidemia hirta* sampled in (a) Hawaii, part of the introduced range, and (b) Costa Rica, part of the native range. Shaded areas around populations in Costa Rica delimit the five regions chosen a priori based on geographic proximity. Numbers correspond to populations described in the Appendix (see Supplemental Data accompanying the online version of this article).

a vigorous resprouter following damage, *C. hirta* does not propagate vegetatively.

Sample collections—In 2000, we collected *C. 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) (Fig. 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 to 800 m a.s.l. and in Costa Rican populations located along pastures, roadsides, and young plantations of *Bactris gasipaes* from 0 to 300 m a.s.l. (see the Appendix in Supplemental Data accompanying the online version of this article). Maternal plants were chosen haphazardly within populations. Seeds from each fruit were separated from the pulp, dried at room temperature, and stored in 1.5-mL microcentrifuge tubes.

Ten to 20 seeds per fruit were surface sown in individual cells of trays filled with Fafard 3B mix (Conrad Fafard, Awawam, Massachusetts, USA).

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 selected 4–6 mo after sowing, when the plants were of sufficient size for enzyme extraction. Seedlings ranged in height from 1 to 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), colorimetric 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 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 for one enzyme—glutamate dehydrogenase (GDH); therefore, this enzyme was not included in our analysis.

Genetic diversity analyses—Analyses were conducted on two data sets. The “individuals” data set used the genotypic data from one randomly chosen fruit per plant ($N = 11\text{--}13$ per population). The “bulk” collection data set used the genotypic data from three fruits per maternal plant that produced at least one seedling ($N = 21\text{--}39$ per population). This data set mimics bulk collections, in which multiple seeds are collected from the same plant (e.g., Novak and Mack, 1993; Chase et al., 1995; Chamberlain, 1998). Analyses refer to the individuals 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 (A_p), 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 by pooling all values for the Hawaiian and Costa Rican populations (the “true” species level for this study). Pooled values were also calculated separately for each area (species level within Hawaii or Costa Rica). We used these pooled values to compare genetic diversity in *C. hirta* to other species of non-native invasive plants, native Hawaiian plants, and widespread tropical woody plants. Population-level values were calculated to statistically compare within-population levels of genetic diversity between Hawaii and Costa Rica and among regions (Costa Rica) or islands (Hawaii).

Population-level fixation indices ($F = 1 - H_o \div H_e$) were calculated for each polymorphic locus and significant deviations from Hardy-Weinberg expectations ($F = 0$) were identified by χ^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, χ^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 $D_{ST} \div H_T$. 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 nonmetric 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. Nonmetric multidimensional scaling 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 the computer program Isolation by Distance (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 $F_{ST} = 1 \div (1 + 4Nm)$, 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} cannot 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 variation—A total of 477 *C. hirta* individuals from Hawaiian populations (239) and Costa Rican populations (238) were analyzed in the individuals data set (see the Appendix in Supplemental Data accompanying the online version of this article). At the species level (across Hawaii and Costa Rica), 43.8% of the 16 loci that were found in both areas 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 1). Total expected heterozygosity for the species was 0.225. The results were similar using the bulk data set, for which we analyzed 652 plants from Hawaiian seed sources and 690 plants Costa Rican seed sources (Table 1).

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-

TABLE 1. Within-population genetic diversity measures for (a) Hawaiian and (b) Costa Rican populations of *Clidemia hirta* in the individuals and bulk data sets.

Population	%P	A_p	Individuals			Bulk		
			A_e (SD)	H_o (SD)	H_e (SD)	A_e (SD)	H_o (SD)	H_e (SD)
(a) Hawaii								
Kauai								
1	12.50	2.5	1.13	0.052 (0.050)	0.060 (0.044)	1.16	0.058 (0.033)	0.067 (0.048)
2	12.50	2.5	1.17	0.068 (0.046)	0.071 (0.049)	1.18	0.066 (0.030)	0.072 (0.050)
3	12.50	2.5	1.17	0.047 (0.049)	0.069 (0.048)	1.17	0.049 (0.028)	0.069 (0.048)
4	12.50	2.5	1.16	0.063 (0.044)	0.069 (0.047)	1.16	0.061 (0.029)	0.069 (0.047)
Oahu								
5	12.50	2.5	1.16	0.047 (0.048)	0.068 (0.047)	1.13	0.049 (0.028)	0.061 (0.043)
6	12.50	2.5	1.14	0.052 (0.050)	0.065 (0.045)	1.17	0.048 (0.029)	0.069 (0.048)
7	12.50	2.5	1.12	0.063 (0.051)	0.062 (0.043)	1.13	0.061 (0.032)	0.061 (0.043)
8	12.50	2.5	1.13	0.074 (0.050)	0.064 (0.044)	1.13	0.065 (0.038)	0.065 (0.044)
9	12.50	2.5	1.10	0.063 (0.050)	0.055 (0.038)	1.10	0.055 (0.031)	0.055 (0.038)
10	12.50	2.0	1.07	0.016 (0.035)	0.041 (0.035)	1.12	0.038 (0.028)	0.059 (0.044)
11	12.50	2.5	1.17	0.073 (0.050)	0.068 (0.049)	1.15	0.077 (0.028)	0.067 (0.048)
Maui								
12	6.25	3.0	1.10	0.026 (0.036)	0.039 (0.039)	1.13	0.038 (0.028)	0.057 (0.043)
13	12.50	2.5	1.15	0.032 (0.040)	0.055 (0.051)	1.14	0.048 (0.030)	0.057 (0.049)
14	12.50	2.5	1.17	0.047 (0.049)	0.070 (0.048)	1.14	0.039 (0.030)	0.064 (0.046)
Hawaii								
15	12.50	2.5	1.09	0.042 (0.041)	0.052 (0.036)	1.12	0.037 (0.023)	0.058 (0.041)
16	12.50	2.5	1.12	0.032 (0.048)	0.054 (0.045)	1.12	0.039 (0.031)	0.056 (0.045)
17	12.50	2.5	1.17	0.047 (0.049)	0.067 (0.050)	1.17	0.034 (0.027)	0.064 (0.050)
18	12.50	2.5	1.09	0.036 (0.046)	0.047 (0.035)	1.13	0.033 (0.028)	0.062 (0.044)
19	12.50	2.5	1.15	0.091 (0.045)	0.066 (0.047)	1.15	0.056 (0.033)	0.065 (0.047)
20	12.50	2.5	1.17	0.047 (0.048)	0.067 (0.048)	1.17	0.050 (0.029)	0.069 (0.049)
(b) Costa Rica								
EARTH								
1	11.76	2.0	1.10	0.093 (0.056)	0.054 (0.031)	1.10	0.080 (0.028)	0.054 (0.049)
2	11.76	2.0	1.07	0.051 (0.029)	0.045 (0.026)	1.08	0.052 (0.030)	0.045 (0.044)
3	11.76	2.0	1.06	0.034 (0.022)	0.042 (0.024)	1.08	0.048 (0.029)	0.046 (0.044)
La Selva								
4	11.76	2.0	1.07	0.034 (0.020)	0.044 (0.026)	1.08	0.044 (0.027)	0.047 (0.044)
5	11.76	2.0	1.04	0.010 (0.008)	0.022 (0.019)	1.05	0.026 (0.025)	0.034 (0.042)
6	0.00	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1.01	0.007 (0.014)	0.007 (0.032)
7	5.88	1.0	1.07	0.068 (0.045)	0.042 (0.026)	1.09	0.052 (0.026)	0.050 (0.043)
San Carlos								
8	11.76	2.0	1.08	0.054 (0.031)	0.046 (0.027)	1.09	0.049 (0.028)	0.052 (0.047)
9	11.76	2.0	1.09	0.053 (0.034)	0.049 (0.030)	1.08	0.034 (0.026)	0.043 (0.044)
10	5.88	1.0	1.10	0.067 (0.040)	0.055 (0.032)	1.11	0.069 (0.026)	0.055 (0.050)
11	11.76	2.0	1.06	0.027 (0.023)	0.029 (0.026)	1.06	0.029 (0.025)	0.033 (0.042)
Los Chiles								
12	11.76	2.0	1.03	0.016 (0.010)	0.023 (0.013)	1.03	0.020 (0.022)	0.026 (0.035)
13	5.88	1.0	1.05	0.015 (0.012)	0.028 (0.023)	1.06	0.021 (0.022)	0.034 (0.041)
14	11.76	2.0	1.09	0.064 (0.049)	0.049 (0.029)	1.11	0.061 (0.013)	0.056 (0.050)
15	11.76	2.0	1.12	0.061 (0.036)	0.058 (0.033)	1.10	0.051 (0.031)	0.049 (0.050)
Quepos								
16	11.76	2.5	1.07	0.036 (0.021)	0.041 (0.024)	1.09	0.042 (0.029)	0.049 (0.045)
17	11.76	2.0	1.11	0.069 (0.048)	0.056 (0.031)	1.11	0.076 (0.019)	0.056 (0.049)
18	11.76	2.5	1.12	0.083 (0.052)	0.059 (0.034)	1.12	0.078 (0.021)	0.060 (0.051)
19	11.76	2.0	1.09	0.078 (0.051)	0.052 (0.030)	1.10	0.079 (0.020)	0.052 (0.048)
20	11.76	2.0	1.12	0.074 (0.050)	0.059 (0.034)	1.11	0.076 (0.021)	0.057 (0.050)
Hawaii mean	12.19	2.50	1.14	0.051	0.060	1.14	0.050	0.063
(SD)	(1.40)	(0.16)	(0.03)	(0.018)	(0.010)	(0.02)	(0.007)	(0.010)
Costa Rica mean	10.29	1.80	1.08	0.049	0.043	1.08	0.050	0.045
(SD)	(3.23)	(0.59)	(0.03)	(0.026)	(0.015)	(0.03)	(0.005)	(0.008)
Species values	43.75	2.57	1.56		0.225	1.56		0.225

Note: %P = percentage polymorphic loci, A_p = mean number of alleles per polymorphic locus, A_e = effective number of alleles per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, SD = standard deviation.

2, which is considered as two monomorphic loci (FE-2 and FE-3). Four loci (AAT-1, LAP-3, FE-4, 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, whereas 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 = 11.76, $A = 1.18$, $A_p = 1.80$, $A_c = 1.12$, $H_c = 0.060$) and Hawaii (%P = 12.50, $A = 1.19$, $A_p = 2.50$, $A_c = 1.17$, $H_c = 0.071$).

Costa Rican populations generally had lower within-population genetic diversity than Hawaiian populations for both the bulk and individuals data sets (Table 1). In the individuals data set, Hawaiian populations had significantly greater %P_{pop} (Mann-Whitney $U = 594$, $P < 0.001$), H_c ($U = 550$, $P < 0.001$), A_p ($U = 583$, $P < 0.001$), and A_c ($U = 570$, $P < 0.001$) than Costa Rican populations. Observed heterozygosity did not differ significantly between the two areas ($U = 403$, $P = 0.86$).

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 the number of alleles per polymorphic locus were almost identical for populations on the four islands. We found no island-unique alleles. Mean observed and expected heterozygosity did not differ significantly among islands (H_o : Kruskal-Wallis $H = 5.37$, $df = 3$, $P = 0.15$; H_e : Kruskal-Wallis $H = 4.15$, $df = 3$, $P = 0.25$). In general, however, mean H_o and H_e were lower for Hawaii (0.049, 0.059) and Maui (0.035, 0.055) than for Kauai (0.058, 0.067) and Oahu (0.055, 0.060). No significant differences were found among the five regions in Costa Rica for any of the within-population diversity measures.

Many of the Hawaiian and Costa Rican populations had significant deviations from Hardy-Weinberg expectations. In each area, we would expect 5%, or two 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. Of the significant indices, all were positive for AAT-2, while four were positive and one was negative for FE-3. In Costa Rica, 20 of the 40 fixation indices had significant differences from HWE. All Costa Rican populations that showed significant deviations had an excess of heterozygotes ($F > 0$) for FE-1 and an excess of homozygotes ($F < 0$) for AAT-2. Most populations, regardless of region, had this pattern, even if the indices were not significantly different from 0. Hawaiian populations had an overall tendency for a deficit of heterozygotes ($F_{IS} = 0.127$), and Costa Rican populations had an excess of heterozygotes ($F_{IS} = -0.205$; Table 2).

Among-population variation—The mean total genetic diversity (H_T) was similar for Hawaiian (0.568) and Costa Rican populations (0.506), and most of this diversity was held within populations (low G_{ST}) in the two areas (Table 2). More genetic diversity was partitioned among populations (greater G_{ST}) in Costa Rica ($G_{STC} = 0.271$) than in Hawaii ($G_{STH} = 0.120$). 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 ($P < 0.001$)

TABLE 2. Among-population genetic diversity statistics for polymorphic loci of *Clidemia hirta* in (a) Hawaii and (b) Costa Rica, using the individuals data set. Results for the bulk data set were similar and are not shown here.

Area/Locus	H_T	H_S	G_{ST}	F_{IS}
(a) Hawaii				
FE-3	0.497	0.422	0.151	-0.061
AAT-2	0.638	0.582	0.089	0.314
Mean	0.568	0.502	0.120	0.127
SD	0.100	0.113	0.044	0.265
(b) Costa Rica				
FE-1	0.557	0.321	0.424	-0.594
AAT-2	0.455	0.401	0.118	0.185
Mean	0.506	0.361	0.271	-0.205
SD	0.072	0.057	0.216	0.551

Note: H_T = total genetic diversity, H_S = within-population genetic diversity, G_{ST} = among-population variation, F_{IS} = Wright's fixation index within populations, SD = standard deviation.

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 Rican and Hawaiian populations ($I = 0.64$) but differed little within areas (Costa Rica: $I = 0.920$ – 0.990 ; Hawaii: $I = 0.955$ – 0.999). Hawaiian populations had higher genetic identities and clustered more tightly together along dimension 2 of the NMDS ordination than Costa Rican populations (Fig. 2a). No geographic differentiation in allele frequencies by island was evident (Fig. 2b), but within Costa Rica, there was some geographic differentiation (Fig. 2c). Four of the five populations on the Pacific side of the Cordillera Central in the Quepos region clustered together in the NMDS ordination because they differed 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 (\hat{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 ($Nm = 0.61$) than in Hawaii ($Nm = 1.19$).

DISCUSSION

The high genetic identity of Hawaiian populations and lack of island-unique alleles suggest a few possibilities for the introduction history: populations on the different islands were founded by a relatively large number of individuals from the same source; multiple founding events occurred on each island; or subsequent gene flow via pollen or seeds following introduction has been substantial. Intraspecific hybridization of genotypes from different parts of the native range seems unlikely given the low level of variation.

Although low levels of genetic diversity may hinder our

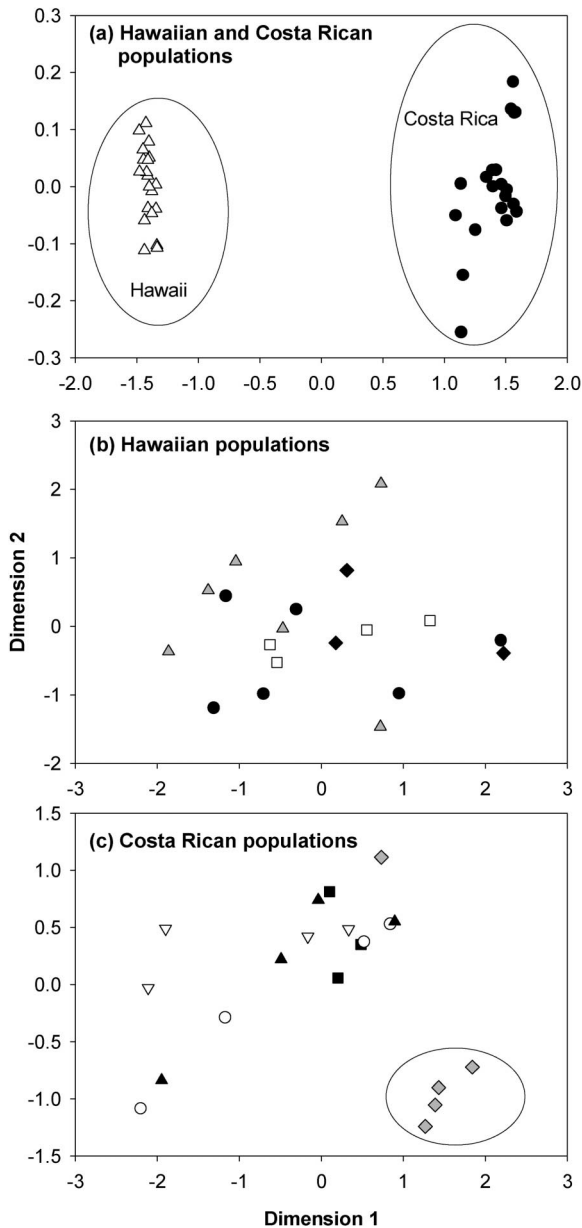


Fig. 2. Nonmetric multidimensional scaling ordination of Nei's (1972) genetic distances for (a) Hawaiian and Costa Rican populations (stress = 0.01), (b) only Hawaiian populations (stress = 0.08), and (c) only Costa Rican populations (stress = 0.08) of the tropical shrub *Clidemia hirta*. Each point represents one population. Different symbols in (b) represent different islands: Kauai (open squares), Oahu (stippled triangles), Maui (filled diamonds), and Hawaii (filled circles). Different symbols in (c) represent different regions chosen a priori: EARTH (filled squares), La Selva (open circles), San Carlos (open inverted triangles), Los Chiles (filled triangles), and Quepos (stippled diamonds).

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. High levels of historic gene flow (Nm) among *C. hirta* populations in Hawaii would suggest that these newly founded populations may not have yet reached equilibrium between genetic drift and gene flow. Additionally, current gene flow through pollen or seed movement among islands may prevent differentiation. It has been

shown that some gene flow must occur among the Hawaiian Islands for the widespread and ecologically variable dominant canopy tree, *Metrosideros polymorpha*. 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 along an altitudinal gradient on Maui (Aradhya et al., 1993). Human-mediated dispersal of small-seeded non-native 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 since ca. 1940 and spread as recently as the 1970s and 1980s to Kauai, Maui, and Hawaii (Smith, 1992).

Clidemia hirta populations in Hawaii had relatively low levels of within-population genetic diversity ($\%P_{\text{pop}} = 12.5\%$, $H_e = 0.071$) compared to many other non-native invasive species, such as *Pueraria lobata* (Pappert et al., 2000), *Lonicera japonica* (Schierenbeck et al., 1995), and *Lathyrus latifolius* (Godt and Hamrick, 1991). These species had an average of $\%P_{\text{pop}} > 40\%$ and $H_e > 0.10$. However, levels of genetic variation of *C. hirta* in Hawaii were comparable to other non-native species, such as *Setaria faberi* (Warwick et al., 1987b), *Bromus tectorum* (Novak et al., 1991), and *Bryonia alba* (Novak and Mack, 1995). These species generally had low levels of genetic diversity within populations (average $\%P_{\text{pop}} < 15\%$ and $H_e < 0.02$).

Population fixation indices showed that there were fewer heterozygotes in Hawaiian populations and more heterozygotes in Costa Rican populations than would be expected under Hardy-Weinberg equilibrium. Such differences could result from a predominance of uniparental reproduction in Hawaii but outcrossing in Costa Rica or from the effects of a population bottleneck in Hawaii. Analyses of progeny arrays would be needed to determine 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 heterozygous for FE-1 and homozygous for AAT-2 followed by predominantly asexual reproduction. The number of heterozygotes and homozygotes for each locus would then reflect the make-up of the original genotypes.

Species-level genetic variation of *C. hirta* in Hawaii ($\%P = 12.5$, $A = 1.19$, $H_e = 0.071$) was lower than many native Hawaiian angiosperms and particularly low compared to *M. polymorpha*. Endemic species of *Brighamia* (Campunulaceae), *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 ($H_e = 0.063$ – 0.076 ; Witter and Carr, 1988; Gemmill et al., 1998) as *C. hirta*. Two genera of Hawaiian Alsinoideae, *Schiedea* and *Alsinidendron* (Caryophyllaceae), were more diverse with 1.84 alleles per locus, 43.6% polymorphic loci, and expected heterozygosity of 0.188 (Weller et al., 1996). *Metrosideros polymorpha* was exceptionally diverse with 2.9 alleles per locus and an expected heterozygosity of 0.36 (Aradhya et al., 1991).

Native Costa Rican populations of *C. hirta* also harbored little within-population allozyme variation ($\%P = 11.76$, $A_e = 1.12$, $H_e = 0.060$), particularly 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%, $A_c = 1.13$ – 1.21 , $H_c = 0.109$ – 0.149 ; Hamrick and Godt, 1989). The proportion of genetic diversity partitioned among native populations of *C. hirta* ($G_{STC} = 0.271$) was somewhat higher than that reported for many outcrossing, animal-dispersed species ($G_{ST} = 0.197$; Hamrick and Godt, 1996). This high among-population variation in *C. hirta* may result partly from reduced long-distance pollen flow because agamospermy occurs, potentially frequently, in natural *C. hirta* populations (Renner, 1989). The only genetic differentiation found among Costa Rican regions was between the Caribbean and Pacific sides of the Cordillera Central. This regional differentiation is consistent with other genetic studies of woody species in Costa Rica (Chase et al., 1995; Gillies et al., 1997).

The markedly large genetic distance between Costa Rican and Hawaiian populations strongly suggests that Costa Rica was not the source of *C. hirta* introduced to Hawaii. We suggest that a South American or Caribbean origin seems more likely than a Central American origin for two reasons. First, genetic distances often are substantial between populations of tropical woody species that are geographically distant (Chamberlain, 1998; Sawkins et al., 2001; Rivera-Ocasio et al., 2002; Cavers et al., 2003). 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 Venezuela coast). Simmonds (1937) postulated that seeds of *C. hirta* were introduced to Fiji as contaminants in nursery stock of coffee plants from mainland South America. 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 as early as the late 1880s) cannot be determined by this study. A phylogeographic study that examined genetic relationships among individuals across the species' range (e.g., Novak and Mack, 1993; Saltonstall, 2002) might help understand the pathways of introduction.

Had *C. hirta* in Hawaii been from Costa Rica, we would have expected to find lower intrapopulation genetic diversity and greater interpopulation differentiation of populations in the introduced part of its range (Hawaii) than in Costa Rica (Brown and Marshall, 1981). In fact, we found slightly greater mean within-population levels of allozyme variation in introduced populations than in native populations of *C. hirta*. Interestingly, many comparisons have failed to find substantially lower genetic diversity within introduced populations compared to native populations for other plant species (Warwick et al., 1987a; Novak and Mack, 1993; Wang et al., 1995; Neuffer and Hurka, 1999; Meekins et al., 2001; Squirrell et al., 2001; Bartlett et al., 2002). We also did not find support for the prediction of greater genetic differentiation among introduced populations than in native populations (Brown and Marshall, 1981). Instead, less of the total genetic diversity was partitioned among the Hawaiian populations ($G_{STH} = 0.120$) than among the Costa Rican populations ($G_{STC} = 0.271$).

Ultimately, a goal of genetic studies of introduced species should be to assess the selective advantage of genetic vari-

ability in their native and introduced ranges. 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 higher levels of genetic variation than would be predicted from their life history traits (mean species level $H_c = 0.258$; range = 0.085–0.489). In contrast, we found little genetic variation in sampled native populations of *C. hirta*. It would be informative to sample *C. hirta* more extensively within its native range to determine geographic variability in this species. 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 et al. (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 noninvasive populations on Madagascar. It may be impossible to generalize about the genetic characteristics of non-native invasive plants (Gray, 1986), just as it has proven difficult to generalize about their morphological and physiological characteristics (Mack et al., 2000).

LITERATURE CITED

- AMSELLEM, L., J. L. NOYER, T. LE BOURGEOIS, AND M. HOSSAERT-MCKEY. 2000. Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology* 9: 443–455.
- ANONYMOUS. 1954. Notes and exhibitions. *Proceedings of the Hawaiian Entomological Society* 15: 263–265.
- ARADHYA, K. M., D. MUELLER-DOMBOIS, AND T. A. RANKER. 1991. Genetic evidence for recent and incipient speciation in the evolution of Hawaiian *Metrosideros* (Myrtaceae). *Heredity* 67: 129–138.
- ARADHYA, K. M., D. MUELLER-DOMBOIS, AND T. A. RANKER. 1993. Genetic structure and differentiation in *Metrosideros polymorpha* (Myrtaceae) along altitudinal gradients in Maui, Hawaii. *Genetical Research* 61: 159–170.
- BAKER, H. G. 1965. Characteristics and modes of origin of weeds. In H. G. Baker and G. L. Stebbins [eds.], *The genetics of colonizing species*, 147–168. Academic Press, New York, New York, USA.
- BARRETT, S. C. H., AND B. J. RICHARDSON. 1986. Genetic attributes of invading species. In R. H. Groves and J. J. Burdon [eds.], *Ecology of biological invasions*, 21–33. Cambridge University Press, Cambridge, UK.
- BARTLETT, E., S. J. NOVAK, AND R. N. MACK. 2002. Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. *American Journal of Botany* 89: 602–612.
- BERG, E. E., AND J. L. HAMRICK. 1997. Quantification of genetic diversity at allozyme loci. *Canadian Journal of Forest Research* 27: 415–424.
- BOHONAK, A. J. 2002. IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity* 93: 153–154.
- BROWN, A. H. D., AND D. R. MARSHALL. 1981. Evolutionary changes accompanying colonization in plants. In G. C. E. Scudder and J. L. Reveal [eds.], *Evolution today*, Proceedings of the Second International Congress of Systematic and Evolutionary Biology, 351–363. Hunt Institute for Botanical Documentation, Pittsburgh, Pennsylvania, USA.
- CAVERS, S., C. NAVARRO, AND A. J. LOWE. 2003. Chloroplast DNA phylogeography reveals colonization history of a neotropical tree, *Cedrela odorata* L., in Mesoamerica. *Molecular Ecology* 12: 1451–1460.
- CHAMBERLAIN, J. R. 1998. Isozyme variation in *Calliandra calothyrsus* (Leguminosae): its implications for species delimitation and conservation. *American Journal of Botany* 85: 37–47.
- CHASE, M. R., D. H. BOSHER, AND K. S. BAWA. 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 1. Genetic variation in natural populations. *American Journal of Botany* 82: 468–475.

- CHELIAK, W. M., AND J. A. PITEL. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National Forestry Institute, Canadian Forestry Service, Chalk River, Ontario, Canada, P1-X-42.
- DEWALT, S. J., J. S. DENSLow, AND J. L. HAMRICK. 2004a. Biomass allocation, growth, and photosynthesis of genotypes from the native and introduced ranges of the tropical shrub *Clidemia hirta*. *Oecologia* 138: 521–531.
- DEWALT, S. J., J. S. DENSLow, AND K. ICKES. 2004b. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology* 85: 471–483.
- ELLSTRAND, N. C., AND K. A. SCHIERENBECK. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA* 97: 7043–7050.
- GEMMILL, C. E. C., T. A. RANKER, D. RAGONE, S. P. PERLMAN, AND K. R. WOOD. 1998. Conservation genetics of the endangered endemic Hawaiian genus *Brighamia* (Campanulaceae). *American Journal of Botany* 85: 528–539.
- GERLACH, J. 1993. Invasive Melastomataceae in Seychelles. *Oryx* 27: 22–26.
- GILLIES, A. C. M., J. P. CORNELIUS, A. C. NEWTON, C. NAVARRO, M. HERNANDEZ, AND J. WILSON. 1997. Genetic variation in Costa Rican populations of the tropical timber species *Cedrela odorata* L., assessed using RAPDs. *Molecular Ecology* 6: 1133–1145.
- GITZENDANNER, M. A., AND P. S. SOLTIS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87: 783–792.
- GODT, M. J. W., AND J. L. HAMRICK. 1991. Genetic variation in *Lathyrus latifolius* (Leguminosae). *American Journal of Botany* 78: 1163–1171.
- GRAY, A. J. 1986. Do invading species have definable genetic characteristics? *Philosophical Transactions of the Royal Society of London, B* 314: 655–674.
- HAMRICK, J. L., AND M. J. W. GODT. 1989. Allozyme diversity in plant species. In A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], *Plant population genetics, breeding and genetic resources*, 43–63. Sinauer, Sunderland, Massachusetts, USA.
- HAMRICK, J. L., AND M. J. W. GODT. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, B* 351: 1291–1298.
- HAMRICK, J. L., M. J. W. GODT, AND S. L. SHERMAN-BROYLES. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.
- HAMRICK, J. L., Y. B. LINHART, AND J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* 10: 173–200.
- HUSBAND, B. C., AND S. C. H. BARRETT. 1991. Colonization history and population genetic structure of *Eichhornia paniculata* in Jamaica. *Heredity* 66: 287–296.
- LI, C. C., AND D. G. HOROVITZ. 1953. Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics* 5: 107–117.
- MACK, R. N., D. SIMBERLOFF, W. M. LONSDALE, H. EVANS, M. CLOUT, AND F. A. BAZZAZ. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689–710.
- MEEKINS, J. F., H. E. BALLARD, AND B. C. MCCARTHY. 2001. Genetic variation and molecular biogeography of a North American invasive plant species (*Alliaria petiolata*, Brassicaceae). *International Journal of Plant Sciences* 162: 161–169.
- MELO, G. F. A., I. C. MACHADO, AND M. LUCEÑO. 1999. Reproducción de tres especies de *Clidemia* (Melastomataceae) en Brasil. *Revista de Biología Tropical* 47: 359–363.
- MINCHIN, P. R. 1987. An evaluation of relative robustness of techniques for ecological ordination. *Vegetatio* 69: 89–107.
- MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of *Ponderosa* pine. *Journal of Heredity* 70: 86–89.
- NEI, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.
- NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.
- NEI, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.
- NEUFFER, B., AND H. HURKA. 1999. Colonization history and introduction dynamics of *Capsella bursa-pastoris* (Brassicaceae) in North America: isozymes and quantitative traits. *Molecular Ecology* 8: 1667–1681.
- NOVAK, S. J., AND R. N. MACK. 1993. Genetic variation in *Bromus tectorum*: comparison between native and introduced populations. *Heredity* 71: 167–176.
- NOVAK, S. J., AND R. N. MACK. 1995. Allozyme diversity in the apomictic vine *Bryonia alba* (Cucurbitaceae): potential consequences of multiple introductions. *American Journal of Botany* 82: 1153–1162.
- NOVAK, S. J., R. N. MACK, AND D. E. SOLTIS. 1991. Genetic variation in *Bromus tectorum*: population differentiation in its North American range. *American Journal of Botany* 78: 1150–1161.
- PAPPERT, R. A., J. L. HAMRICK, AND L. A. DONOVAN. 2000. Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. *American Journal of Botany* 87: 1240–1245.
- PARKER, I. M., J. RODRIGUEZ, AND M. E. LOIK. 2003. An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology* 17: 59–72.
- RENNER, S. S. 1989. A survey of reproductive biology in neotropical Melastomataceae and Memecylaceae. *Annals of the Missouri Botanical Garden* 76: 496–518.
- RIVERA-OCASIO, E., T. M. AIDE, AND W. O. MCMILLAN. 2002. Patterns of genetic diversity and biogeographical history of the tropical wetland tree, *Pterocarpus officinalis* (Jacq.), in the Caribbean basin. *Molecular Ecology* 11: 675–683.
- SAKAI, A. K., ET AL. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332.
- SALTONSTALL, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences, USA* 99: 2445–2449.
- SAS INSTITUTE. 2000. SAS/STAT user's guide, version 8, vols. 1–3. SAS Institute, Cary, North Carolina, USA.
- SAWKINS, M. C., B. L. MAASS, B. C. PENGELLY, H. J. NEWBURY, B. V. FORD-LLOYD, N. MAXTED, AND R. SMITH. 2001. Geographical patterns of genetic variation in two species of *Stylosanthes* Sw. using amplified fragment length polymorphism. *Molecular Ecology* 10: 1947–1958.
- SCHIERENBECK, K. A., J. L. HAMRICK, AND R. N. MACK. 1995. Comparison of allozyme variability in a native and an introduced species of *Lonicera*. *Heredity* 75: 1–9.
- SHEIL, D. 1994. Naturalized and invasive species in the evergreen forests of the East Usambara Mountains, Tanzania. *African Journal of Ecology* 32: 66–71.
- SIMMONDS, H. W. 1933. The biological control of the weed *Clidemia hirta*, D. Don., in Fiji. *Bulletin of Entomological Research* 24: 345–348.
- SIMMONDS, H. W. 1937. The biological control of the weed *Clidemia hirta*, commonly known in Fiji as “The Curse.” *Fiji Agricultural Journal* 8: 37–39.
- SINGHAKUMARA, B. M. P., R. S. J. P. UDUPORUWA, AND P. M. S. ASHTON. 2000. Soil seed banks in relation to light and topographic position of a hill dipterocarp forest in Sri Lanka. *Biotropica* 32: 190–196.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39: 53–65.
- SLATKIN, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47: 264–279.
- SMITH, C. W. 1992. Distribution, status, phenology, rate of spread, and management of *Clidemia* in Hawaii. In C. P. Stone, J. T. Tunison, and C. W. Smith [eds.], *Alien plant invasions in native ecosystems of Hawaii: management and research*, 241–253. University of Hawaii Cooperative National Park Resources Studies Unit, Honolulu, Hawaii, USA.
- SOLTIS, D. E., C. H. HAUFLE, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27.
- SQUIRELL, J., P. M. HOLLINGSWORTH, R. M. BATEMAN, J. H. DICKSON, M. H. S. LIGHT, M. MACCONAILL, AND M. C. TEBBITT. 2001. Partitioning and diversity of nuclear and organelle markers in native and introduced populations of *Epipactis helleborine* (Orchidaceae). *American Journal of Botany* 88: 1409–1418.
- STEYERMARK, J. A., AND B. HUBER. 1978. Flora del Avila: flora y vegetación de las montañas del Avila, de la Silla y del Naiguatá. Sociedad Venezolana de Ciencias Naturales, Caracas, Venezuela.
- STRAHM, W. 1999. Invasive species in Mauritius: examining the past and

- charting the future. In O. T. Sandlund, P. J. Schei, and A. Viken [eds.], *Invasive species and biodiversity management*, 325–347. Kluwer, Dordrecht, Netherlands.
- TRESEDER, K. K., AND P. M. VITOUSEK. 2001. Potential ecosystem-level effects of genetic variation among populations of *Metrosideros polymorpha* from a soil fertility gradient in Hawaii. *Oecologia* 126: 266–275.
- WANG, R.-L., J. F. WENDEL, AND J. H. DEKKER. 1995. Weedy adaptation in *Setaria* spp. I. Isozyme analysis of genetic diversity and population genetic structure in *Setaria viridis*. *American Journal of Botany* 82: 308–317.
- WARWICK, S. I., B. K. THOMPSON, AND L. D. BLACK. 1987a. Genetic variation in Canadian and European populations of the colonizing weed species *Apera spica-venti*. *New Phytologist* 106: 301–307.
- WARWICK, S. I., B. K. THOMPSON, AND L. D. BLACK. 1987b. Life history and allozyme variation in populations of the weed species *Setaria faberi*. *Canadian Journal of Botany* 65: 1396–1402.
- WELLER, S. G., A. K. SAKAI, AND C. STRAUB. 1996. Allozyme diversity and genetic identity in *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae) in the Hawaiian Islands. *Evolution* 50: 23–34.
- WENDEL, J. F., AND C. R. PARKS. 1982. Genetic control of isozyme variation in *Camellia japonica* L. (Theaceae). *Journal of Heredity* 7: 197–204.
- WESTER, L. 1992. Origin and distribution of adventive alien flowering plants in Hawaii. In C. P. Stone, J. T. Tunison, and C. W. Smith [eds.], *Alien plant invasions in native ecosystems of Hawaii: management and research*, 99–154. University of Hawaii Cooperative National Park Resources Studies Unit, Honolulu, Hawaii, USA.
- WESTER, L., AND H. B. WOOD. 1977. Koster's curse (*Clidemia hirta*), a weed pest in Hawaiian forests. *Environmental Conservation* 4: 35–41.
- WHITLOCK, M. C., AND D. E. MCCAULEY. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity* 82: 117–125.
- WITTER, M. S., AND G. D. CARR. 1988. Adaptive radiation and genetic differentiation in the Hawaiian silversword alliance (Compositae: Madinae). *Evolution* 42: 1278–1287.
- WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on Southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* 22: 24–29.
- WRIGHT, S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323–354.
- WURDACK, J. J. 1980. Melastomataceae. In G. Harling and B. Sparre [eds.], *Flora of Ecuador*, 1–406. Swedish Natural Science Research Council, Stockholm, Sweden.