Morphological and Genetic Variation in the Endemic Sea Grass Halophila hawaiiana (Hydrocharitaceae) in the Hawaiian Archipelago

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Morphological and Genetic Variation in the Endemic Seagrass *Halophila hawaiiana* (Hydrocharitaceae) in the Hawaiian Archipelago[1]

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Abstract: The endemic seagrass *Halophila hawaiiana* Doty & Stone is found in discrete populations throughout the Hawaiian Archipelago. Morphological characteristics of plants from Midway Atoll, Pearl and Hermes Reef, Kaua'i, O'ahu, Moloka'i, and Maui were measured and compared. Striking variation in leaf length, leaf width, leaf length to width ratio, and internode length was evident among the 18 collection sites sampled at depths ranging from 0.32 to 18 m. DNA sequence analyses of a chloroplast-genome, single-base repeat locus in ramets from nine different collections found only two repeat haplotypes. Repeat haplotypes were fixed at all collection sites and for all islands except O'ahu.

THERE ARE TWO species of seagrass found in the Hawaiian Islands. *Halophila hawaiiana* Doty & Stone was described as a new, endemic species in 1966. A second species, *Halophila decipiens* Ostenfeld, was recently reported from Midway Atoll, O'ahu, and the island of Hawai'i (McDermid et al. 2002). Research on *Halophila* began with Doty and Stone (1966), who recorded collections of *H. hawaiiana* from the islands of Kaua'i, O'ahu, Moloka'i, and Maui. Herbert (1984, 1986) studied the growth dynamics of one population of *H. hawaiiana;* estimated biomass, productivity, and turnover; and developed a growth model. High productivity levels were observed and applied to a hypothesis about the pioneer-type successional characteristics in this *Halophila* species (Herbert 1986). A study by Michael-Taxis (1993) focused on the surface of *H. hawaiiana* leaves as a primary site for recruitment of epiphytes. She extensively described ecological contributions of *H. hawaiiana* meadows on O'ahu, chemical content of the leaves, leaf ultrastructure, and the anatomy of *H. hawaiiana. Halophila hawaiiana* plants support a native grazing snail, *Smaragdia bryanae* Pilsbry (Unabia 1984). The leaves of *H. hawaiiana* also serve as a food source for other grazers such as the threatened Hawaiian green sea turtle, *Chelonia mydas* (Linnaeus) Schweigger (Balazs 1980, Russell and Balazs 2000, Balazs et al. in press). However, knowledge of many other aspects of *H. hawaiiana* biology and ecology is limited.

The goals of this research were (1) to systematically document the morphological variation among *Halophila hawaiiana* populations, both within and among islands in the Hawaiian Archipelago, and (2) to conduct DNA analyses on specimens from different islands to assess genetic diversity among populations. Sequences of the intergenic spacers and intron of the *trnL* region of the chloroplast genome in *Halophila* species have a number of single-base nucleotide repeats (D.W.F., Robert A. York, and J.W.R., unpubl. data). In this study, variation at a repeat within the *trnL* intron was determined for separate ramets of *H. hawaiiana.*

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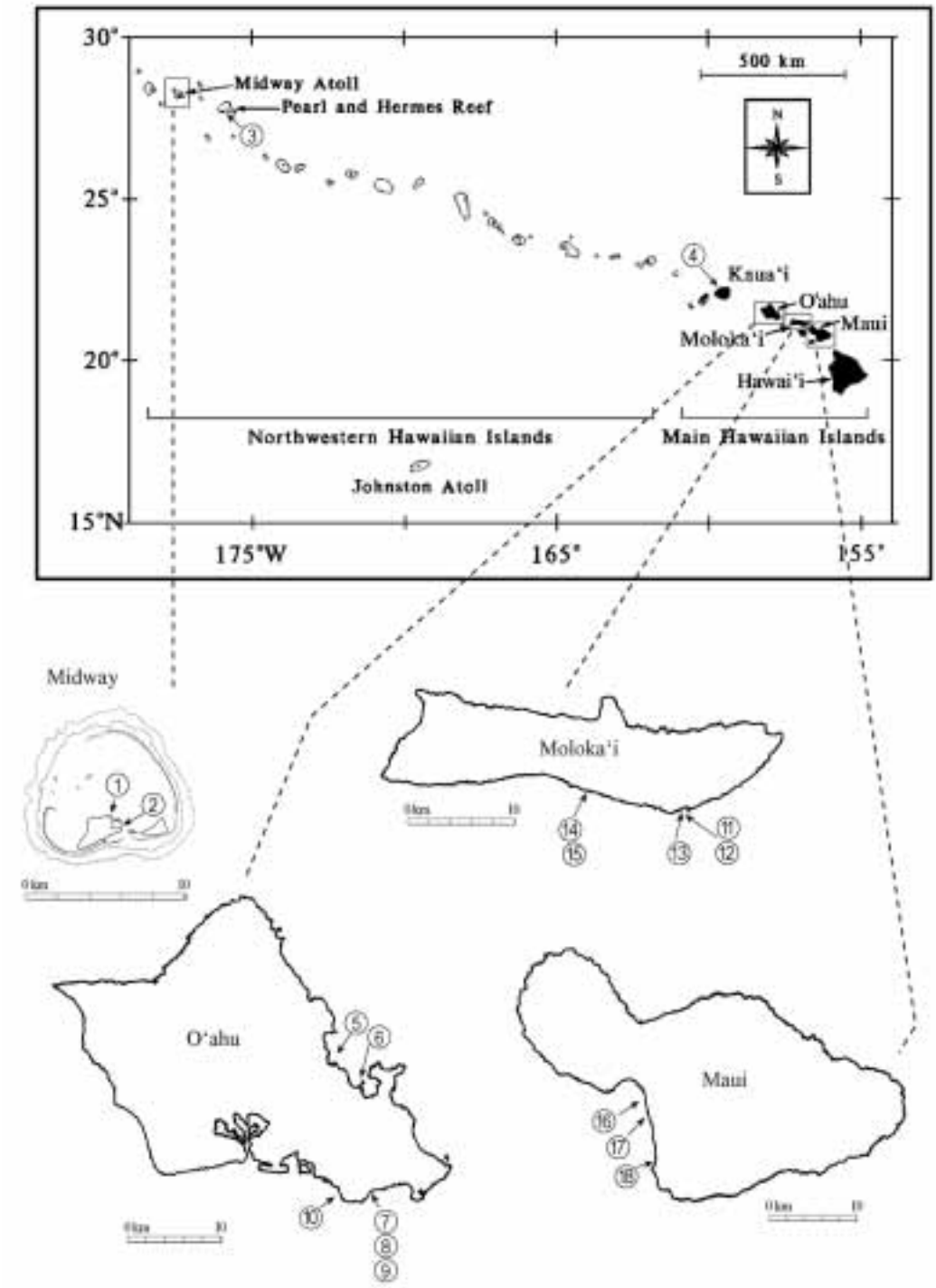


FIGURE 1. Map of the Hawaiian Archipelago. Locations of collection sites are numbered in order from north to south and correspond to site numbers in Table 1.

TABLE 1

List of 19 Collection Sites Throughout the Hawaiian Archipelago by Island from North to South

Collection Site	Collection No.[a]	Collection Date	Depth (m)
1 Midway, cargo pier	KM 4317	20 September 1996	10
2 Midway, harbor breakwall	KM 4666	11 June 1999	1.5–3
M Midway, inner harbor	KM 5411	27 July 2001	Drift
3 Pearl & Hermes Reef	RO 74	9 October 2000	8
4 Kaua'i, 'Anini Beach Road	Bishop 8464	21 February 2000	1–1.5
5 O'ahu, Mark's Reef, Kāne'ohe	MG 35	29 January 2001	3
6 O'ahu, Coconut Island	MG 31	10 November 2000	0.6
7 O'ahu, Kahala	Bishop 14171	21 May 1964	—
8 O'ahu, Kahala	KM 16	28 September 1983	<1
9 O'ahu, Kahala	KM 5355	8 June 2001	2
10 O'ahu, Waikīkī Natatorium	D&S 3208	—	1
11 Moloka'i, Keawa Nui Pond	KM 915	29 November 1985	<1
12 Moloka'i, Keawa Nui Pond	KM 916	29 November 1985	<1
13 Moloka'i, 'Ōhi'a Bridge Pond	KM 5347	23 March 2001	0.32
14 Moloka'i, Hotel Moloka'i	KM 5349	24 March 2001	1.5
15 Moloka'i, Hotel Moloka'i	KM 5348	24 March 2001	1.5
16 Maui, Kīhei	MG 32	15 January 2001	1
17 Maui, Līpoa Street	MG 33	15 January 2001	1
18 Maui, Mākena Beach	KM 5357	9 July 2001	18

[a] KM, K. McDermid; RO, R. Okano; Bishop, Bishop Museum; MG, M. Gregoritza; D&S, Doty and Stone.

MATERIALS AND METHODS

Morphometrics of plants from 18 collection sites on six islands, including Midway Atoll, Pearl and Hermes Reef, Kaua'i, O'ahu, Moloka'i, and Maui (Figure 1, Table 1) were analyzed using techniques based on Tomlinson (1982), Williams and Dennison (1990), Larkum (1995), and Procaccini et al. (1999). From November 2000 to July 2001, collection sites were visited by wading, snorkeling, or with SCUBA, and live voucher specimens were uprooted by hand or with a trowel, rinsed in seawater to remove sediment and debris, placed in seawater in a watertight container, and transported in a cooler to the laboratory. Plants were photographed, preserved as herbarium specimens that will be donated to the Bishop Museum (BISH), and on some occasions preserved in 4% formalin-seawater solution. Individual plants ($n = 20$ whenever possible) were chosen haphazardly from the collections, as well as from herbarium sheets of collections made before November 2000. The plants were measured using calipers to obtain values for leaf length (extending from the node on the rhizome to the outer edge of the blade tip), leaf width (at the widest point), and the internode length between leaf pairs. Means of these measurements were calculated. In addition, a mean leaf length : width (L : W) ratio was calculated for each collection site or meadow. One-way analysis of variance (ANOVA) was conducted on the morphometric data.

Seagrass samples for DNA analysis were taken from nine collection sites between November 2000 and July 2001. Individual sections of plants including a growing tip and three leaf pairs along the rhizome were hand-collected at least 5 m apart, rinsed in seawater, cleaned of any epiphytes, and then placed in a plastic bag containing silica gel desiccant (28–200 mesh) (Chase and Hills 1991). Dried samples in silica were mailed to two of the authors (J.W.R. and D.W.F.) at the Center for Marine Science, Wilmington, North Carolina, for testing. Total genomic DNA was extracted from two to three leaf pairs using the DNeasy Plant Mini Kit and protocol (Qiagen, Inc., Valencia, California). An approximate 400–base pair (bp) section of the *trnL* intron was amplified using the forward primer 5′-GCA AAT CGG TAG ACG

CTA CG-3′ and reverse primer 5′-GCA ATT TCG ATA GAA AGA TCC-3′. Amplification reactions were set up in 50-μl volumes containing 40–100 ng DNA template, 1.25 mM $MgCl_2$, 80 μM each dNTP, 0.2 μM each flanking primer and HotStarTaq DNA polymerase and reaction buffer as suggested by the manufacturer (Qiagen, Inc., Valencia, California). The thermocycling protocol followed that of Freshwater et al. (2000) with the addition of an initial enzyme activation step of 15 min at 95°C. Reaction products were cleaned of excess dNTPs, enzymes, and primers using a GeneClean II Kit (Qbiogene, Carlsbad, California) and used as templates in sequencing reactions set up using the Big Dye Ready Reaction Kit and protocol (Applied Biosystems, Foster City, California). Sequencing reactions were run on an ABI Prism 377 DNA Sequencer (DNA Analysis Core Facility, Center for Marine Science). Sequence reactions were generated through the repeat locus in both the forward and reverse directions, and the separate reactions combined and repeat number determined using Sequencher (Gene Codes Corp., Ann Arbor, Michigan).

RESULTS

Morphometrics

The leaves and rhizomes showed great variability among collection sites of *Halophila hawaiiana* (Figures 2–9). Samples from the Northwestern Hawaiian Islands of Midway Atoll and Pearl and Hermes Reef (Figures 2 and 3) displayed similar spatulate leaf shape and size. O'ahu and Maui specimens (Figures 4 and 5) had more elongated leaves. However, on one island, Moloka'i, leaves varied from short and spatulate to paddle-shaped (Figures 6 and 7) to long and narrow and straplike (very uncharacteristic for the genus) (Figures 8 and 9). Mean leaf lengths among meadows ranged from 2.03 to 5.36 cm, leaf widths ranged from 0.12 to 0.68 cm, and internode length ranged from 0.93 to 2.45 cm (Table 2, Figure 10). However, the mean leaf length, leaf width, and internode length within the meadows showed little variation, as demonstrated by the small standard error values. The leaf L:W ratios showed the greatest intermeadow variation. Plants in a Midway Atoll meadow (site 2) had the shortest leaves with the smallest L:W ratio (5.21), and plants from Maui (site 18) had the longest, narrow leaves resulting in the largest L:W ratio (41.23). The greatest variety of leaf forms was observed among collection sites on Moloka'i. Plants from site 11 on Moloka'i displayed the narrowest mean leaf width, and plants collected at site 13 (less than 3 km away) the widest.

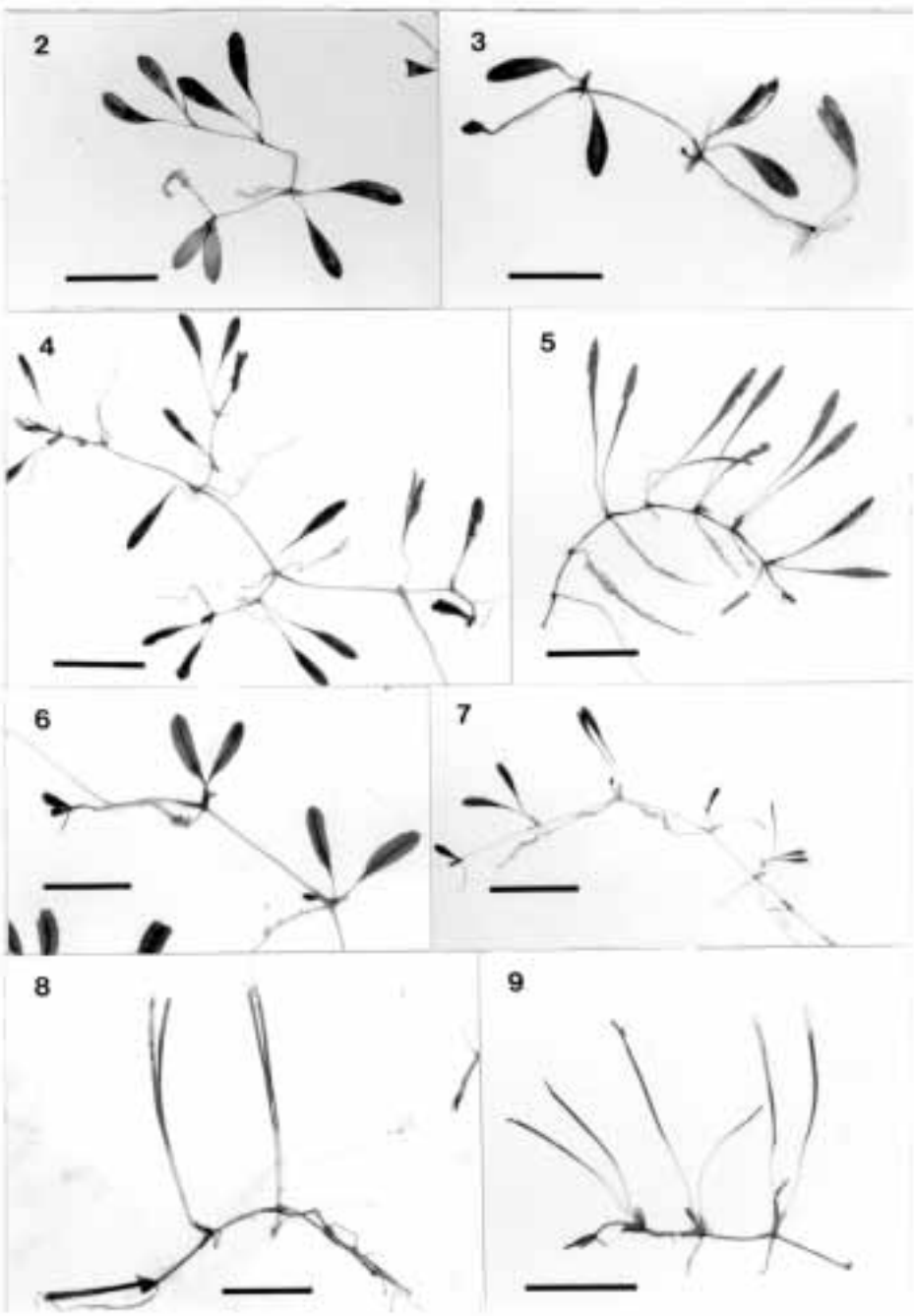
No correlation was found between latitude and any of the morphological characteristics. Yet ANOVA showed that for each of the traits (leaf length, leaf width, L:W ratio, and internode length) there were significant differences ($P < 0.001$) among the 18 collection sites (Table 3).

DNA Analysis

Variation in nucleotide repeat number at an adenine repeat within the *trnL* intron was tested in 64 *H. hawaiiana* samples from nine collection sites within the Hawaiian Archipelago. This particular locus is 104 bp from the 3′ end of the first *trnL* exon. Only two repeat haplotypes, 11 adenines (11A) and 12 adenines (12A), were detected in the 64 samples. No variation was found in the adjacent intron sequence 100 bp 5′ or 225 bp 3′ of the repeat. Repeat haplotypes were found to be fixed at a collection site and from all collection sites from a particular island except O'ahu (Table 4). Samples from Kahala on the south shore of O'ahu had the 12A haplotype, whereas samples from Coconut Island on the northeast side of O'ahu had the 11A haplotype.

DISCUSSION

The results of this study document the amazing diversity of morphologies in *Halophila hawaiiana*, characterized by great variation in leaf length, leaf width, leaf L:W ratio, and internode length among 18 collection sites occurring in the Hawaiian Archipelago from Midway Atoll at 28°N latitude to Maui at



FIGURES 2–9. *Halophila hawaiiana*. Scale bar in each figure = 2 cm. 2, Cargo pier, Midway Atoll, site 1, 20 September 1990 (KM 4317); 3, Pearl and Hermes Reef, site 3, 9 October 2000 (RO 74); 4, Kahala, O'ahu, site 8, 28 September 1983 (KM 16); 5, Lipoa Street, Maui, site 17, 15 January 2001 (MG 33); 6, 'Ohi'a Bridge Pond, Moloka'i, site 13, 23 March 2001 (KM 5347); 7, Hotel Moloka'i, Moloka'i, site 14, 24 March 2001 (KM 5349); 8, Keawa Nui Pond, Moloka'i, site 11, 29 November 1985 (KM 915); 9, Hotel Moloka'i, Moloka'i, site 15, 24 March 2001 (KM 5348).

TABLE 2

Results of Mean Leaf Length, Leaf Width, Length:Width Ratio, and Internode Length Measurements of *Halophila hawaiiana* from 18 Meadows Throughout the Hawaiian Archipelago (Collection Sites Correspond to Locations in Table 1)

Collection Site (sample size)	Mean Length (cm)	Mean Width (cm)	Mean L:W Ratio (cm)	Mean Internode Length (cm)
1 Midway ($n = 20$)	2.60	0.43	6.05	2.23
2 Midway ($n = 10$)	2.03	0.39	5.21	1.81
3 Pearl & Hermes Reef ($n = 20$)	3.02	0.50	6.04	2.22
4 Kaua'i ($n = 5$)	3.29	0.30	10.87	0.93
5 O'ahu ($n = 20$)	2.79	0.46	6.07	2.24
6 O'ahu ($n = 20$)	2.38	0.25	9.52	1.28
7 O'ahu ($n = 5$)	2.91	0.27	10.78	1.50
8 O'ahu ($n = 20$)	2.34	0.24	9.75	1.35
9 O'ahu ($n = 20$)	2.98	0.35	8.51	2.45
10 O'ahu ($n = 5$)	2.85	0.25	11.40	2.35
11 Moloka'i ($n = 20$)	4.58	0.12	38.17	1.18
12 Moloka'i ($n = 20$)	3.22	0.53	6.08	1.59
13 Moloka'i ($n = 20$)	3.70	0.68	5.44	2.43
14 Moloka'i ($n = 20$)	3.16	0.26	12.15	1.91
15 Moloka'i ($n = 20$)	3.29	0.15	21.93	1.43
16 Maui ($n = 20$)	4.18	0.37	11.30	0.94
17 Maui ($n = 20$)	4.18	0.25	16.72	1.04
18 Maui ($n = 20$)	5.36	0.13	41.23	1.29

21°N. The leaf L:W ratios showed the greatest variation between collection sites; however, the within-meadow leaf length, leaf width, and internode length values were highly consistent. Leaf shape was so unusual and un-*Halophila*-like in some Moloka'i plants that previous collectors had mislabeled their specimens as *Halodule* sp.! Careful examination of all the specimens in this study revealed that they are all *Halophila hawaiiana*, and our DNA analyses confirmed their common taxonomic identity. The apparently plastic phenotype of *H. hawaiiana* may be influenced by environmental conditions. Previous studies by Williams and Dennison (1990), Ralph and Burchett (1995), Dawson and Dennison (1996), Benjamin et al. (1999), Longstaff and Dennison (1999), and Schwarz et al. (2000) demonstrated the effects of some abiotic factors on morphology, physiology, and survival in various *Halophila* species. The influence of environmental conditions on leaf width in North American seagrasses was shown by Phillips and Lewis (1983). Powell et al. (1989) reported longer and wider leaves in *Thalassia testudinum* Banks ex König at nutrient-enriched collection sites. During our study, differences in depth, salinity, water temperature, photosynthetically active radiation levels, and sediment grain size were noted in the *H. hawaiiana* habitats, and further study will assess possible cause-effect relationships among abiotic factors, morphology, and *Halophila* abundance.

Morphological diversity in a species may be accompanied by genetic diversity, but even when this occurs the two are not always directly correlated. In Fiji and Western Samoa, *Halophila* plants that differed in leaf structure (i.e., smooth versus bullate) had identical isozyme patterns for five enzyme systems; however, differences in isozymes indicating genetic variation were seen in between-island comparisons of plants (McMillan and Bridges 1982). Similarly, Sicilian populations of *H. stipulacea* (Forsskål) Ascherson exhibited both significant morphological variations with depth and collection location, and high genetic polymorphism between sample sites and among depths (Procaccini et al. 1999). Al-

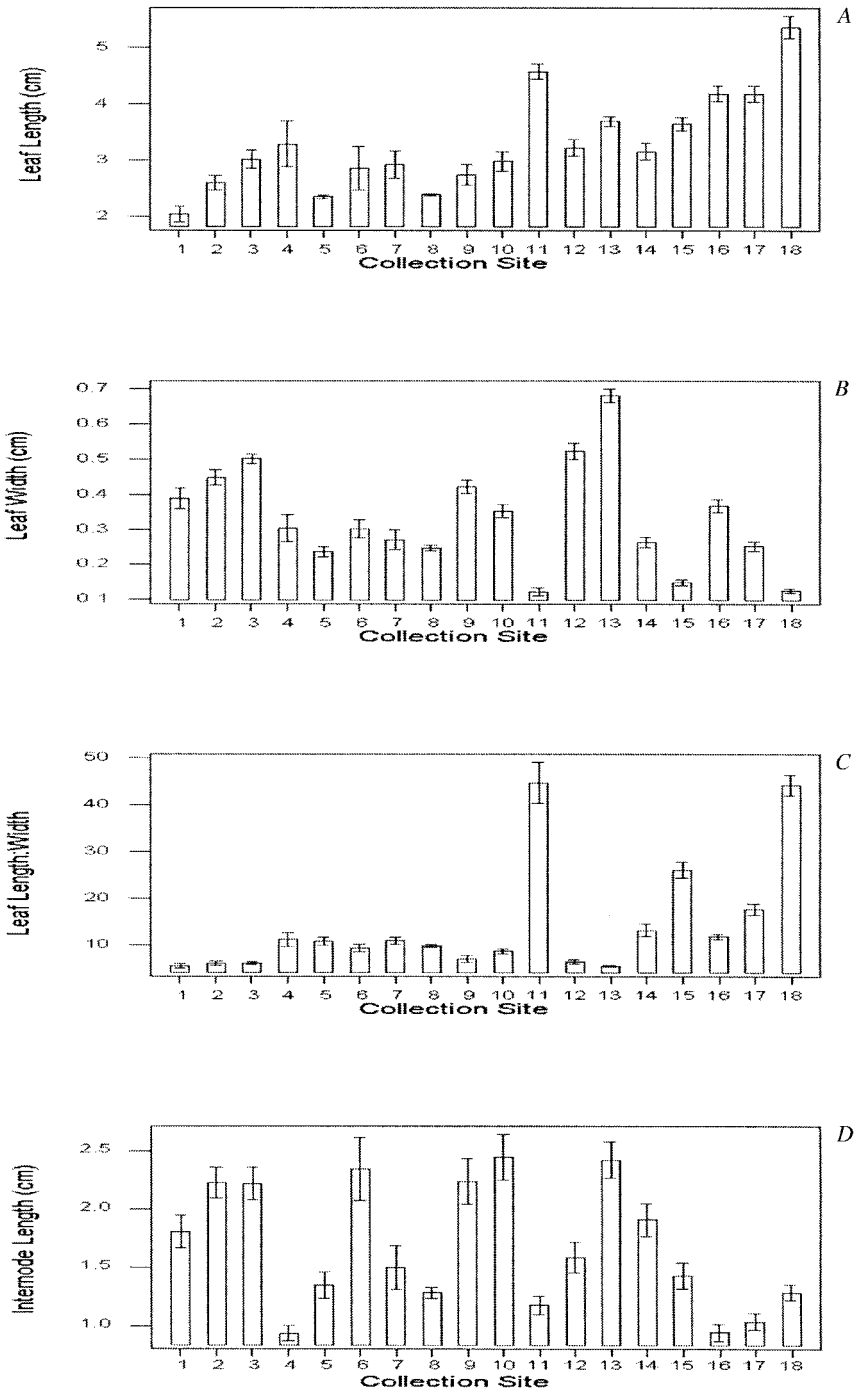


FIGURE 10. Mean measurements of *H. hawaiiana* leaves from 18 populations. Locations of collection sites are numbered in order from north to south and correspond to site numbers in Table 1. Bars represent the standard error of the mean (SE). *A*, mean leaf lengths; *B*, mean leaf widths; *C*, mean values for leaf length : width ratios; *D*, mean internode lengths.

TABLE 3

Results of ANOVA Performed on Leaf Length, Leaf Width, Length:Width Ratio, and Internode Length of *Halophila hawaiiana* from 18 Collection Sites

Source of Variation	DF	Leaf Length			Leaf Width			Length:Width Ratio			Internode Length		
		SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
Coll. Site	17	226.185	13.305	34.7	7.225	0.425	85.0	48,263	2839	65.6	81.804	4.812	15.47
Error	287	109.921	0.383		1.435	0.005		12,427.1	43.3		89.257	0.311	
Total	304	336.106			8.660			60,690.1			171.061		
Critical value for $F_{0.001(1)17,200} = 2.56$						$P < 0.001$							
Critical value for $F_{0.001(1)17,300} = 2.50$													

Note: DF, degrees of freedom; SS, sum of squares; MS, mean squared deviation from the mean; F, collection site MS/error MS.

TABLE 4

Distribution of Chloroplast *trnL* Intron Single-Base Repeat Haplotypes (11 Adenines = 11A, 12 Adenines = 12A) in 64 Sampled Ramets from Nine Collection Sites within the Hawaiian Archipelago

Collection Site	Collection No.	Sample Sizes (*n*)	Repeat Haplotypes
M Midway, inner harbor	KM 5411	3	12A
6 O'ahu, Coconut Island	MG 31	10	11A
9 O'ahu, Kahala	KM 5355	6	12A
13 Moloka'i, 'Ōhi'a Bridge Pond	KM 5347	8	11A
14 Moloka'i, Hotel Moloka'i	KM 5349	8	11A
15 Moloka'i, Hotel Moloka'i	KM 5348	10	11A
16 Maui, Kīhei	MG 32	8	12A
17 Maui, Līpoa	MG 33	7	12A
18 Maui, Mākena	KM 5357	4	12A

Note: All samples from a collection site shared the same repeat haplotypes.

though both morphological and genetic variations were detected, there was no significant correlation between the two.

In our study, despite significant variation in morphology, we detected only a low level of variation in the *trnL* intron: only two repeat haplotypes in 64 samples from nine different populations of *Halophila hawaiiana*. Previous studies generally have revealed a high level of intraspecific chloroplast repeat polymorphism (for review see Provan et al. 2001). Both high and low levels of intraspecific genetic variation have been reported in seagrasses (Waycott 1995, Procaccini et al. 1996, Waycott et al. 1997, Kirsten et al. 1998, Reusch et al. 1999, 2000, Reusch 2000). Examination of more loci with random amplified polymorphic DNAs (RAPDs) (e.g., Procaccini et al. 1999) or with nuclear microsatellite DNA (e.g., Reusch et al. 1999) is needed to make a full assessment of the genetic diversity of *H. hawaiiana* and its possible correlation to the observed morphological variation.

If the low variability indicated by our analyses of this particular locus is an accurate reflection of genetic diversity in *Halophila hawaiiana*, then we need to consider the possible factors maintaining genetic homogeneity within and between many collection sites/islands, yet also maintaining a genetic discontinuity between other collection sites/islands. Patterns of genetic relatedness among *Posidonia oceanica* (Linnaeus) Delile populations in Italy were found to be in accord with the direction of dominant currents (Procaccini et al. 2001). The North Hawaiian Ridge Current in the Hawaiian Islands flows northwestward along the northeastern coast

of the main Hawaiian Islands at an average speed of 0.1–0.15 m/sec and then flows westward northwest of Kaua'i (Qiu et al. 1997). In the lee of the islands, eddies, gyrepairs, and the Hawaiian Lee Counter Current at 19°N have been mapped (Qiu et al. 1997). The role of these currents in pollen and seed dispersal, and vegetative fragment transport is unknown. However, our *trnL*-intron repeat data suggest that there is a discontinuity in gene flow, as evidenced by the repeat haplotypes being "fixed" at a collection site and nearly so between islands. Some marine animal species with high dispersal potential (e.g., with planktonic larvae or long-distance migrating adults) have a practical dispersal limit, and gene flow can be affected by island position, ocean currents, random long-distance dispersal events, larval behavior, natal homing, and selection (Palumbi et al. 1997). Gene flow has been estimated in only a few seagrass populations (Williams and Davis 1996, Procaccini and Mazella 1998, Ruckelshaus 1998, Schlueter and Guttman 1998, Procaccini et al. 2001, Reusch 2001). The practical dispersal limit of *H. hawaiiana* remains undetermined.

Despite the apparent discontinuity found in the distribution of repeat haplotypes, there were only two haplotypes detected overall and no variation among ramets at a collection site. *Halophila hawaiiana* is a dioecious seagrass species (Doty and Stone 1966), and dioecy should enhance outcrossing and genetic variability (Procaccini and Mazella 1996, Reusch 2001). However, male flowers of *H. hawaiiana* are rarely observed, and the apparent low genetic diversity may be being maintained by vegetative or asexual reproduction. Low genetic diversity in *H. hawaiiana* may be the result of founder effect during initial colonization of the isolated Hawaiian Islands. In addition, this endemic seagrass may have suffered genetic erosion due to population-level inbreeding, and genetic drift within populations over evolutionary time.

The important ecological roles of *Halophila hawaiiana* as psammophytic primary producer, sediment stabilizer, and microhabitat builder in the coastal and coral reef ecosystems of the Hawaiian Islands call for further studies on the population dynamics and genetics of this species. Information on population growth and persistence, dispersal, genetic diversity, effective population size, mating system patterns, outcrossing rates, and clone size and dominance has implications for the conservation and management of *H. hawaiiana* populations, especially in terms of transplantation or restoration. The populations of *H. hawaiiana* are small, fragmented, and isolated between and within islands in the archipelago. Genetic structure of these populations may be critical to their survival in the face of natural ecological events, as well as anthropogenic disturbances and changing environmental conditions.

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