Genetic Population Structure of the Hawaiian Alien Invasive Seaweed *Acanthophora spicifera* (Rhodophyta) as Revealed by DNA Sequencing and ISSR Analyses[1]

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Abstract: *Acanthophora spicifera* (Vahl) Børgesen is the most widespread and invasive alien macroalga on coral reefs throughout the main Hawaiian Islands. This alga disperses from harbors and ports to coral reefs throughout the state, producing high quantities of biomass that affect a wide range of reef flora and fauna. Population samples of *A. spicifera* from across the main Hawaiian Islands were collected and compared through two kinds of analyses: DNA sequencing (based on a variable region of the nuclear large subunit ribosomal RNA gene, and the mitochondrial cox 2-3 spacer region) and fragment techniques (Inter-Simple Sequence Repeats [ISSRs]). DNA sequencing revealed no variation for the two markers, even when collections from other areas of the Pacific and Australia were included. In contrast, ISSR analyses revealed highly structured Hawaiian populations of *A. spicifera* with a substantial range of both within- and among-population variation, with individual plants forming discrete clusters corresponding to geographic locality.

THE INVASION OF coral reef ecosystems by nonnative species can alter ecosystem structure and reduce indigenous biodiversity (Pandolfi et al. 2005). In Hawai'i, the number of documented alien algal species has increased from 18 in 1992 (Russell and Balazs 1994) to 24 species in 2003 (Godwin 2003). Of the five notably invasive macroalgae, *Acanthophora spicifera* (Vahl) Børgesen is the most common species throughout the main Hawaiian Islands (Smith et al. 2002). Successful invaders such as *A. spicifera* have substantial impacts on Hawai'i's coral reef ecosystems (Godwin 2003). In addition to damaging native ecosystems, invasive algal blooms have caused economic losses to the state exceeding 20 million dollars (Smith et al. 2004). The widespread distribution and rapid growth of *A. spicifera* make it a particularly dangerous threat to Hawai'i's coral reefs and a priority species for control efforts.

The vegetative characteristics of *A. spicifera* contribute to it being a successful colonizer that is capable of attaching to, and competing with, native algal species (Russell and Balazs 1994). The spiny thalli of this brittle alga are easily fragmented by wave action (Kilar and McLachlan 1986) and readily snag on suitable substrates, including other macroalgae. Kilar and McLachlan (1986) found that plants growing on exposed reef in Panama were able to generate up to 74 kg of vegetative fragments per month, and each fragment had the potential to reattach after only 2 days. Although the reproductive phenology of alien algae in Hawaiian waters has not been thoroughly investigated, *A. spicifera* is the only species in this category that has been consistently observed in a sexually reproductive state (Smith et al. 2002). Genetic recombination may result in increased population-level variation that allows *A. spicifera* to be more resistant to control efforts than clonal invasive species. Reproductive species may pose an additional threat through the release of copious quantities of microscopic spores that

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disperse over great distances. As with many invasive organisms, the physical and reproductive characteristics of *A. spicifera* foster widespread dispersal and enable successful competition for resources within native reef communities.

Algal invasions are often related to anthropogenic factors, including aquaculture (Abbott 1999), eutrophication (Stimson et al. 2001), hull fouling (Smith et al. 2002), ballast water discharge (Godwin 2003), and overfishing (McClanahan et al. 2001, Pandolfi et al. 2005). The major phase shifts involving primary producers on coral reefs associated with these factors have substantial and often catastrophic impacts on macroalgal, coral, and fish assemblages (Littler and Littler 1985, Hughes 1994, Russell and Balazs 1994, Stimson et al. 2001, Pandolfi et al. 2005). *Acanthophora spicifera* is implicated in major phase shifts on Hawaiian reefs because it has been found to produce high biomass throughout the main Hawaiian Islands (Russell and Balazs 1994). In Hawai'i, alien algae have been documented to compete with native species of algae (Russell and Balazs 1994) and corals (Stimson et al. 2001). However, the ecological interactions involving *A. spicifera* on Hawaiian reefs may be complex. For example, the regular consumption of *A. spicifera* by Hawaiian sea turtles and herbivorous fish has been documented by Russell and Balazs (1994). Although the effects of alien algae ingestion on the diets of native herbivores are unknown (Smith et al. 2004), a large increase in edible algal biomass at a particular locale would be expected to have numerous impacts throughout the reef community.

Although research concerning the effects of invasive macroalgae on tropical reefs is still limited (e.g., Coles and Eldredge 2002, Smith et al. 2004), even fewer studies have examined the genetic structure of invasive algae in tropical waters. In temperate latitudes, DNA sequencing and fragment techniques have been used to investigate invasions of green and red seaweeds. These molecular tools have been used to determine the possible geographic sources and the genetic diversity of notable invasive algae such as *Caulerpa taxifolia*, *Grateloupia doryphora*, and *Asparagopsis taxiformis* (Famà et al. 2002, Marston and Villalard-Bohnsack 2002, Chualáin et al. 2004). In the study reported here, DNA sequence analyses of a region of the nuclear ribosomal large subunit gene and the mitochondrial cox 2-3 spacer region, and Inter-Simple Sequence Repeats (ISSRs) were used to investigate the population structure of *A. spicifera* throughout the main Hawaiian Islands. ISSRs are a cost-effective method for the resolution of intraspecific genetic variation and have been used to study a wide variety of plant taxa (Li and Ge 2001, Sica et al. 2005), as well as other red algae (Vis 1999, Sherwood et al. 2002). Here we illustrate the utility of ISSR analyses for revealing the degree of genetic structure within and between populations, and use these data to provide insight and recommendations for management of this alien invasive seaweed.

MATERIALS AND METHODS

Sample Collection

Specimens were collected from tide pools and reef flats by snorkeling or wading. Individuals of *A. spicifera* were collected from O'ahu ($n = 30$), Moloka'i ($n = 7$), Maui ($n = 15$), and Hawai'i ($n = 15$) (Figure 1, Table 1). For each population, individuals were collected from an area no more than 30 m on the longest dimension. Freshly collected samples from O'ahu were immediately cooled and transported to the laboratory for processing. Samples from other Hawaiian islands were transported to O'ahu frozen in seawater or desiccated in silica gel.

DNA Extraction and Morphological Voucher Preparation

Before DNA extraction, 10 of the cleanest individuals from each location were examined under a stereomicroscope to determine reproductive status and to remove visible epiphytes. When reproductive status (tetrasporangial, spermatangial, or carpogonial) was not discernible using stereomicroscopy,
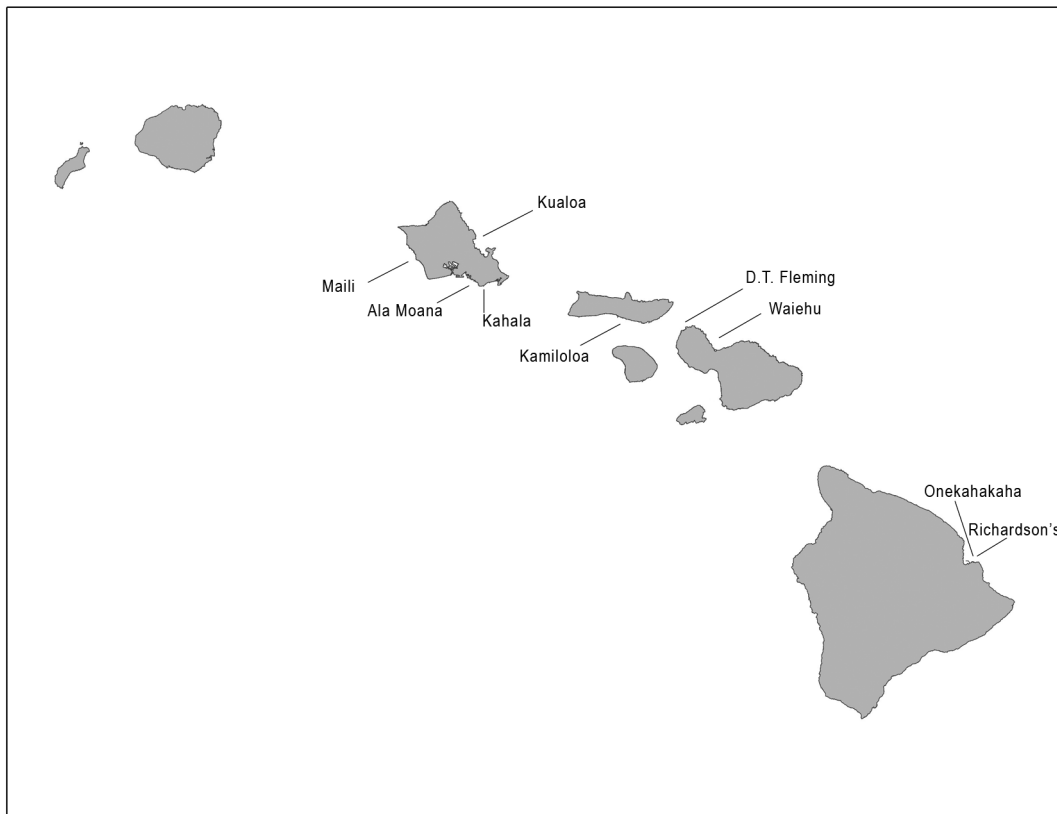
FIGURE 1. Map of collection localities for *Acanthophora spicifera* samples used in this study.

specimens were mounted, sectioned, stained in 1% aniline blue, and examined with a compound microscope (Olympus BX51). Total genomic DNA was extracted by freezing in liquid nitrogen, grinding using a mortar and pestle, and processing approximately 50 mg of fresh material or 10 mg of silica-dried material using the Qiagen DNeasy Plant Mini Kit (Valencia, California). All DNA extracts were stored in labeled screw-cap vials at $-20°C$. Remaining plants were processed as morphological vouchers, either stored in 4% formalin/seawater or pressed onto herbarium sheets. Voucher specimens of each population are maintained in the Sherwood Laboratory at the University of Hawai'i at Mānoa (DCO001–DCO009).

DNA Sequence Analyses

Genetic regions representing the nuclear and mitochondrial genomes were investigated through DNA sequencing for a subset of the *A. spicifera* collections from the main Hawaiian Islands. The same DNA extracts were used as for the ISSR study. The mitochondrial cox 2-3 spacer region was sequenced for 43 individuals (20 from O'ahu, two from Moloka'i, 11 from Maui, and 10 from Hawai'i) using previously published reaction and cycling conditions (Zuccarello et al. 1999). Several additional *A. spicifera* collections from other parts of the Pacific were added to the DNA sequencing study as a comparison across a broader geographical

TABLE 1

Collection Details for Populations of *Acanthophora spicifera* Analyzed in ISSR and DNA Sequence Analyses

Collection Location[a]	Collection Date	Collector	Reproductive Status	Exposure
Richardson's BP, Hawai'i	13 Jan. 2005	R. Okano	Unknown	Partially protected cove: windward
Kahala BP, O'ahu	16 Jan. 2005	A.R.S. & G. Presting	Unknown	Exposed reef: leeward
Kualoa BP, O'ahu	05 Feb. 2005	D.C.O.	Sterile	Exposed reef: windward
Waiehu BP, Maui	21 May 2005	A.R.S. & G. Presting	Sterile	Exposed reef: windward
Ala Moana BP, O'ahu	08 July 2005	V. Nestor	Tetrasporangial	Sheltered/exposed reef: leeward
Onekahakaha BP, Hawai'i	13 Jan. 2005	R. Okano	Unknown	Sheltered tide pool: windward
Maili BP, O'ahu	26 May 2005	D.C.O.	Tetrasporangial	Exposed reef: leeward
D. T. Fleming BP, Maui	21 May 2005	A.R.S. & G. Presting	Sterile	Exposed reef: leeward
Kamiloloa BP, Moloka'i	28 Apr. 2005	B. Puleloa	Unknown	Exposed reef: leeward
*Uken Beach, Okinawa Is., Japan	08 Mar. 2005	R. Terada	Unknown	Unknown
*Pago Bay, Guam	03 Oct. 2004	R. Tsuda	Unknown	Unknown
*Bangi Is., Guam	01 Apr. 2005	K. Peyton & L. Basch	Unknown	Unknown
*Townsville, Australia	24 Mar. 2005	P. Skelton	Unknown	Unknown

[a] BP = beach park.
* Collections used only for DNA sequencing.

range. Samples from Guam (Pago Bay), Australia (Townsville), and Japan (Uken Beach, Okinawa) were sequenced for the cox 2-3 spacer and the LSU-Y fragment to provide a context for comparing the DNA sequence variation in the Hawaiian collections. This spacer region has been successfully used to discern population structure for red algae (Zuccarello et al. 1999) and thus was a logical candidate for our study. A variable region of the nuclear ribosomal large subunit gene ("LSU-Y," according to the terminology of Harper and Saunders [2001]) was sequenced for seven *A. spicifera* individuals (five from O'ahu and two from Hawai'i), again using previously published reaction and cycling conditions (Harper and Saunders 2001). Although in entirety the LSU gene is quite conserved and is useful for distinguishing species in red algae (Harper and Saunders 2001), this variable region has the potential to detect intraspecific variation.

Polymerase chain reaction (PCR) products were verified for size and concentration by gel electrophoresis. PCR products were direct purified using the Qiagen PCR Purification Kit (Valencia, California) and sequenced on an automated DNA sequencer (ABI 377XL). Resulting sequence chromatograms were examined and consensus sequences assembled from both forward and reverse reads using BioEdit v.7.0.4.1 (Hall 1999), and consensus sequences were aligned by eye. DNA sequence data are available via GenBank (accession numbers DQ500955 for cox 2-3 spacer and DQ500956 for LSU-Y).

ISSR Analyses

Fourteen ISSR primers were screened for successful amplification. Five of these 14 primers produced clear and well-separated bands, and were used to generate the population data set (see Table 2). Because some DNA extracts were too dilute or degraded for reproducible ISSR amplification, only seven or eight individuals from each population were included in the final analyses. To account for variable extract concentration, 0.1–1.0 µl of DNA extract was added to each 25-µl reaction containing 2.5 µl of buffer (Promega), 2.0 µl 2.0 mM magnesium chloride (Promega), 1.5 µl of 1.0% bovine serum albumin, 1.0 µl (0.4 mM) of primer, 0.25 µl (20 mM)

of each dNTP, 0.1 μl of Promega Taq Polymerase (Madison, Wisconsin), and 12.8 μl autoclaved nanopure water. DNA amplification was carried out in a thermocycler (Eppendorf GradientS, Stuttgart, Germany) with the following parameters: 94°C for 2 min, followed by 45 cycles of 94°C for 45 sec, 37°C for 45 sec, 72°C for 2 min, and a final extension of 72°C for 5 min. PCR products and 150 base pair (bp) DNA ladders were resolved electrophoretically on 1.5% agarose gels run at 100 V for 2.5 hr in 0.5X TBE buffer. DNA fragments were visualized by staining with ethidium bromide, and photographed under UV light. ISSR bands were scored as present (1) or absent (0) for each DNA sample, and these binary data were entered into a spreadsheet. Fragments of the same molecular weight were assumed to be homologous. The Multi-Variate Statistical Package (MVSP) was employed to evaluate the genetic similarity between all accessions (Kovach Computing Services 1986–1999). MVSP was used to create a similarity matrix based on Jaccard's coefficient and to conduct a cluster analysis with the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) algorithm and a Principal Co-Ordinates (PCO) analysis based on the similarity matrix. Popgene software v.1.32 (Yeh et al. 1997) was used to calculate Nei's genetic distance and genetic diversity within each population as Percentage Polymorphic Bands (PPB) and Shannon's Index. GenAlEx was used to conduct an Analysis of Molecular Variance (AMOVA) and estimate population differentiation (Φ_{ST}) as measures of genetic relationships within and among populations.

RESULTS

DNA Sequencing Analyses

No sequence-level variation was observed in either the Hawaiian collections of *Acanthophora spicifera* or the more broadly distributed Pacific collections of the alga. A single haplotype for the cox 2-3 spacer (305 nucleotides [nt]) was observed for all sequenced collections from the main Hawaiian Islands ($n = 43$ samples), as well as collections from

TABLE 2

ISSR Oligonucleotide Primers That Produced Clear and Reproducible Bands for Population-Level Analyses of the Red Alga *Acanthophora spicifera*

Primer Name	Sequence	Total No. of Bands
ISSR 1	$(CA)_6GG$	8
ISSR 2	$(CT)_8AC$	7
ISSR 5	$(CT)_8GC$	6
ISSR 10	$(GAG)_3CC$	13
ISSR 12	$(CAC)_3GC$	11

Guam ($n = 5$), Australia ($n = 3$), and Japan ($n = 2$). Similar results were obtained for sequence data of the LSU-Y region. Again, a single haplotype (531 nt) was recovered for all *A. spicifera* collections from the main Hawaiian Islands ($n = 7$). In addition, this same haplotype was sequenced for the samples from Guam ($n = 1$), Australia ($n = 1$), and Japan ($n = 1$).

ISSR Fragment Polymorphism and Genetic Structure

Of the 14 ISSR primers tested, five generated a total of 45 bright and reproducible bands ranging in size from approximately 375 to 1,600 bp (Table 2). The vast majority (91.1%) of fragments produced from the 67 individuals in eight populations were polymorphic. Analyses of PPB and Shannon's diversity index ranged from 4.4% and 0.030 (D. T. Fleming Beach, Maui) to 44.4% and 0.278 (Onekahakaha Beach, Hawai'i), indicating a wide range of genetic diversity within populations (see Table 4).

Cluster analysis with Jaccard's coefficient was used to visualize the degree of similarity between accessions of *A. spicifera* (Figure 2). The cluster dendrogram exhibits a high degree of population structure and clearly shows the distinct grouping of individuals collected at each location. When all individuals are included in the comparison, the average genetic similarity measured by Jaccard's coefficient is 0.493. The coefficient among populations ranged from a low of 0.424 (Ala Moana Beach, O'ahu, and Richardson's

UPGMA

Ala Moana Beach
Park, Oahu

Kamiloloa,
Molokai

D.T. Fleming,
Maui

Maili Beach Park,
Oahu

Waiehu, Maui

Kualoa, Oahu

Kahala, Oahu

Onekahakaha, Hawaii

Richardson's Beach
Park, Hawaii

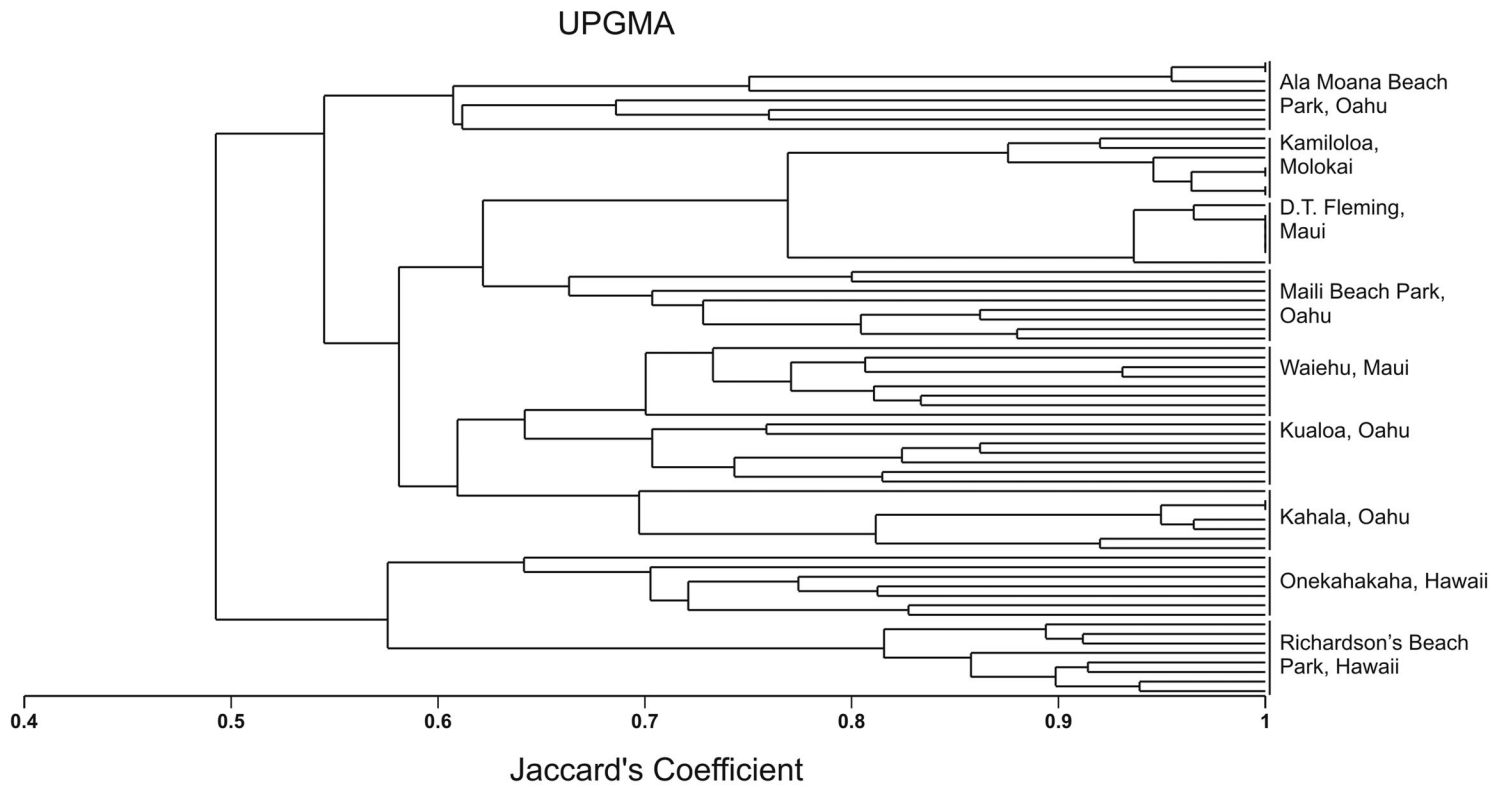0.4   0.5   0.6   0.7   0.8   0.9   1

Jaccard's Coefficient

FIGURE 2. UPGMA dendrogram (Jaccard's coefficient) of *Acanthophora spicifera* collections from the main Hawaiian Islands.

TABLE 3

Genetic Distance Presented as Jaccard's Coefficient of Distance (above Diagonal) and Nei's Genetic Distance (below Diagonal) among Eight Hawaiian Populations of *Acanthophora spicifera* Used in ISSR Analyses

Collection Location:	P1	P2	P3	P4	P5	P6	P7	P8	P9
P1 - Richardson's Beach, Hawai'i		0.515	0.506	0.465	0.576	0.424	0.564	0.414	0.464
P2 - Kahala Beach, O'ahu	0.463		0.409	0.374	0.507	0.586	0.534	0.394	0.448
P3 - Kualoa Beach, O'ahu	0.466	0.256		0.358	0.446	0.511	0.446	0.348	0.376
P4 - Waiehu Beach, Maui	0.404	0.218	0.133		0.441	0.542	0.412	0.369	0.431
P5 - Ala Moana Beach, O'ahu	0.501	0.302	0.187	0.187		0.559	0.453	0.449	0.434
P6 - Onekahakaha Beach, Hawai'i	0.306	0.506	0.349	0.443	0.319		0.518	0.453	0.412
P7 - Maili Beach, O'ahu	0.533	0.379	0.196	0.179	0.132	0.324		0.411	0.351
P8 - D. T. Fleming Beach, Maui	0.394	0.310	0.257	0.295	0.313	0.396	0.280		0.231
P9 - Kamiloloa, Moloka'i	0.420	0.377	0.270	0.335	0.248	0.281	0.198	0.147	

Beach, Hawai'i) to a high of 0.769 (Kamiloloa Beach, Moloka'i, and D. T. Fleming Beach, Maui) (Table 3). Nei's measure of genetic distance revealed similar trends of genetic relationships among populations (Table 3). The PCO analysis generated a biplot visually representing the associations of individuals within and between populations. The relationships among individuals in the PCO analysis were similar to those revealed by both cluster analysis and Nei's measure of genetic distance, and only the cluster dendrogram based on Jaccard's coefficient is shown (Figure 2). All measures of genetic distance placed the population samples from the island of Hawai'i at a considerable distance from populations collected from all other islands. The AMOVA yielded high values ($\Phi_{ST} = 0.609$) that were significantly different ($P = .001$) from zero with 999 random permutations, indicating a substantial degree of differentiation among populations. Although individuals within populations from all islands formed discrete clusters, for some populations we observed a substantial amount of within-population variation.

Seasonality of Reproduction of *A. spicifera*

Acanthophora spicifera samples from all shores of O'ahu were regularly collected and observed for reproductive status between February 2005 and late October 2005. No reproduction was visible for any of these collections until the final week of May 2005.

Many of these tetrasporophyte thalli contained spores in various stages of development. Nearly every subsequent collection contained at least one tetrasporangial plant. Ongoing collections have revealed tetraspore production into October 2005.

The isomorphic life history of many red algae, including *A. spicifera*, makes it impossible to visually distinguish sterile tetrasporophytes and gametophytes. Although carpogonial specimens have been observed in the past (Smith et al. 2002), no spermatangial or carpogonial plants were observed in our collections, and tetrasporangia represent the only reproductive cells detected in this study.

DISCUSSION

Although the cox 2-3 spacer has been used to resolve genetic variation at the intraspecific level (Zuccarello et al. 1999), no sequence variation was found for samples from even distant locations (Hawai'i, Guam, Australia, and Japan). In contrast, our ISSR analyses demonstrate a substantial degree of population structure that is related to geographic distance, with populations forming discrete groups. This high degree of genetic structure suggests that currently used population-level DNA sequence markers (e.g., cox 2-3 spacer, LSU-Y) do not possess resolution high enough to detect population structure in all algal species.

Patterns of genetic variation in *A. spicifera* are likely influenced by a myriad of natural

TABLE 4

Percentage of Polymorphic Bands (PPB) and Shannon Index (*I*) for Nine Populations of *Acanthophora spicifera* Used in ISSR Analysis

Collection Location	No. of Polymorphic Bands	PPB	*I* (SD)
Richardson's Beach, Hawai'i	12	26.67	0.1676 (0.2846)
Kahala Beach, O'ahu	13	28.89	0.1496 (0.2571)
Kualoa Beach, O'ahu	18	40.00	0.2320 (0.2981)
Waiehu Beach, Maui	19	42.22	0.2478 (0.3048)
Ala Moana Beach, O'ahu	20	44.44	0.2556 (0.3045)
Onekahakaha Beach, Hawai'i	20	44.44	0.2779 (0.3116)
Maili Beach, O'ahu	16	35.56	0.2134 (0.2967)
D. T. Fleming Beach, Maui	2	4.44	0.0301 (0.1411)
Kamiloloa Beach, Moloka'i	5	11.11	0.0704 (0.2042)

SD, standard deviation.

and anthropogenic variables. The degree of genetic similarity found both within and among populations in our current study ranged widely (Figure 2, Tables 3 and 4). The genetic differentiation and relationships among populations suggest that a limited amount of genetic exchange takes place within the main Hawaiian Islands. Populations collected near Hilo, Hawai'i (Onekahakaha Beach and Richardson's Beach), are the most geographically and genetically distant from populations surrounding the suspected site of introduction (Pearl Harbor, O'ahu) (Smith et al. 2002). The highest within-population variation was observed for well-established populations (e.g., Ala Moana Beach, O'ahu, and Onekahakaha Beach, Hawai'i) that are proximal to intense boat traffic (Honolulu Harbor, O'ahu, and Hilo Bay, Hawai'i) and are thus likely to be exposed to incoming propagules from hull-fouled vessels and ballast water discharge.

Acanthophora spicifera is a hardy species that is capable of flourishing under a wide range of environmental conditions. The natural and anthropogenic conditions that vary among collection sites likely affect the quantity of sexual and asexual propagules produced, as well as the likelihood of dispersal to other sites. Occasional events such as severe weather systems and large ground swells would certainly generate unusually high numbers of fragments. Anthropogenic influences such as boat traffic are implicated in the ini-

tial introduction of *A. spicifera* to Hawai'i (Doty 1961), and it is likely that hull-fouled interisland vessels act as occasional dispersal vectors. Due to an abundance of dispersal mechanisms, this species is widely distributed across Hawai'i. Our analyses indicate that it possesses a surprising amount of population structure. The discrete population groups revealed by cluster analysis and AMOVA support the hypothesis of Smith et al. (2002) that *A. spicifera* is occasionally introduced to new localities by hull fouling, where it readily spreads to nearby reefs through fragmentation or spore production.

Tetrasporangia are typically produced by sexually reproducing populations of red algae as one of the three life history stages, but instances are known in which the gametophyte stages are omitted and the algae live continuously as tetrasporophytes (e.g., the marine species of the red alga *Hildenbrandia* [Sherwood et al. 2002]). Because gametophytes were never observed in our own collections of *A. spicifera* in the main Hawaiian Islands, it is possible that this species also has, or is transitioning to, such a truncated life history in Hawai'i. However, detailed collections from throughout the year are necessary to determine this possibility. This scenario would account for the abundance of tetrasporophytic plants in our collections and the lack of gametophytic plants. Thus, although evidence of sexual reproduction has been reported in the past (Smith et al. 2002), we

cannot confirm these data based on our own observations. The molecular data are only partially congruent with our observations of reproductive status. A large amount of genetic variation was recovered based on the ISSR analyses (Figure 2), but no variation was observed in the cox 2-3 spacer or LSU-Y DNA sequences of *A. spicifera* across the Pacific. Thus, it is not clear whether *A. spicifera* is commonly employing sexual reproduction as a strategy in the main Hawaiian Islands. Population-level sequence markers have not always revealed variation within populations of invasive seaweeds (Marston and Villalard-Bohnsack 2002). However, dominant genetic markers, including ISSR and Random Amplified Polymorphic DNA (RAPD), have been used to confirm suspected clonal invasions (Hollingsworth et al. 1998) and reveal population structure in invasive species (Meekins et al. 2001, Marston and Villalard-Bohnsack 2002, Sun et al. 2005). The high degree of genetic structure found with our ISSR analyses suggests that *A. spicifera* in Hawai'i is reproducing sexually, but that gene flow between geographically distant populations is rather limited.

Management Recommendations

Of the many invasive organisms in Hawai'i, introduced red algae appear to be the most pressing threat to reef habitats (Coles and Eldredge 2002). Among all the invasive seaweeds, *A. spicifera* is the most widespread and successful (Smith et al. 2002). Therefore, this species should be considered a priority for invasive species managers. Although complete eradication of this alien alga is highly unlikely, the prevention or reduction of further spread is a feasible goal.

PREVENTION OF SPREAD VIA BOAT TRAFFIC. Because boat traffic is the primary vector involved in the spread of invasive marine species in Hawai'i (Godwin 2003), increased public awareness and more stringent regulations regarding hull fouling and ballast water discharge are necessary to prevent or slow the recruitment of new populations.

SITE-SPECIFIC CONTROL EFFORTS. The results of our research should help invasive species managers target control efforts to specific sites. Populations that are not yet well established may lack "genetic flexibility" (i.e., abundant genetic variation) and thereby be less likely to resist control measures such as large-scale removal or introduced herbivores. Control efforts should thus target locations that have strong potential to recruit new populations through the frequent production and dispersal of propagules. Populations on exposed reefs, when compared with those in protected bays, lagoons, and tide pools, would be expected to produce and disperse a greater number of propagules because of higher wave action. Efforts at particular sites should be focused on outer areas of the reef that experience relatively high wave action and therefore likely generate a greater quantity of fragments. Being much smaller propagules than vegetative fragments, tetraspores would be expected to be carried farther by ocean currents. Thus, removal efforts should be timed to predate the release of tetraspores (likely in the late spring, although detailed year-round observations are needed to confirm this). Other local conditions associated with increased dispersal, such as proximity to boat harbors and strong currents, need to be investigated to best select these target localities around the state.

CONCLUSIONS

Although molecular tools have been used to investigate invasive algae in the North Atlantic (Chualáin et al. 2004, Provan et al. 2005) and Mediterranean Sea (Famà et al. 2002, Meusnier et al. 2002, Piazza and Cinelli 2003), this study is the first to examine the population genetics and biogeography of an alien seaweed in Hawai'i. Our analyses indicate a surprising amount of within- and among-population variation based on ISSR markers, but DNA sequence analyses of the mitochondrial cox 2-3 spacer and a fragment of the nuclear ribosomal large subunit gene failed to reveal any variation. This genetic analysis provides a "first look" at the genetic structure of *A. spicifera* and a basis for future analyses to monitor dispersal and gene flow over time. The current research also presents

a foundation upon which to develop further research regarding the sexual strategies and dispersal mechanisms that influence the spread of other alien algae in Hawai'i.

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