MULTI-SCALE GENETIC ANALYSIS OF *UNIOLA PANICULATA* (POACEAE): A COASTAL SPECIES WITH A LINEAR, FRAGMENTED DISTRIBUTION[1]

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Geographic and fine-scale population genetic structures of *Uniola paniculata*, the dominant coastal dune grass in the southeastern USA, were examined. The linear, naturally fragmented distribution of this native perennial was hypothesized to lead to high genetic structure and lower genetic diversity at the margin of the species range. The extensive ramet production and low seed germination of this species were also expected to cause populations to be dominated by a few large clones. At 20 sites throughout the range of the species, leaf tissue was collected from 48 individuals. Clonal structure was examined using leaf tissue collected from an additional 60 individuals, each in four patches at two sites. Starch gel electrophoresis was used to resolve 27 allozyme loci. The results indicated that *Uniola* had greater genetic structure ($G_{ST} = 0.304$) than most other outcrossing species, indicating moderate barriers to gene flow. There was a weak but significant positive relationship between genetic distance and geographic distance, supporting an isolation-by-distance model of gene flow. There were no obvious disjunctions between regions. Genetic diversity (H_e) was relatively uniform throughout most of the range of the species but was lower in all western Gulf of Mexico populations. Clonal diversity varied both within and among sites, but clones were often small, suggesting that sexual reproduction and recruitment from seeds are important factors maintaining genetic diversity.

Key words: allozymes; clonal structure; coastal plants; gene flow; population genetics; sea oats; southeastern United States; *Uniola paniculata*.

Information on the distribution of genetic variation across the range of a species is fundamental to our understanding of ecological and evolutionary processes. Partitioning genetic variation into geographical components (e.g., among regions, among and within populations) and examining genetic patterns at a range of spatial scales (Reusch et al., 2000; Stehlik and Holderegger, 2000; Suyama et al., 2000; Nassar et al., 2001) can provide information on founder events and bottlenecks, on patterns of gene flow, and on fine-scale processes such as clonal reproduction. Genetic structuring among populations may result from limitations to gene flow, genetic drift, spatial variability in selection, or a combination of these factors (Hedrick, 2000). Patterns of genetic structuring may be continuous, as in isolation-by-distance models of gene flow or discontinuous, as in stepping stone models (Futuyma, 1986, pages 136–139). Genetic structuring may also occur among geographic regions. Such large-scale patterns may result from major barriers to gene flow in populations with discontinuous distributions or, in populations with continuous distributions, from processes such as glaciation that have played a role in colonization processes (Avise, 2000).

Large-scale population structuring may also occur if genetic patterns differ between the center of a species' range and the margins of its distribution. There is currently some debate as to whether populations near the center should display more or less genetic variability than those at the edge of the range. The classical view, often referred to as the Central–Marginal Model, is that central populations are more genetically variable than marginal populations because of fewer ecological niches at the margins (da Cunha and Dobzhansky, 1954) or a greater importance of heterosis in central populations (Carson, 1959; Wallace, 1984). In contrast, Brussard (1984) proposed that selection at environmentally variable margins may promote genetic diversity. In reviewing the literature on *Drosophila* species, Brussard (1984) found declines in inversion polymorphisms, which are presumably under selection, from the center to the margins of the species' ranges, but no change in supposedly neutral allozyme variation. Patterns of genetic variation between central and marginal populations may thus depend on the degree to which the markers are under selection, rates of gene flow into marginal populations, and the amount of habitat variability and rates of population growth in central and marginal areas.

Coastal plants are particularly appropriate for addressing questions regarding patterns of spatial genetic structuring and comparisons of genetic variation between central and marginal areas because of their long, narrow, and naturally fragmented habitat. How and whether the unique geographic distribution of coastal plants affects patterns of gene flow remains largely unknown because of the paucity of population genetic studies in these species. In addition, many coastal dune plants reproduce both asexually and sexually (Ranwell, 1972). While vegetative propagation (Pemadasa and Lovell, 1976) and flowering (Pemadasa and Lovell, 1974) have been examined separately for some species, the relative importance of asexual vs. sexual reproduction in coastal dune plants is generally un-
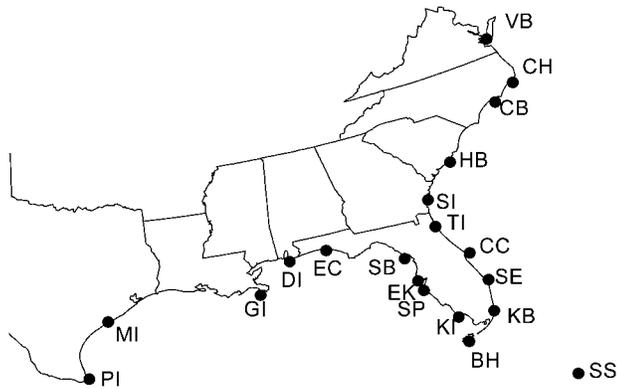
Fig. 1. Locations of the 20 sampling sites for *Uniola paniculata* on the Atlantic and gulf Coasts of North America and the Bahamas.

known. The objective of this study was to investigate the population genetics of the coastal dune grass *Uniola paniculata* L. at three spatial scales: among regions, within and among populations, and among clones within patches.

Uniola paniculata (sea oats) is the dominant dune-stabilizing grass in southeastern USA. This native plant occurs on the primary dunes of beaches and barrier islands from Virginia, south along the Atlantic Ocean, into the Bahamas, and along the Gulf of Mexico coast to Veracruz, Mexico (Wagner, 1964). This range spans more than 5000 km of coastline. However, the width of the habitat within a dune is quite narrow—generally less than 200 m. Thus, this species has an essentially linear distribution that is naturally fragmented: patches of beach occur on barrier islands or on mainland beaches that are isolated by waterways. Additional fragmentation occurs both by natural disturbances such as hurricanes and anthropogenic disturbances such as coastal housing developments.

Uniola is outcrossing and wind-pollinated, and its seeds (caryopses) are dispersed by wind and water (Harper and Seneca, 1974; Hester and Mendelssohn, 1987). While these factors should promote gene flow and decrease genetic differences among populations, its linear, fragmented distribution should limit gene flow and increase genetic structure. In addition,

Seneca (1972) found that Virginia and North Carolina populations of *Uniola* required cold stratification for germination, while Florida populations did not. This suggests that selection may differ among climatically distinct regions of the range of *Uniola*, further increasing among-population differences. Based on this information, we hypothesized that *Uniola* would have more genetic structure than other outcrossing species, that genetic distance would increase with geographic distance, that genetically similar populations would cluster within regions, and that genetic diversity would be lowest at the margin of the species' range.

In addition to sexual reproduction, *Uniola* reproduces clonally by the production of rhizomes (Wagner, 1964). Clonal reproduction is thought to be extensive for this species (Harper and Seneca, 1974), and rates of germination and seedling emergence and establishment in the field tend to be low (Wagner, 1964; Franks, 2003). We hypothesized that extensive ramet production and low seed germination and establishment would cause populations to be dominated by a few large clones.

MATERIALS AND METHODS

Geographic survey—For the geographic survey, 20 sampling sites (populations) were selected (Fig. 1, Table 1). These sites spanned the entire range of *Uniola paniculata* in the USA and the Bahamas. We attempted to choose sites that were natural populations and to avoid any areas that were replanted or included any cultivated individuals, and we verified these criteria with land managers at each site. At each site, live leaf tissue was collected from 48 individuals each separated by 10 m along a transect. The results of the clonal study (see Clonal study next) indicated that the 10-m distance between plants was sufficient to avoid sampling genetically identical individuals. The transect was established in the foredune, parallel to the high tide mark, on the shoreward edge of the distribution of *Uniola*.

Clonal study—Leaf tissue samples were collected at two locations: Cape Hatteras, North Carolina (CH), and Emerald Coast (Grayton Beach) Florida (EC). Four patches (subpopulations) of 60 individuals each were sampled at each of the two locations. Of these four patches, two were on foredunes and two were in low-lying areas between dunes (swales). Plants were collected on an 8 × 8 m grid (patch) at every 1-m mark, excluding the four corners or any bare sand areas for a total of 60 plants sampled per patch.

TABLE 1. Geographic locations and regional distributions of the 20 sites for collecting *Uniola paniculata*.

Site	State	Code	Latitude	Longitude	Coast	Region
Kiptopeke	Virginia	VB	37°09′57″	75°59′08″	Atlantic	1
Cape Hatteras	North Carolina	CH	35°13′28″	75°31′50″	Atlantic	1
Carolina Beach	North Carolina	CB	34°02′06″	77°53′38″	Atlantic	1
Huntington Beach	South Carolina	HB	32°21′56″	80°27′57″	Atlantic	1
Sapelo Island	Georgia	SI	31°28′38″	81°14′31″	Atlantic	2
Little Talbot Island	Florida	TI	30°27′08″	81°25′08″	Atlantic	2
Cape Canaveral	Florida	CC	28°28′11″	80°33′46″	Atlantic	2
Sebastian Inlet	Florida	SE	27°51′36″	80°26′51″	Atlantic	3
Key Biscane	Florida	KB	25°41′36″	80°09′47″	Atlantic	3
San Salvador	Bahamas	SS	24°03′47″	75°31′26″	Atlantic	3
Bahia Honda	Florida	BH	24°39′46″	81°15′50″	Atlantic	3
Keywadin Island	Florida	KI	26°08′30″	81°47′42″	Gulf	4
Fort DeSoto	Florida	SP	27°37′10″	82°43′40″	Gulf	4
Egmont Key	Florida	EK	27°35′30″	82°45′46″	Gulf	4
Cape San Blas	Florida	SB	29°48′26″	85°24′42″	Gulf	4
Grayton Beach	Florida	EC	30°19′29″	86°09′15″	Gulf	5
Dauphin Island	Alabama	DI	30°14′58″	88°11′02″	Gulf	5
Grande Isle	Louisiana	GI	29°14′26″	89°59′17″	Gulf	5
Mustang Island	Texas	MI	27°44′21″	97°07′53″	Gulf	5
Padre Island	Texas	PI	26°50′39″	97°22′03″	Gulf	5

TABLE 2.   Estimates of genetic diversity and structure for *Uniola paniculata*, for outcrossing grasses, for grasses with a narrow distribution, for all grasses, and for all plants.

Taxa	No. species	p_s	AP_s	H_{es}	G_{ST}	Source[a]
Uniola paniculata	1	77.8	2.57	0.151	0.304	1
Outcrossing	69	69.1	2.69	0.212	0.112	2
Narrow distribution	36	56.9	2.16	0.204	0.231	2
Poaceae	161	60.0	2.38	0.191	0.272	2
All plants	655	51.3	1.97	0.150	0.228	3

Note: p_s = percentage of polymorphic loci, AP_s = mean number of alleles per polymorphic locus, H_{es} = mean Hardy-Weinberg expected heterozygosity, G_{ST} = proportion of total genetic diversity due to differences among populations.
[a] 1, This study; 2, Godt and Hamrick (1998); 3, Hamrick and Godt (1996).

Electrophoresis—Leaf tissue samples were kept cool, taken back to the laboratory, and crushed for protein extraction using liquid nitrogen, sea sand, and Camellia buffer (Wendel and Parks, 1982). The extracted material was absorbed onto Whatman 3MM filter paper wicks. These wicks were stored at −70°C until needed for electrophoresis. Extracted proteins were run on 9.5% starch gels. Seventeen enzymes were examined—PGI, MNR, PGM and ADH on buffer system 34; MDH, IDH, AK, SKDH, MPI and F16 on buffer system 11; DIA, AAT, TPI, FE, GDH, and ME on buffer system 8−; and 6-PGD on buffer system 4 (Soltis et al., 1983 for all buffers and stains except AAT, ADH, AK, DIA, MNR, MPI [Cheliak and Pitel, 1984]). Gels were then stained with enzyme-specific stains according to the protocols above and scored for allozyme banding patterns. Interpretations of the banding patterns follow the recommendations of Weeden and Wendel (1989) and Kephart (1990) for polyploid species.

Analyses—Population genetic statistics were calculated using the program LYNSPROG developed by M. D. Loveless (College of Wooster, Wooster, Ohio, USA) and A. F. Schnabel (University of Indiana, South Bend, Indiana, USA). At the species (subscript *s*) and population (subscript *p*) levels, we estimated percentage polymorphic loci (*p*), mean number of alleles per polymorphic locus (AP), observed heterozygosity (H_o), and expected heterozygosity ($H_e = 1 − \Sigma p_i^2$), which estimates genetic diversity. Standard errors for within-population parameters were obtained by averaging across all populations.

Nei's (1972) genetic distance and identity statistics were calculated for each pair of populations. Genetic distance was used to perform a cluster analysis using the unweighted pairwise groups method using arithmetic averages (UPGMA) with the program Phylip (version 3.2; Felsenstein, 1989). Geographic distance was calculated between all pairs of populations. For adjacent populations, we calculated the distance between the sites using the latitude and longitude coordinates of each site. For nonadjacent populations, we calculated the distance along the coast between them by summing the distances connecting adjacent populations between them. This method assumes that the relevant geographic distance among populations is the distance along the coast, because noncoastal habitat is unsuitable for this species. To examine the relationship between geographic distance and Rousset's (1997) genetic distance ($F_{ST}/[1 − F_{ST}]$), we performed Mantel tests and reduced major axis (RMA) regression on natural log-transformed data using the program IBD (version 2.03) by A. J. Bohonak (Bohonak, 2002). We used Rousset's genetic distance and RMA regression because these statistics are appropriate for genetic/geographic distance comparisons (Bohonak, 2002).

To partition genetic diversity into geographical components, we conducted a hierarichal analysis of genetic structure. Genetic structure, G_{ST}, was calculated following Nei (1987). Populations were grouped by two coasts (Atlantic and Gulf) or by five smaller regions, and total G_{ST} was partitioned into components among regions and among populations within regions.

We examined fine-scale genetic structure by investigating both clonal structure and patterns of relatedness among individuals within a patch. To assess clonal structure, we first determined the multilocus genotypes for each individual within each patch. We assessed genetic diversity first as the number of genets per number of ramets sampled (*G/N*). Our second measure of genotypic diversity is Simpson's index: $D = 1 − \Sigma([n_i\{n_i − 1\}]/[N\{N − 1\}])$; $n_i =$ number of ramets of the *i*th genet and *N* = total number of ramets (Pielou, 1969). We also mapped multilocus genotypes to examine spatial patterns of clonal structure. To examine fine-scale patterns of genetic relatedness, we estimated the coefficient of coancestry (Cockerham, 1969) between all pairs of genetic individuals within patches using the program fijAnal3D developed by J. Nason (Department of Botany, Iowa State University, Ames, Iowa, USA). Coancestry (ρ_{ij}) was estimated following Parker et al. (2001). We generated 95% confidence intervals around the hypothesis of no spatial genetic structure by bootstrapping using the program BS_fij3D developed by J. Nason.

RESULTS

Geographic survey—A total of 27 allozyme loci were resolved, 21 of which were polymorphic. Estimates of genetic diversity and structure at the species level were compared to other outcrossing grasses, grasses with a narrow distribution, all grasses, and all plants included in the literature reviews by Godt and Hamrick (1998) and Hamrick and Godt (1996) (Table 2). *Uniola* had 77.8% polymorphic loci, which is higher than the average for other plants in these groups. There were 2.57 alleles per polymorphic locus, which was comparable to other plants in these groups. Expected heterozygosity was 0.151, which was slightly lower than average compared to the other groups with similar life history traits. The proportion of genetic variation due to differences among populations (G_{ST}) was 0.304 for *Uniola*, which was greater than average for any of the other groups. Within populations, observed and expected heterozygosities tended to be similar, indicating allele frequencies were generally in Hardy-Weinberg equilibrium (Table 3).

There was a significant but weak relationship between geographic and genetic distances (Mantel test $Z = −86.78$, $r = 0.225$, $P = 0.015$). To further investigate geographic patterns of genetic structure, the 20 populations were grouped into regions. When the 20 populations were divided into Atlantic and Gulf regions (Table 1), G_{ST} for the two regions was only 0.060 compared to the G_{ST} of 0.304 for the 20 populations, indicating that only approximately 20% of the overall differentiation occurred between the two regions. Grouping the populations into five smaller regions (Table 1) produced a G_{ST} of 0.158, indicating that approximately half of the total differentiation occurred among the five regions.

A similar lack of strong regional genetic pattern was found by organizing the populations into a UPGMA tree (Fig. 2). This clustering dendrogram based on Nei's genetic distances revealed that populations spatially near each other tended to be genetically similar. However, there were notable exceptions. For example, Virginia Beach, Virginia, and South Padre Island, Texas, were the two most widely separated populations geographically, yet are fairly similar genetically.

TABLE 3. Within-population estimates of genetic diversity and structure for *Uniola paniculata* at each site.[a]

Site[b]	p_P (%)	AP_P	A_p	A_{ep}	H_o	SD	H_{ep}	SD
VB	16.00	2.00	1.16	1.08	0.064	0.028	0.051	0.025
CH	36.00	2.00	1.36	1.16	0.113	0.034	0.100	0.035
CB	28.00	2.00	1.28	1.12	0.087	0.028	0.072	0.030
HB	36.00	2.00	1.36	1.14	0.107	0.039	0.085	0.034
SI	23.08	2.00	1.23	1.09	0.066	0.024	0.050	0.034
TI	36.00	2.00	1.36	1.16	0.083	0.030	0.090	0.035
CC	18.18	2.00	1.18	1.07	0.035	0.021	0.045	0.033
SE	19.23	2.20	1.23	1.13	0.069	0.028	0.070	0.032
KB	48.15	2.00	1.48	1.17	0.080	0.034	0.105	0.034
SS	32.00	2.00	1.32	1.15	0.097	0.032	0.089	0.033
BH	18.52	2.00	1.19	1.13	0.100	0.022	0.066	0.034
KI	33.33	2.00	1.33	1.14	0.055	0.032	0.079	0.036
SP	22.22	2.00	1.22	1.10	0.052	0.027	0.060	0.031
EK	28.00	2.43	1.40	1.16	0.088	0.033	0.088	0.035
SB	28.00	2.14	1.32	1.12	0.059	0.029	0.071	0.030
EC	24.00	2.33	1.32	1.14	0.065	0.030	0.078	0.032
DI	20.83	2.40	1.29	1.09	0.042	0.025	0.052	0.027
GI	24.00	2.17	1.28	1.11	0.049	0.028	0.069	0.028
MI	8.00	2.00	1.08	1.02	0.015	0.018	0.012	0.020
PI	19.23	2.40	1.27	1.07	0.028	0.021	0.035	0.031
Mean	25.94	2.10	1.28	1.12	0.068		0.068	
SD	1.91	0.16	0.09	0.04	0.006		0.007	

[a] p_P (%) = percentage of polymorphic loci, AP_P = mean number of alleles per polymorphic locus, A_p = mean number of alleles per locus, A_{ep} = expected number of alleles per locus, H_o = observed heterozygosity, H_{ep} = mean Hardy-Weinberg expected heterozygosity, SD = standard deviation.

[b] See Table 1 and Fig. 1 for site locations.

Genetic diversity (expected heterozygosity [H_e]) did not increase with increasing distance south from the northern edge of the species' range in Virginia. However, all populations along the Gulf of Mexico west of the Florida panhandle had low levels of genetic diversity ($H_e < 0.07$). While overall genetic diversity did not have a strong geographic pattern, there were specific loci at which allele frequencies had a geographic trend. These loci include AAT, DIA, PGD1, PGI1, PGI2, PGI3, TPI3, and TPI4. For diaphorase (DIA), the locus with the most pronounced geographic cline, the frequency of allele 4 was 1.00 in Virginia, decreased to 0.50 in Bahia Honda in the Florida Keys, and was 0.05 in the south Texas populations.

Clonal study—Clonal structure and diversity varied widely among the eight patches sampled (Table 4). One patch (Cape Hatteras A) contained only two multilocus genotypes for 60 ramets sampled. Another patch (Emerald Coast A) had 13 genotypes. The average number of ramets/genet was 11.8 ($G/N = 0.13$), and mean clonal diversity (measured as Simpson's diversity index) was 0.60 (Table 4). The scale of spatial autocorrelation tended to be fairly small (<3 m) based on spatial analysis of coancestry coefficients. At distances greater than 3 m, ramets were not any more likely to be genetically similar
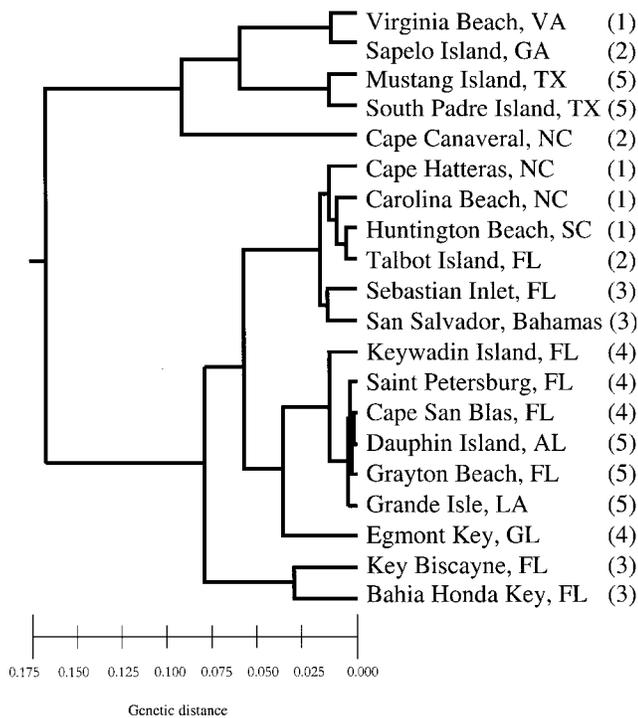

Fig. 2. The UPGMA clustering dendrogram based on Nei's (1987) genetic distances for all 20 populations of *Uniola paniculata*. The five regions in the hierarchal analysis are indicated by the numbers in parentheses.

TABLE 4. Statistics of clonal structure and diversity for *Uniola paniculata*.

Site	Patch	G/N[a]	Clonal diversity[b]
CH	A	0.033	0.064
CH	B	0.149	0.686
CH	C	0.182	0.600
CH	D	0.167	0.610
EC	A	0.263	0.851
EC	B	0.067	0.656
EC	C	0.050	0.463
EC	D	0.013	0.742
Mean		0.131	0.600

[a] Genets per ramet, with 60 ramets sampled per patch.

[b] Clonal diversity = Simpson's diversity index (D). See Materials and Methods: Analyses for descriptions.

than expected by chance. The probability that two individuals shared a multilocus genotype by chance alone (Harada et al., 1997) was fairly small (approximately 0.097 for both sites).

DISCUSSION

Population structure and gene flow—*Uniola paniculata* had greater genetic structuring ($G_{ST} = 0.304$) than expected for an outcrossing, wind-dispersed grass. Despite the fact that the outcrossing breeding system and wind- and water-driven dispersal system of this species should reduce genetic differences among populations, genetic differentiation did occur and tended to increase with increasing geographic distance. This pattern is likely due to the fact that the linear, fragmented distribution of this species may limit gene flow among populations (Berg and Hamrick, 1997). While the results gave moderate support for an isolation-by-distance model of gene flow, the relationship between genetic and geographic distance was weak (Mantel test; $r = 0.225$), and populations grouped by UPGMA did not always follow geographic patterns (Fig. 2). Thus, gene flow among populations is fairly high and does not always match the predictions of an isolation-by-distance model. This pattern could be due to rare long-distance dispersal of pollen or seeds by air or water currents or to human transfer of plant materials. While we attempted to choose sites that had natural populations of *U. paniculata* and avoided areas that had been planted, it is possible that, because this species is used in restoration projects, some of the plants could have been cultivated or have arisen from crosses with cultivars, which would potentially complicate the interpretation of geographic patterns of genetic structure.

In one of the few genetic studies of coastal plants, Clausing et al. (2000) examined genetic variation in two coastal species (*Cakile maritima* and *Eryngium maritimum*) in Europe. For these species, genetic distance and geographic distance along the coast were well correlated. They also found regional differences between the Atlantic and Mediterranean populations of both species, which contrasts with the lack of regional differences found for *Uniola*.

Regional genetic patterns—Based on the Central–Marginal Model (da Cunha and Dobzhansky, 1954), we predicted that genetic diversity should increase southward from the northern edge of the species range. Our results did not support this prediction. Instead, some populations near the northern margin of the species range (e.g., Cape Hatteras) were among the most diverse. Also, diversity was low in all populations from the Gulf of Mexico west of the Florida panhandle, possibly due to natural or anthropogenic disturbance.

The results of this study contrast with predictions of the Central–Marginal Model as well as with some empirical work. For example, Robinson et al. (2002) showed a decline in genetic diversity toward the edge of the range of a mite species. A possible explanation for this discrepancy is that the Central–Marginal Model, developed for mobile insect species, does not hold for plants that are sessile except for dispersal of pollen and seeds. It is also possible that only loci under selection increase in diversity away from the margin. This hypothesis is supported by Brussard (1984), who found that in *Drosophila*, inversion polymorphisms, which are presumably under selection, supported the model but that allozyme data did not. Other theoretical work (Mayr, 1970; Kirkpatrick and Barton, 1997) suggests that gene flow into marginal populations may

offset drift and directional selection, minimizing genetic differences between central and marginal populations. Our results are consistent with the hypothesis of relatively high gene flow into marginal populations.

Previous studies have examined genetic patterns for several vertebrate and invertebrate species specifically in the region of the Florida peninsula. Many of these studies have found genetic disjunctions between populations that are located on the Gulf of Mexico and the Atlantic coasts and between south Florida and more northern populations, though other studies failed to find such a disjunction (reviewed in Avise, 2000, pages 224–242). Avise (2000) argues that historical biogeographic factors were responsible for establishing barriers to gene flow and creating the patterns of genetic disjunctions seen in this region. In our study, we could explain interpopulation genetic variation by differences among regions, but we did not find a regional disjunction between Atlantic and Gulf coasts or between northern and southern areas, as has been found for other species. There are several possible reasons for this discrepancy. First, studies in this region cited in Avise (2000) were all of animals, while our study was of a coastal plant. Although many of the animals studied were highly mobile, the outcrossing and wind-dispersed plant we examined may be better able to overcome potential geographic barriers to gene flow than animal species. Second, our study utilized biparentally inherited markers (allozymes) rather than uniparentally inherited markers, such as mtDNA, used in the animal studies. Uniparentally inherited markers allow additional power to trace genetic lineages, so it is possible that a study using mtDNA or cpDNA would find genetic disjunctions in *Uniola* that were not detected using allozymes. Third, while the overall genetic patterns did not show a strong Gulf–Atlantic or north–south disjunction for *Uniola*, at least one locus, DIA, did show a geographic cline in allele frequencies.

Fine-scale genetic structure—The results of the clonal study indicate that patterns of clonal diversity and structure can vary widely within a relatively small area. Both sites (Cape Hatteras, North Carolina, and Grayton Beach, Florida) had patches with both low and high clonal diversity. Overall, clonal diversity tended to be higher than expected, averaging 0.600 (Simpson's diversity index, Table 4). In most areas, there were many different genotypes, and ramets spaced more than 4 m apart were unlikely to belong to the same clone. This result indicates that both clonal and sexual reproduction are likely important for this species.

The finding that many of the patches had a high diversity of clones was somewhat surprising in light of the fact that clonal reproduction in this species is known to be extensive (Harper and Seneca, 1974) and that, while germination rates can be high under laboratory conditions (Seneca, 1972; Hester and Mendelssohn, 1987), seedling emergence in the field is often very low (Wagner, 1964; Franks, 2003). There are, however, occasional periods in which a large number of seedlings establish. Such a localized flush of seedlings has been observed on Keywadin Island (S. J. Franks, personal observation). This pattern of sporadic and patchy establishment could also help to explain geographic patterns of genetic structure, with rare long-distance dispersal events reducing genetic structure but clonal reproduction and recruitment of seedlings into localized patches maintaining differences among populations.

Widen et al. (1994) surveyed clonal structure in 45 plant species and found an average of 26% multilocus genotypes,

which is lower than was found in this study for *Uniola*. Other studies have shown that clonal structure varies widely both among species and among populations within species (Kudoh et al., 1999; Reusch et al., 2000; Stehlik and Holderegger, 2000; Suyama et al., 2000). Variation in clonal diversity can be related to such biotic factors as breeding system and dispersal patterns and to such abiotic factors as nutrient heterogeneity and disturbance (Kudoh et al., 1999; Stehlik and Holderegger, 2000; Suyama et al., 2000; Parker et al., 2001). Kudoh et al. (1999) found that for the temperate woodland herb *Uvularia perfoliata*, clonal structure was strongly dependent on disturbance patterns, with patches in canopy gap habitats containing a few large clones but many different genotypes and patches in closed canopy habitats comprised of a single multilocus genotype. In contrast, Parker et al. (2001) found that although fire history altered population age structure and genetic structure in seedlings of *Pinus clausa*, disturbance did not detectably affect adult genetic structure. Disturbance may play a role in the clonal structure of *Uniola*, especially because such factors as burial, erosion, and storm overwash are important in its distribution and population dynamics (Wagner, 1964; Franks and Peterson, 2003). However, causes of variation in clonal structure among *Uniola* patches could not be determined from this study and remain uncertain.

In summary, several conclusions can be drawn from the patterns of genetic variation and structure in *Uniola paniculata*. First, the degree of genetic differentiation among populations and the relationship between geographic and genetic distance indicate that this species experiences moderate barriers to gene flow, which is consistent with expectation for a species with a linear, fragmented distribution. Second, there were no discreet disjunctions among regions, and patterns of genetic similarity did not always follow geographic location, potentially due to occasional long-distance dispersal, disturbance events, or to anthropogenic factors. Third, genetic diversity was not lowest at the margins of the species' range, which does not support the predictions of the Central–Marginal Model. Finally, fine-scale genetic patterns indicate that clonal diversity is highly variable and that both asexual reproduction via ramets and sexual reproduction via seeds are likely important for regeneration in this species. These results aid our understanding of the population genetics of a species with a unique distribution and can be used in conservation and restoration efforts involving this important coastal plant. Experiments designed to elucidate the degree of local adaptation and the optimum genotypes for use in restoration would complement the findings of this study and aid coastal management efforts.

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