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MICROGEOGRAPHIC GENETIC STRUCTURE AND GENE FLOW IN *HIBISCUS MOSCHEUTOS* (MALVACEAE) POPULATIONS[1]

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Microgeographic genetic variation in populations of a wetland macrophyte, *Hibiscus moscheutos* L. (Malvaceae), was investigated using allozyme polymorphism. The species is a self-compatible insect-pollinated perennial, and seeds are water dispersed (hydrochory). Six hundred plants were analyzed from eight brackish and two freshwater populations within the Rhode River watershed/estuarine system. The genetic structure of the populations was assessed by fixation indices and spatial autocorrelation analyses. The degree of genetic differentiation among sites and gene flow between all paired combinations of sites ($\hat{M}$) was analyzed using three hypothetical gene flow models. Fixation indices indicated almost complete panmixia within populations, and spatial autocorrelations showed that genotypes were randomly distributed within sites, most likely the result of water dispersal of seeds. Allele frequencies were significantly different among sites, and estimated F_{ST} indicated moderate genetic differentiation ($\theta = 0.062$). Genetic differences between populations were mostly explained by a gene flow model that accounted for the location of populations relative to the tidal stream. The importance of hydrochory in affecting spatial genetic structure was thus suggested both within and among *H. moscheutos* populations.

Key words: allozyme variation; gene flow; genetic structure; *Hibiscus moscheutos*; hydrochory; isolation by distance; Malvaceae; population differentiation; wetland plants.

The movement of pollen and seeds influences the genetic structure of plant populations and determines the extent to which local populations are independent evolutionary units (Levin and Kerster, 1974; Slatkin, 1987, 1994a; Heywood, 1991). If there are few impediments to gene flow, local populations will evolve together and there will be few genetic differences between them. If gene flow is restricted, distant populations or remote areas within a population can become genetically differentiated (Wright, 1943; Kimura and Weiss, 1964). The distribution of allozyme variation within and among populations has been used as a method to evaluate the combined effects of breeding systems and dispersal of pollen and seeds (Brown, 1979; Loveless and Hamrick, 1988; Hamrick, 1989). These genetic data often represent the only information to assess aspects of population demography, such as migration among multiple populations (Slatkin, 1987). In this paper we describe microgeographic genetic variation, using allozyme polymorphism, in populations of *Hibiscus moscheutos* L. (Malvaceae) along a freshwater to brackish gradient. We selected *H. moscheutos* for several reasons.

Within the Rhode River watershed/estuarine system, *Hibiscus moscheutos* occurs in physically isolated populations in nontidal herbaceous freshwater wetlands and in brackish intertidal wetlands along a tidal stream, locally known as Muddy Creek. Along Muddy Creek, populations of *H. moscheutos* typically occur in two types of locations. In the upstream portions of the intertidal zone, where salinities of a tidal stream are lower (Whigham, Jordan, and Miklas, 1989), *H. moscheutos* populations are adjacent to the tidal channel and are regularly inundated at high tide. In the lower portion of the estuary, where salinities are higher (Whigham, Jordan, and Miklas, 1989), *H. moscheutos* populations are found mostly near the upland–wetland border in sites that receive freshwater input from adjacent uplands and are only infrequently flooded at high tide. While *H. moscheutos* populations are physically isolated, they are all hydrologically connected by freshwater flow and/or by tidal exchange. Physical isolation in combination with hydrologic connectivity and the presence of a salinity gradient allowed us to evaluate the importance of three factors (pollen flow, water dispersal of seeds, and salinity) on microgeographic genetic variation in *H. moscheutos*.

Previous research on the reproductive ecology of *H. moscheutos* (Snow and Spira, 1991a, b, 1993; Spira et al., 1992; Spira, Snow, and Puterbaugh, 1996) allowed us to evaluate the importance of pollen flow within and between isolated populations. *H. moscheutos* is an obligate outcrosser pollinated by a specialist anthophorid bee, *Ptilothrix bombiformis*, and also by bumble bees, *Bombus pensylvanicus* (Rust, 1980; Spira, 1989; Spira et al., 1992). Both pollinators have short flight distances between flower visits, which results in a leptokurtic pollen dispersal pattern (Heinrich, 1976; Eickwort and Ginsberg, 1980; Rust, 1980; Waddington, 1983). Based on pollinator behavior, we predicted deficiency of allozyme heterozygosity relative to expectations under random mating, presumably due to biparental inbreeding (Brown,

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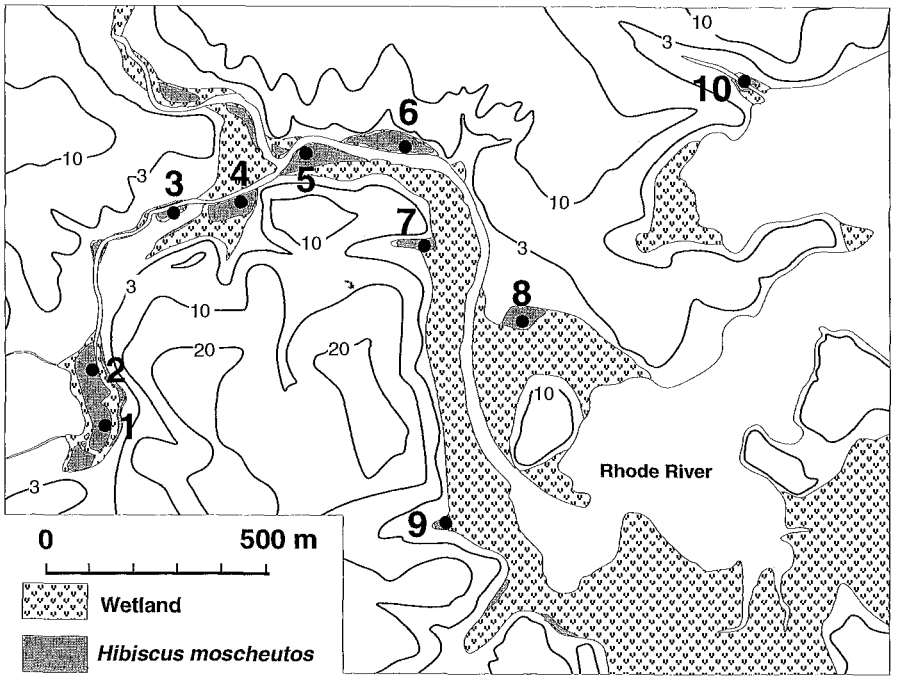


Fig. 1. Map showing the distribution of *Hibiscus moscheutos* in the research area and the locations of ten sampling sites (closed circle). Contours in the map indicate altitude above sea level (m). The tidal creek that connects Sites 1 and 2 with the other populations is called Muddy Creek.

1979). We also predicted that pollen flow between physically isolated populations would be limited and would lead to genetic differentiations between populations (e.g., Levin, 1988; Heywood, 1991).

Seeds are often dispersed by water (hydrochory) in wetlands (Waser, Vickery, and Price, 1982; Schneider and Sharitz, 1988; Leck, 1989; Edwards, Wyatt, and Sharitz, 1994) and seeds of *H. moscheutos* are thick-coated and buoyant (Blanchard, 1976). Genetic structuring within and between populations in wetlands may be weak or absent if hydrochory effectively mixes seeds spatially. However, little is known about the effect of hydrochory on the genetic structure of plant populations in wetlands. We predicted that seed dispersal by water would minimize genetic structuring within populations. Seed exchange between populations would prevent genetic differentiation between them, but only for populations adjacent to the tidal stream that were regularly flooded by high tides. Seed exchange rate to other populations would be much lower in populations in nontidal freshwater habitats and in brackish habitats near the upland/wetland boundary.

H. moscheutos occurs in a wide range of freshwater habitats, but its distribution within the intertidal zone is limited to low-salinity brackish wetlands (Tiner and Burke, 1995). If selection by salinity has acted on allozyme loci or genes closely linked to them, allele frequencies would be correlated with differences in interstitial salinity.

MATERIALS AND METHODS

Species—*Hibiscus moscheutos* is native to freshwater and brackish wetlands in the eastern United States (Blanchard, 1976; Beal, 1977; Brown and Brown, 1984; Cahoon and Stevenson, 1986; Spira, 1989). In Maryland, wetlands dominated by *H. moscheutos* cover 4.9 and 0.2% of the freshwater and brackish wetland habitats (McCormick and Somes, 1982), respectively. *H. moscheutos* is a long-lived perennial; older plants may form compact multistemmed clumps, but they are not rhizomatous or stoloniferous and cannot spread away from the point of establishment except by seed dispersal. The flowers are large (10–15 cm across) and showy, with one or two open flowers per shoot each day during the flowering period. Spatial separation of anthers and stigmas prevents autopollination (Spira, 1989), but pollinators transfer pollen within and among flowers of the same plant, and seeds can be sired by inbreeding (Spira, 1989).

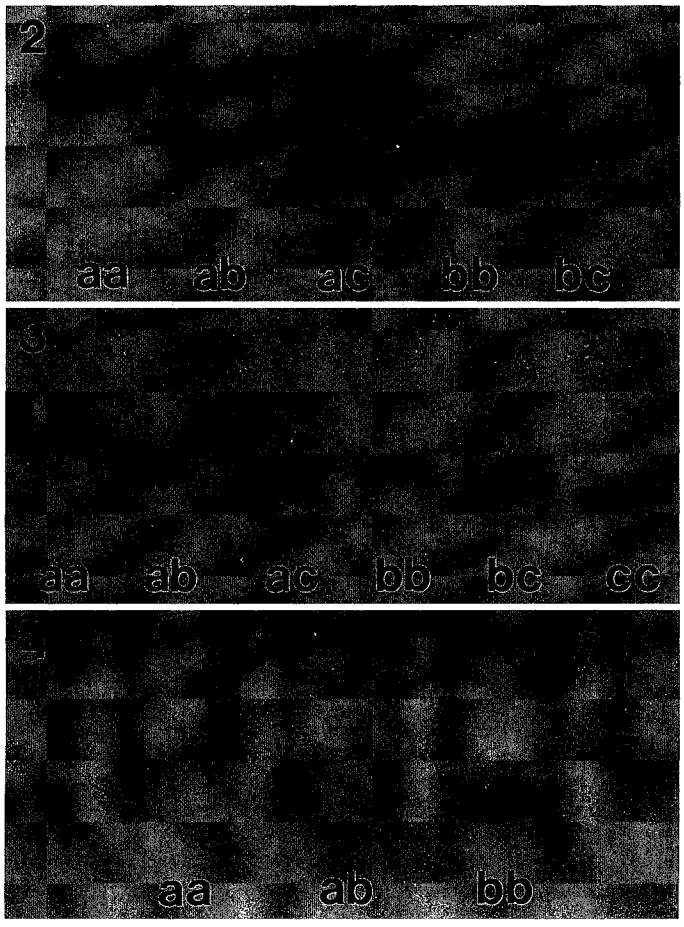
Study sites—We sampled ten sites (Fig. 1) within an area of ~1.5 km^2 along a fresh to brackish water gradient (Whigham, Jordan, and Miklas, 1989; Jordan et al., 1991a, b) in the Rhode River subestuary (38°53′N, 76°33′W) of Chesapeake Bay. Eight of the populations (Sites 3–10; Fig. 1) were physically isolated but hydrologically linked within the intertidal zone. At the brackish sites, *Typha angustifolia, Spartina cynosuroides, Scirpus olneyi,* and *Polygonum punctatum* were the most commonly associated species. Sites 1 and 2 (Fig. 1) were not physically isolated from each other and both were in a freshwater nontidal wetland, locally known as Mill Swamp. The Mill Swamp sites were hydrologically linked by freshwater flow from Site 1 to Site 2. The Mill Swamp sites have been used for previous studies of the reproductive ecology of *H. moscheutos* (Spira, 1989; Snow and Spira, 1991a, b, 1993; Spira et al., 1992; Spira, Snow, and Puterbaugh, 1996). *Saururus cernuus, Polygonum arifolium, Polygonum punctatum,* and *Leersia oryzoides* were the most commonly associated species in Mill Swamp. The shapes and sizes of the areas used to sample *H. moscheutos* at each site are shown in Table 1. At each site, we counted the number of plants and the number of shoots on each plant in five 2 × 2 m^2 quadrats. The height of the tallest shoot in each quadrat was measured. Leaves used for isozyme analysis were collected from 60 plants at each site and all plants sampled were at least 2 m apart. In Sites 2 and 8, we mapped the locations of all plants sampled for isozyme analysis to perform the spatial autocorrelation analyses (described in data analysis section). We

TABLE 1. Profiles of the ten *Hibiscus moscheutos* sites. Site numbers correspond to those shown in Fig. 1. The size and shape of the area sampled to obtain 60 plants for isozyme analysis are given. Means and standard deviations are given for numbers of shoots/4 m^2, plants/4 m^2, shoots/plant, and the maximum shoot height of *H. moscheutos* in five 2 × 2 m^2 subquadrats sampled at each site. Means and standard deviations are given for salinity, conductivity, and pH of interstitial water.

Site	Size (m^2)	Shape (m × m)	No. of shoots/4 m^2	No. of plants/4 m^2	No. of shoots/plant	Shoot height (cm)	Salinity (‰)	Conductivity (mS/cm)	pH
1	350	25 × 14	90.6 ± 46.3	7.6 ± 4.3	13.6 ± 4.5	239 ± 7	0.0	0.19 ± 0.07	6.0 ± 0.6
2	360	24 × 15	77.6 ± 22.8	8.8 ± 4.7	10.4 ± 3.9	253 ± 24	0.0	0.16 ± 0.03	5.4 ± 0.4
3	336	28 × 12	36.2 ± 6.0	19.4 ± 5.2	1.9 ± 0.4	189 ± 8	4.7 ± 0.8	7.9 ± 0.7	5.9 ± 0.3
4	360	30 × 12	21.8 ± 10.8	11.8 ± 5.4	1.9 ± 0.3	167 ± 12	3.8 ± 0.6	6.9 ± 0.8	6.0 ± 0.2
5	360	30 × 12	50.6 ± 19.0	25.4 ± 8.9	2.0 ± 0.4	170 ± 11	3.3 ± 0.6	5.9 ± 0.6	6.1 ± 0.4
6	560	80 × 7	19.8 ± 9.1	5.6 ± 2.4	3.7 ± 1.2	160 ± 17	2.3 ± 0.4	4.9 ± 0.7	6.3 ± 0.4
7	364	28 × 13	22.2 ± 17.6	7.2 ± 3.4	3.0 ± 1.5	169 ± 13	2.9 ± 0.9	5.4 ± 1.4	6.7 ± 0.3
8	360	24 × 15	15.4 ± 10.6	3.8 ± 1.3	3.7 ± 1.9	137 ± 13	4.4 ± 0.7	7.1 ± 1.2	6.3 ± 0.1
9	350	25 × 14	62.4 ± 35.0	23.2 ± 8.6	2.6 ± 0.6	175 ± 21	2.5 ± 0.9	5.4 ± 1.5	5.9 ± 0.5
10	200	20 × 10	36.2 ± 28.2	8.6 ± 4.4	3.9 ± 1.0	181 ± 17	6.9 ± 0.9	10.7 ± 1.6	6.5 ± 0.5

sampled interstitial water at all sites in August at 10–13 sampling locations to determine salinity [parts per thousand (‰)], conductivity [millisiemens per centimeter (mS/cm)], and pH. Interstitial salinity typically reaches a maximum in August in the Rhode River subestuary (Whigham, Jordan, and Miklas, 1989).

Isozyme analyses—Leaves were placed in an ice box in the field and then stored in the laboratory at 4°C prior to electrophoresis. We initially tested 23 enzymes on 180 plants selected randomly from the total set of 600. We analyzed all 600 samples for three enzymes (esterase [EST], phosphoglucoisomerase [PGI], phosphoglucomutase [PGM]) that showed consistently clear and genetically interpretable banding patterns. Approximately 80 mg of leaf tissue was homogenized in 1.2 mL of a modified Shiraishi (1988) extraction buffer [0.1 mol/L tris-HCl (pH 7.5), 20 %(v/v) glycerol, 1 %(v/v) tween 80, 10 mmol/L dithiothreitol (DTT), 0.1 %(v/v) β-mercaptoethanol, and 75 mg/mL polyvinylpolypyrrolidone (PVPP)]. The extracts were loaded on polyacrylamide vertical slab gels (Davis, 1964; Ornstein, 1964) after refining by centrifugation (15000 rpm for 45 min at 1°C). Electrophoresis was carried out at 4°C, 11 mA/cm^2 for ~ 180 min. Enzymes were stained following the protocols of Shiraishi (1988).



Figs. 2–4. Zymograms for three enzyme systems, (2) esterase (EST), (3) phosphoglucoisomerase (PGI), and (4) phosphoglucomutase (PGM). Letters in the zymograms indicate putative genotypes for corresponding banding patterns.

Interpretation of isozyme banding patterns—The three putative loci (*Est, Pgi,* and *Pgm*) were scored for all individuals (Figs. 2–4). *Est* and *Pgm* showed monomeric banding patterns with three and two alleles, respectively. *Pgi* showed a dimeric variation pattern with three alleles. Allelic variation at these loci was coded alphabetically with the most slowly migrating allozyme designated *a.* The interpretations of zymograms in Figs. 2–4 were consistent with patterns seen in the available literature on inheritance of plant isozymes (Gottlieb, 1982; Weeden and Wendel, 1989; Kephart, 1990). Even though the band for allele *c* in *Est* was weak compared with bands for alleles *a* and *b* and we could not find *cc* homozygote in the samples, we treated this band as an allele because the band was consistently absent in *ab* genotypes. Because the frequency of *Est-c* was low, whether or not the *Est-c* band was taken as an allele had little effect on the interpretation of the data. Following interpretation of the zymograms, we counted the genotype frequencies and calculated allele frequencies for each site.

Data analyses—*Hardy-Weinberg equilibrium within populations*—Deviation from Hardy-Weinberg expectations was analyzed with a χ^2 test (Workman and Niswander, 1970) for each site, and deviations were expressed as the fixation index: $F = 1 - (Ho/He)$, where *Ho* is the observed number of heterozygotes and *He* is the expected number of heterozygotes for populations in Hardy-Weinberg equilibrium (Wright, 1921, 1969). Spearman's rank correlations of *F* across the ten sites were calculated against plant density, shoot density, number of shoots per plant, and sampling area, to test whether any of the habitat characteristics explain variation in *F.*

Spatial genetic structuring within populations—We subjected the isozyme data from Sites 2 and 8, to spatial autocorrelation analysis (Sokal and Oden, 1978a, b; Heywood, 1991) to determine whether there was genetic structuring within sites. Moran's *I* was calculated for each allele separately across a range of distances using the Spatial Analysis Programmes written by R. Duncan (1990). We used distance intervals of

0–3, 3–6, 6–9, and 9–12 m in the spatial autocorrelation analysis. Statistical significance of the observed Moran's I was tested under the null hypothesis of spatial independence of a given allele by comparison with a set of values calculated with random permutations of the data.

Genetic subdivision between populations—We used Weir and Cockerham's (1984) estimates of Wright's (1951) F statistics (F, θ, and f for estimates of F_{IT}, F_{ST}, and F_{IS}, respectively) to characterize overall genetic subdivision. The extent of inbreeding in all populations combined (F) was partitioned into inbreeding due to nonrandom mating in each site (f) and inbreeding due to the correlation among alleles caused by their occurrence in the same site (θ). Calculations were made using FSTAT (Version 1.2, 1994 by J. Goudet, see Goudet, 1995). The significances of F, θ, and f per locus and overall loci were tested (see detail in Goudet, 1995) against the distribution of the null hypothesis, namely F (or θ, f) not >0, obtained by permutations (Manly, 1991; Excoffier, Smouse, and Quattro, 1992; Hudson, Boss, and Kaplan, 1992). Whether or not there is significant microgeographic structure in genetic variation among sites can be assessed by determining whether θ is significantly greater than zero. In addition to calculating F statistics, we assessed variation in allele frequencies across sites using χ^2 contingency table analysis. We analyzed each locus separately, combining rare alleles to keep expected values above 5 (Zar, 1984).

Gene flow between sites—We calculated the average level of gene flow (Nm) among sites using the equation $Nm = (1/\theta - 1)/4$, which was based on the infinite island model (Wright, 1951). However, in the infinite island model, every site has an equal probability of exchanging migrants with any other, a situation that is not likely to occur in natural plant populations (Levin and Kerster, 1974; Brown, 1979). Thus, we estimated gene flow between each pair of sites ($\hat{M}$) from a matrix of θ (Slatkin and Maddison, 1990; Slatkin, 1993) using FSTAT (Goudet, 1995). Slatkin (1993) has shown theoretically that $\hat{M}$ depends on geographic distance between pairs of locations in the one- and two-dimensional stepping stone models (Kimura and Weiss, 1964) in which gene flow occurs only between immediate neighbor populations. The simulation studies indicated that, in the regression analyses of $\log\hat{M}$ on $\log k$ where k is the distance separating two locations, the predicted values of regression slopes are -1 for the one-dimensional model and -0.5 for the two-dimensional model (Slatkin, 1993, 1994b). In our study, however, hydrological relationships between populations may influence gene flow (e.g., seed dispersal) more than geographic distances between populations (Schneider and Sharitz, 1988; Nilsson, Gardfjell, and Grelsson, 1991; Johansson and Nilsson, 1993). We thus tested three hypothetical models of the gene flow pathways (Fig. 5) to interpret variation in $\hat{M}$. Two of the three models are based on distance dependency of gene flow and the third model was based on hydrological relationships between sites. In the *two-dimensional stepping stone distance model* (TSD model, Fig. 5A), gene flow depends on geographic distance between sites. We measured the geographic distance between any two sites as the Euclidean distance separating them. In the second model, *one-dimensional stepping stone distance model* (OSD model, Fig. 5B), gene flow depends on geographic distance between sites measured along the freshwater stream (Sites 1–2), tidal stream (Sites 3–9), and estuary (Site 10). This model assumes that the differences in the types of hydrological connections, i.e., freshwater stream or tidal stream and main channel or water flow through marsh vegetation, does not influence seed dispersal. In the third model, *stream accessibility model* (STA model, Fig. 5C), sites were divided into two categories based on the distances between *H. moscheutos* populations and the stream channel of Muddy Creek (thick line in Fig. 5C). In the STA model we predicted that there would be almost no barrier to gene flow among populations adjacent to the tidal stream channel (Sites 3–6 and 10 in Figs. 1 and 5C), and that the populations located at the upland/wetland boundary (Sites 7–9) and in the nontidal freshwater habitats (Sites 1 and 2) would be relatively isolated from other populations because they are not frequently flooded.

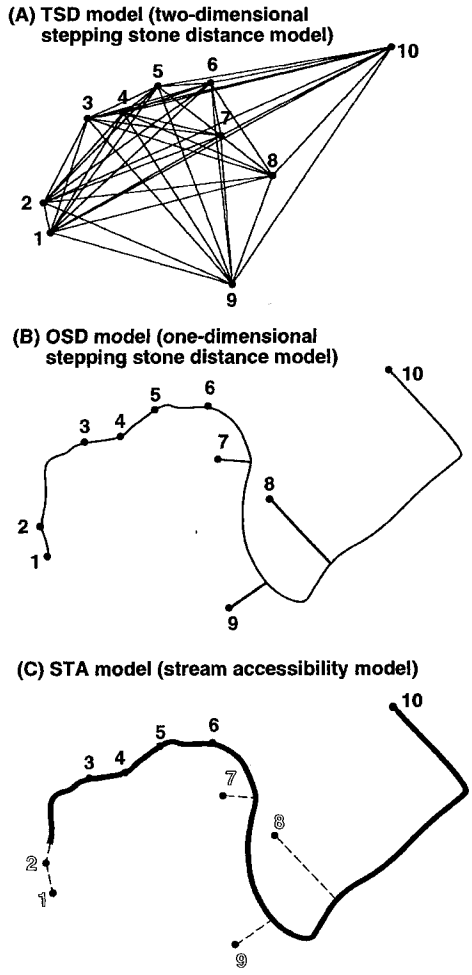


Fig. 5. Three hypothetical models of the actual pathways of gene flow among the ten *H. moscheutos* populations. **(A) TSD model** (*two-dimensional stepping stone distance model*); gene flow depends on the Euclidean distance between sites (indicated by lines). **(B) OSD model** (*one-dimensional stepping stone distance model*); gene flow depends on the distance between sites as measured along the tidal creek and the distance between the tidal stream and the study sites (indicated by lines). **(C) STA model** (*stream accessibility model*); sites were divided into two categories based on proximity to the tidal creek (the thick line). Sites 3, 4, 5, 6, and 10 are adjacent to the tidal creek (noted by boldface letters). Sites that are not adjacent to the tidal creek (Sites 1, 2, 7, 8, and 9) are indicated by open letters. Dashed lines indicate that water flow goes through marsh vegetation along these lines.

This model assumes that there is effective long-distance seed dispersal along the tidal channel. The TSD model predicts the relative importance of gene flow through pollen dispersal among sites, and the OSD and STA models emphasize the relative importance of hydrochory in gene dispersal among sites. The TSD and OSD models were tested by regressing log $\hat{M}$ on log distance between sites where distances were measured based on the model assumptions. The STA model was tested by calculating Weir and Cockerham's (1984) estimates of Wright's (1951) F statistics separately using data for the populations adjacent to the tidal stream and the populations in nontidal habitats or habitats at the upland/wetland boundary.

Salinity and allele frequencies—Gradients in allele frequencies for the ten sites along the salinity gradient were tested by Spearman's rank correlations with both salinity and conductivity of interstitial water in August.

TABLE 2. Allele frequencies and Wright's fixation indices (F) of the ten sites for the three enzyme loci. The results of chi-square contingency table tests are also shown.

Enzyme locus	Site 1	2	3	4	5	6	7	8	9	10
Est										
a	0.42	0.26	0.20	0.19	0.28	0.60	0.09	0.57	0.42	0.28
b	0.58	0.74	0.72	0.79	0.72	0.40	0.91	0.43	0.55	0.70
c	0.00	0.00	0.08	0.02	0.00	0.00	0.00	0.00	0.03	0.02
						$\chi^2_{(12)} = 182$		$P < 0.001$		
Pgi										
a	0.21	0.13	0.27	0.32	0.32	0.18	0.23	0.42	0.15	0.30
b	0.12	0.06	0.17	0.08	0.10	0.12	0.13	0.12	0.24	0.17
c	0.67	0.81	0.56	0.60	0.58	0.70	0.64	0.46	0.61	0.53
						$\chi^2_{(12)} = 72.3$		$P < 0.001$		
Pgm										
a	0.82	0.78	0.92	0.87	0.83	0.97	0.98	0.92	0.97	0.88
b	0.18	0.22	0.08	0.13	0.17	0.03	0.02	0.08	0.03	0.12
						$\chi^2_{(6)} = 182$		$P < 0.001$		
F[a]										
Est	−0.234	0.087	−0.062	−0.041	0.343**	0.028	0.099	0.050	−0.020	0.107
Pgi	0.111	−0.021	0.034	−0.157	0.060	−0.258*	−0.154	0.168	−0.093	−0.032
Pgm	−0.002	0.214	0.159	−0.143	−0.080	−0.026	−0.017	0.159	−0.034	0.314*
All loci	−0.039	0.103	0.021	−0.110	0.130	−0.110	−0.081	0.142	−0.048	0.085

[a] Asterisks indicate significant deviation of F from zero (* $P < 0.05$; ** $P < 0.01$).

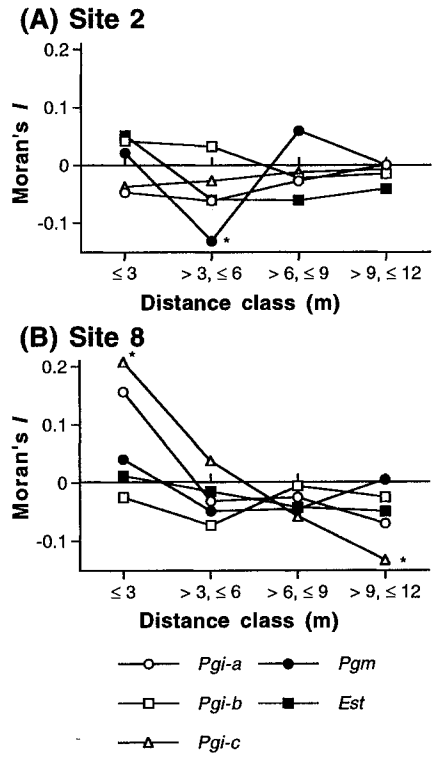


Fig. 6. Correlograms of Moran's I for three loci at Site 2 (A) and Site 8 (B). Sites 2 and 8 were selected to represent freshwater (Site 2) and brackish (Site 8) habitats. For diallelic loci (*Pgm, Est*), the autocorrelation coefficients for each allele were identical.

RESULTS

Interstitial salinity, plant density, and plant size—Interstitial salinity and conductivity were lowest at Sites 1 and 2, where maximum shoot height was greatest (~ 250 cm) (Table 1). Shoot density was three to seven times higher at Sites 1 and 2 than at the other sites (Table 1). In the brackish sites, *H. moscheutos* populations are much patchier and plant densities were lower (Table 1). Interstitial water salinity and conductivity ranged from 2.3 to 6.9 ‰ and 4.9 to 10.7 mS/cm, respectively (Table 1).

Genetic variation within sites—*Est, Pgi,* and *Pgm* were polymorphic at all sites (Table 2). The *Pgi-c* and *Pgm-a* alleles were the most common at all sites (Table 2). For *Est,* allele *a* was most frequent at Sites 6 and 8, and allele *b* had the highest frequencies at the other sites (Table 2). Genotype frequencies were consistent with Hardy-Weinberg equilibrium for all but three of the 30 combinations of locus and site, and F calculated for all loci combined indicated no deficiency of heterozygosity relative to Hardy-Weinberg expectations at all sites (Table 2). Overall, an f of 0.017 also indicated no significant inbreeding effect within sites (Table 3). None of the rank correlations of F across the ten sites calculated against shoot density, plant density, shoot /plant, and shoot height were significant at $P < 0.05$ (data not shown). Spatial autocorrelation analysis indicated spatial independence of allele distribution for all alleles at Site 2 except for *Pgm* in the >3 to ≤6 m class (Fig. 6A). At the Site 8, *Pgi-c* exhibited positive autocorrelation in the shortest distance

TABLE 3. Weir and Cockerham (1984) estimates of Wright's F statistics ($F = F_{IT}$, $\theta = F_{ST}$, $f = F_{IS}$) calculated for all ten sites. Estimates of gene flow (Nm) based on θ are also listed.

Enzyme locus	F	θ	f	Nm
Est	0.138**	0.107**	0.034	3.12
Pgi	0.013	0.030**	−0.018	8.08
Pgm	0.121**	0.044**	0.080	5.43
Overall loci	0.079**	0.062**	0.017	3.78

*, ** Significant deviation of F statistics from zero (* $P < 0.05$, ** $P < 0.01$).

class (≤3 m) and negative autocorrelation in the most distant class (>9–≤12 m), but no spatial structure was detected for the other alleles and locus (Fig. 6B).

Genetic variation and gene flow among sites—Significantly positive F for *Est, Pgm,* and overall loci indicated an overall excess of homozygotes (Table 3). Significantly positive θ and no significant f for each locus and for overall loci (Table 3) indicate that the overall excess of homozygotes was mostly attributable to reduced gene flow among sites. For all three loci, allele frequencies differed across sites (contingency analysis, $P < 0.001$; Table 2). Estimates of θ ranged from 0.030 to 0.107 with a value of 0.062 for overall loci (Table 3), indicating moderate genetic differentiation of allele frequencies among sites.

Average gene flow among sites (Nm) based on θ calculated for overall loci and sites was 3.78 (Table 3). Gene flows between pairs of sites ($\hat{M}$), however, were highly variable (Table 4). All populations adjacent to the tidal stream (STA model), except for Site 6, had higher gene flow ($\hat{M}$ >30) with each other (Table 4; boldface numbers below the diagonal) than those between other combinations of sites. None of the F statistics calculated for populations adjacent to the tidal stream, excluding Site 6, were significantly different from zero, indicating that there was no genetic structuring among Sites 3, 4, 5, and 10, and gene flow was large (Nm = 250; Table 5). Significant genetic structuring was detected (θ = 0.091) among the nontidal populations and sites that were not adjacent to the tidal stream (STA model), and gene flow was reduced between these sites (Nm = 2.50; Table 5). The regressions of log $\hat{M}$ on log distances between sites (k) based on the TSD model (log $\hat{M}$ = 0.013log$k$ + 0.733, $r^2 < 0.001$, F ratio in ANOVA = 0.002) and the OSD model (log $\hat{M}$ = −0.122log$k$ + 1.141, r^2 = 0.008, F ratio = 0.344) were not significant at $P < 0.05$ (Fig. 7).

TABLE 5. Weir and Cockerham (1984) estimates of Wright's F statistics ($F = F_{IT}$, $\theta = F_{ST}$, $f = F_{IS}$) for overall loci calculated for the populations (S) adjacent to the tidal stream (Sites 3, 4, 5, 6, and 10), and the populations (I) that were not adjacent to the Muddy Creek (Sites 1, 2, 7, 8, and 9) in the STA model. Refer to Fig. 1 for site locations. Estimates of gene flow (Nm) based on θ are also listed.

Site category	F	θ	f	Nm
S sites	0.060*	0.048**	0.013	4.96
S sites (exclude Site 6)	0.038	0.001	0.036	250
I sites	0.111**	0.091**	0.022	2.50

*, ** Significant deviation of F statistics from zero (* $P < 0.05$, ** $P < 0.01$).

Two *Pgi* alleles (*a* and *c*) had significant rank correlations with interstitial salinity (r = 0.750, $P < 0.05$, and r = −0.918, $P < 0.01$ for *Pgi-a* and *-c,* respectively) and conductivity (r = 0.750, $P < 0.05$, and r = −0.936, $P < 0.01$ for *Pgi-a* and *-c,* respectively). There were no significant rank correlations between allele frequency and interstitial salinity/conductivity for the other alleles (data not shown).

DISCUSSION

Gene flow within sites—Random mating is rarely realized in natural plant populations of insect-pollinated species due to breeding between genetically related individuals caused by leptokurtic patterns of seed dispersal and of pollen movement (Levin and Kerster, 1974; Brown, 1979). Fixation indices (F, Table 2) indicate, however, that random mating occurs within the ten *H. moscheutos* populations. We anticipated that mating, based on pollen flow, would not be random at our study sites because *H. moscheutos* is mainly pollinated by bumble bees and by anthophorid bees that have high visitation rates (4.1 ± 0.5 and 2.0 ± 0.3 visits per 15-min periods in 2 yr of observation; Spira et al., 1992) but only travel short distances between flowers (Rust, 1980). Even with a high level of pollinator activity, however, pollinator behavior cannot solely explain the almost complete panmixia within *H. moscheutos* populations. The almost complete panmixia suggests that seeds are widely dis-

TABLE 4. Pairwise estimates of Weir and Cockerham's (1984) θ (above diagonal) and gene flow between sites ($\hat{M}$) (below diagonal).[a] Values of $\hat{M}$ for pairs within the same site categories in the STA model are shown in boldface for populations adjacent to the tidal stream and in italics for nontidal freshwater sites and tidal sites at the upland wetland boundary.

Site	1	2	3	4	5	6	7	8	9	10
1	—	0.025	0.037	0.041	0.014	0.043	0.112	0.054	0.021	0.019
2	*9.83*	—	0.053	0.039	0.031	0.130	0.084	0.158	0.077	0.047
3	6.45	4.44	—	0.004	0.007	0.116	0.029	0.090	0.034	−0.001
4	5.88	6.22	**58.79**	—	−0.001	0.145	0.025	0.110	0.067	0.004
5	18.31	7.86	**34.13**	**83.08**	—	0.102	0.055	0.069	0.044	−0.005
6	5.63	1.68	**1.90**	**1.47**	**2.19**	—	0.228	0.048	0.028	0.095
7	*1.99*	*2.74*	8.32	9.83	4.32	0.85	—	0.208	0.114	0.051
8	*4.39*	*1.34*	2.53	2.01	3.38	4.97	*0.95*	—	0.053	0.059
9	*11.83*	*3.02*	7.06	3.46	5.44	8.46	*1.95*	*4.45*	—	0.029
10	13.29	5.05	**83.08**	**65.56**	**83.08**	**2.38**	4.70	3.96	8.50	—

[a] θ = 0.003 was used in the calculation of $\hat{M}$ for the site pairs with negative values of θ.

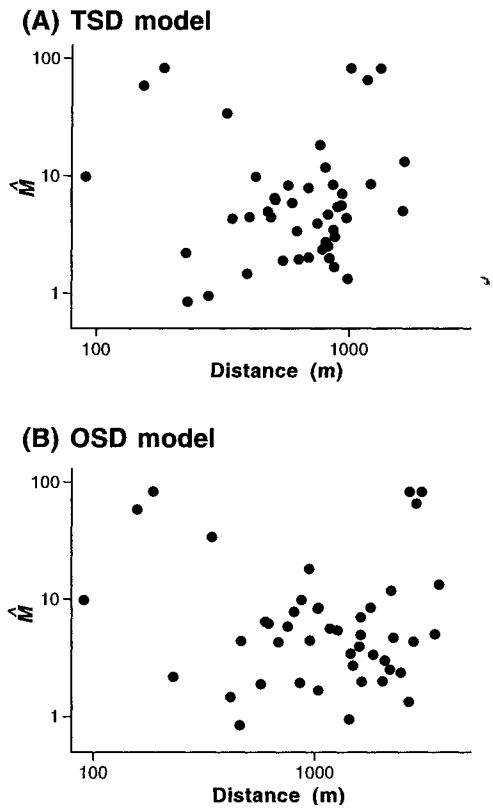


Fig. 7. Log $\hat{M}$ (gene flow between pairs of sites) plotted against log distance between sites for the (A) TSD and (B) OSD models. Geographic distance between sites was measured by the Euclidean distance separating sites in the TSD model (A), and as distance along Muddy Creek and lateral distance between Muddy Creek and the sites in the OSD model (B). $\hat{M}$ is based on the Weir and Cockerham's (1984) estimate of F_{ST}, θ.

persed when sites are flooded. Spatial mixing of genotypes by hydrochory may, thus, minimize biparental inbreeding in *H. moscheutos* populations. Results of spatial autocorrelation analyses for Sites 2 and 8 support this conclusion (Fig. 6). There were no spatial patterns at either site except for one allele at one site, suggesting that groups of related individuals are spatially mixed and randomly distributed within sites. A lack of spatial genetic structures within populations is typically not found in terrestrial herbaceous species (reviewed in Heywood, 1991; Williams, 1994).

Plants and shoot densities might also influence gene flow. Average density within the ten sites ranged from 3.8 to 25.4 plants /4 m^2, and shoot densities ranged from 15.4 to 90.6 /4 m^2 (Table 1). A negative correlation between mean flight distance of pollinators and the densities of open flowers has been reported in other species (Levin and Kerster, 1969; Beattie, 1976; Ellstrand, Torres, and Levin, 1978; Pyke, 1978; Heinrich, 1979), suggesting stronger effects of biparental inbreeding in populations with higher densities. Across the ten *H. moscheutos* populations, we did not detect any significant correlations between fixation indices (F) and plant or shoot densities. This indicates that the mechanisms responsible for random mating within *H. moscheutos* populations are consistently effective over a wide range of plant densities. Our findings are not consistent with Snow et al. (1996) who estimated a selfing rate of 36% for *H. moscheutos* in the area included by our Sites 1 and 2. Snow et al. (1996) based their findings on an analysis of variation in seeds from 38 maternal families. The high selfing rate observed by Snow et al. (1996) may have been due to a positive correlation between the degree of geitonogamy and number of flowers per plant. In *H. moscheutos,* the majority of flowering shoots have only one open flower per day (Spira, 1989), but the number of shoots/plant was much higher in Sites 1 and 2 (13.6 ± 4.5 and 10.4 ± 3.9, respectively), which would lead to higher inbreeding rates. A possible explanation for the difference between our results and those of Snow et al. (1996) is that we sampled mature plants that may have became established many years earlier when the number of flowers per plant was lower and inbreeding rate was lower, whereas seeds sampled by Snow et al. were produced under high flower density per plant with a high inbreeding rate. Seedling establishment rates of *H. moscheutos* in Mill Swamp are very low, and recruitment of new individuals from seeds is almost always associated with habitat disturbance (H. Kudoh and D. F. Whigham, personal observations). Infrequent recruitment of plants may result in changes in the ratio of seeds produced by inbreeding to those produced by outcrossing. As the size of individual plants increase and as the number of flower per plant increases selfing may become more dominant. An alternative explanation for the results of our study and those of Snow et al. (1996) may be differential survival of seedlings that are produced by inbreeding and by outcrossing. Inbred progeny may be less successful than outcrossed plants and mature plants that survive in Mill Swamp may be the result of establishment of outcrossed progeny. Inbred progeny of *H. moscheutos* were reported to have lower growth rates than outcrossed progeny (Snow and Spira, 1993). Hardy-Weinberg equilibrium among mature plants and an excess of homozygotes in their offspring were also reported in *Gentiana pneumonanthe* (Raijmann et al., 1994).

Gene flow and genetic differentiation among sites— Although the ten sites were distributed over a relatively small area (~ 1.5 km^2), there were significant differences in allele frequencies among sites (Table 2). The significantly positive θ (Table 3) also indicates that the range of sampling sites that we selected is subdivided into groups of plants that experience reduced gene exchange. Estimates of F_{ST} for overall loci (θ = 0.062) indicates moderate genetic differentiation among sites. Despite the high average gene flow among populations (Nm = 3.78, Table 3), gene flow between pairs of sites ($\hat{M}$) were highly variable, ranging from 0.95 to more than 80 (Table 4), which suggests that gene flow is sensitive to local conditions.

Of the three models tested, the STA model (Fig. 5C) explained most of the variation in gene flow between pairs of sites, suggesting the relative importance of hydrochory in gene flow compared to gene flow by pollen dispersal, which appears to be localized in *H. moscheutos.* Except for Site 6, gene flow was greatest (Nm = 250; Table 5) between populations that were adjacent to the tidal stream. Although the number of studies in which

hydrochory has been investigated is limited (Waser, Vickery, and Price, 1982; Schneider and Sharitz, 1988; Nilsson, Gardfjell, and Grelsson, 1991; Lonsdale, 1993; Edwards, Wyatt, and Sharitz, 1994), the general pattern appears to be that hydrochory results in effective long-distance seed dispersal (e.g., a scale of hundreds of meters or kilometers). In a seed bank study of a freshwater tidal marsh in New Jersey, Leck and Graveline (1979) found that seedlings of *H. moscheutos* were abundant along the stream bank, suggesting effective hydrochory. In our study, populations that were not close to the tidal stream appeared to be relatively isolated ($\theta = 0.091$) with reduced gene flow ($Nm = 2.50$) with other sites (Table 5). Although all of the sites within the estuarine area (e.g., Sites 3–10) are periodically inundated, the velocity of the water is very low (D. F. Whigham, personal observations) and seed movement into or out of the sites that are not near the tidal stream may be reduced because of a dense litter layer that acts to trap seeds. Among the stream-side sites, Site 6 showed relatively low gene flow with the other sites (Tables 4 and 5). Plant density at Site 6 was much lower compared to the other sites that were adjacent to Muddy Creek (Table 1). Low levels of seedling establishment at Site 6 may explain the relative isolation of the site.

Population differentiation in allozyme frequencies along an environmental gradient indicates that selection can cause population differentiation in allozymes (Silander, 1984). The frequencies of *Pgi-a* and *Pgi-c* were significantly correlated with the salinity of interstitial water at sampling sites in August, suggesting that salinity may select different alleles of *Pgi* or of a locus closely linked to them. *Pgi-c* had a significant spatial autocorrelation within Site 8, a brackish habitat, while there is no spatial structure of this allele at Site 2, a freshwater habitat (Fig. 6). This result suggests that selection by salinity may be important in determining small-scale spatial heterogeneity of the *Pgi* alleles in brackish habitats. This aspect of selection in *H. moscheutos* must, however, be evaluated more rigorously before any conclusion can be reached. First, our salinity data are only for August and temporal fluctuation of salinity (Jordan et al., 1991b) needs to be assessed over a longer period of time. Second, the influence of salinity on growth and survival of *H. moscheutos* plants with different *Pgi* alleles needs to be determined.

In conclusion, the importance of hydrochory in determining the spatial genetic structure was suggested both within and among *H. moscheutos* populations. Hydrochory presumably reduces spatial genetic structuring within populations and reduces the effect of biparental inbreeding. Despite the evidence for gene flow between populations of *H. moscheutos,* significant genetic structuring among populations occurs, primarily when populations are somewhat isolated from the tidal creeks. It remains to be determined whether hydrochory influences the genetic structure of other populations of wetland plant species. Our study suggests, however, that local conditions can influence gene flow of *H. moscheutos* by reducing the efficiency of seed dispersal by water. In such circumstances, local differentiation can occur in other characters, including life history traits. Further research is required to identify the cause of population differentiation in *H. moscheutos,* and it remains to be determined what life history traits are genetically differentiated along the fresh to brackish gradient.

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