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HYDROCHORY AS A DETERMINANT OF GENETIC DISTRIBUTION OF SEEDS WITHIN *HIBISCUS MOSCHEUTOS* (MALVACEAE) POPULATIONS[1]

RYOUJI SHIMAMURA,[2,3] NAOKI KACHI,[2] HIROSHI KUDOH,[3,5] AND DENNIS F. WHIGHAM[4]

[2]Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Minami-osawa 1–1, Hachioji 192-0397, Japan; [3]Department of Biology, Graduate School of Science, Kobe University, Nada-Ku, Kobe 657-8501, Japan; and [4]Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, Maryland 21037 USA

Seed dispersal is a major determinant of the spatial genetic structure of plant populations. In this study, we evaluated the role of distinct hydrologic regimes in determining the spatial genetic structure of the seed bank of the wetland plant *Hibiscus moscheutos*. We analyzed seeds in surface soil samples collected in the autumn and the following spring by determining their allozyme genotypes and estimated the pattern in seed movements during flooding. We selected study sites in nontidal and tidal wetlands with different flooding regimes. One nontidal site had no flooding, while the second nontidal site was inundated for most of the year. One tidal wetland site flooded with almost every tide, and a second tidal site was inundated at moderate frequency. Genetic makeup of the seed bank at the nonflooded site changed little between seasons. Secondary seed dispersal altered absolute allele frequencies at the other three sites, with the greatest change occurring at the two tidally influenced sites. This study demonstrates that secondary hydrochory influences the genetic composition of the seed bank and that hydrologic conditions play an important role in determining the local patterns in seed movements.

Key words: flooding; genetic structure; *Hibiscus moscheutos*; hydrochory; Malvaceae; Maryland; secondary seed dispersal; seed bank.

Wetland plants often appear as discrete patches, reflecting spatial heterogeneity in microgeographic conditions or responses to variation in hydrology and biotic and abiotic disturbances (e.g., van der Valk, 1981; Bertness, 1999). If plant distributions are viewed in terms of genetic variation, patchiness of genetically related individuals may be observed even within patches of single species (Escudero et al., 2003). The genetic variation within patches or populations depends on a variety of factors, but seed dispersal is important (Howe and Smallwood, 1982). Most seeds disperse very close to the source plants, and thus spatial aggregation of seeds with shared lineages is expected in most situations. However, a number of factors that influence secondary seed dispersal are known to alter seed distribution patterns (Hart and Cox, 1995; Peakall and Beattie, 1995; Alvarez-Buylla et al., 1996; Kalisz et al., 1999). Because seed dispersal is the initial template for shaping the genetic structures of plant populations (Cabin et al., 1998; McCue and Holtsford, 1998; Mahy et al., 1999; Koch et al., 2003; Shimono et al., 2006), it is important to understand the factors that influence seed dispersal and subsequently the genetic composition of the seed bank populations.

Secondary dispersal of seeds by water (i.e., hydrochory) may be an especially important factor in wetland ecosystems where seeds of many species are transported by water (Ridley, 1930; Howe and Smallwood, 1982; van der Pijl, 1982; Edwards et al., 1994; Huiskes et al., 1995; Griffith and Forseth, 2002, 2005). Hydrochory has the potential to affect short- and long-distance transport (Cain et al., 2000; Kudoh et al., 2006). Seeds are often buoyant for long periods in many wetland species (Ridley, 1930; Schneider and Sharitz, 1988; Edwards et al., 1994), and such long periods for secondary dispersal may allow seeds to move between and within plant populations. Hydrochorous seeds may disperse several meters to kilometers (Waser et al., 1982; Hart and Cox, 1995; Craddock and Huenneke, 1997; Kudoh and Whigham, 1997), and the distribution of seeds within and between wetlands is influenced by seed buoyancy (Leck and Graveline, 1979; Schneider and Sharitz, 1988; Griffith and Forseth, 2005) and hydrologic conditions in tidal (Huiskes et al., 1995; Griffith and Forseth, 2002) and nontidal wetlands (Schneider and Sharitz, 1988; Hart and Cox, 1995). In wetland plants, spatial aggregation of genetic variation in the seed bank may be less pronounced if hydrochory effectively transports seeds. Earlier work on our study species, *Hibiscus moscheutos* L., has also suggested that secondary seed dispersal occurred (Kudoh and Whigham, 2001) and likely played an important role in determining the spatial genetic structure of populations (Kudoh and Whigham, 1997; Kudoh et al., 2006). We are unaware of any studies of the effects of secondary dispersal of seeds on the genetic composition of the seed bank, although the potential importance of secondary dispersal on population and metapopulation genetics has been documented (e.g., Waser et al., 1982; Gornall et al., 1998; Cain et al., 2000; Griffith and Forseth, 2002). What is less clear is the importance of different hydrologic regimes in the dispersal of seeds of wetland species (Kudoh and Whigham, 1997, 2001; Sork et al., 1999; Kudoh et al., 2006). The mosaic nature of wetlands probably provides a complex set of hydrologic conditions, resulting in variation in important factors such as frequency of flooding, duration of each flooding event, changes in the depth of water during a flooding event, and the directions and velocity of water flow. These factors and others may directly affect seed movements

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[5]Author for correspondence (e-mail: kudoh@kobe-u.ac.jp)

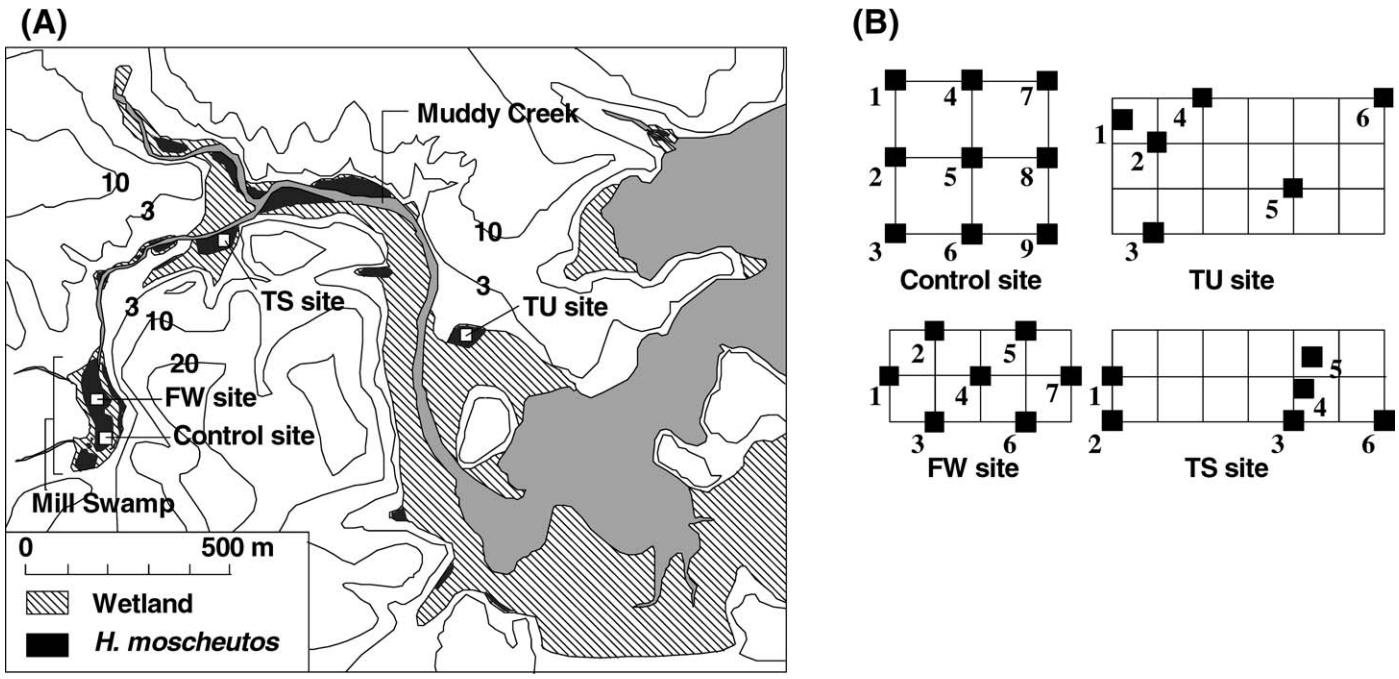

Fig. 1. Map of (A) the study area and (B) spatial arrangements of sampling points in the control and three flooded sites. In (A), *Hibiscus moscheutos* populations (shown by black pattern) occur in a freshwater marsh, Mill Swamp, and in a tidal wetland along Muddy Creek that starts from Mill Swamp. Numbers on contour lines indicate elevation above sea level (m). The locations of the control and three study sites are shown by open squares. In (B), each sampling point is shown by a closed square and a number. These numbers correspond with those in the Tables and the Appendix. Intervals of grid lines are 5 m for the control site and 3 m for the three flooded sites (FW, TU, and TS).

and change the degree of spatial aggregation of genetically related seeds.

In this study, we evaluated the role of different hydrologic regimes on the spatial genetic structure of the seed bank of *H. moscheutos*. Seeds of *H. moscheutos* are dispersed primarily by gravity near to the mother plants, but they are secondarily dispersed by water during winter flooding (Kudoh and Whigham, 1997, 2001).

We quantified seasonal changes in the composition of the *H. moscheutos* seed bank in tidal and nontidal habitats by collecting surface soil samples in the autumn and following spring and estimating the pattern in seed movement by determining the allozyme genotypes of seeds. Previous studies of allozyme variation in established populations (Kudoh and Whigham, 1997) and in floating seed populations (Kudoh and Whigham, 2001) of *H. moscheutos* indicated that the genetic characteristics of populations were influenced by both short- and long-distance seed dispersal. We expected to observe different temporal changes in the distribution of seed genotypes in tidal and nontidal habitats because of contrasting hydrologic regimes. We tested two specific predictions. (1) No secondary seed dispersal of *H. moscheutos* occurs in habitats with no flooding, resulting in no changes in the genetic composition of the seed bank following primary dispersal. (2) Secondary seed dispersal changes the absolute allele frequencies either by exporting or importing seeds, and the magnitude of the change is greater in tidal vs. nontidal habitats because of more frequent and bidirectional flows in the tidal habitats. We quantified the temporal changes in the seed bank by quantifying differences in absolute allele frequency between seasons at multiple sampling points in tidal and nontidal wetlands in the same watershed–estuarine system (Fig. 1).

MATERIALS AND METHODS

Plants and study area—*Hibiscus moscheutos* L. (Malvaceae) is a perennial macrophyte native to freshwater and brackish wetlands in eastern North America. Although *H. moscheutos* does not spread clonally, individual plants can become quite large by producing multiple stems from a perennial root stock. The flowering season extends from late July to early September, and seeds are dispersed from September to November (Spira, 1989; Kudoh and Whigham, 1998). In the field, seeds begin to germinate in May of the following year, and most seeds germinate within a year. Seeds of *H. moscheutos* are short lived in the soil seed bank (Leck and Graveline, 1979; Leck and Simpson, 1995; R. Shimamura et al., unpublished data). Seeds are initially dispersed by gravity and fall below the mother plants during autumn (preflooding). Seeds are buoyant because of an air space inside the water-impermeable seed coat and are subsequently capable of being dispersed by water (secondary dispersal). Intact seed coats provide physical dormancy to the seeds because germination occurs readily if the seed coat is broken (Baskin et al., 2000).

The study was conducted at the Smithsonian Environmental Research Center (SERC; 38°53′ N, 76°33′ W), Edgewater, Maryland, USA, in a nontidal freshwater wetland, locally known as Mill Swamp, and in a brackish tidal wetlands, locally called Muddy Creek, that is part of the Rhode River subestuary of Chesapeake Bay. *Hibiscus moscheutos* is common in both wetland areas. Water flowing through Mill Swamp enters Muddy Creek (Fig. 1A). General hydrologic conditions in Mill Swamp are described in Whigham et al. (1986). In a typical year, the stream that flows through Mill Swamp will become dry by the end of the summer. Water begins to flow again in the autumn, and most of the site is inundated throughout the winter, spring, and early summer. The tidal regime in Muddy Creek and the Rhode River estuary have been described (e.g., Jordan et al., 1984). Muddy Creek is under the influence of tidal exchanges, and wetlands near to the stream are periodically inundated at high tide. Salinity in Muddy Creek varies seasonally and spatially

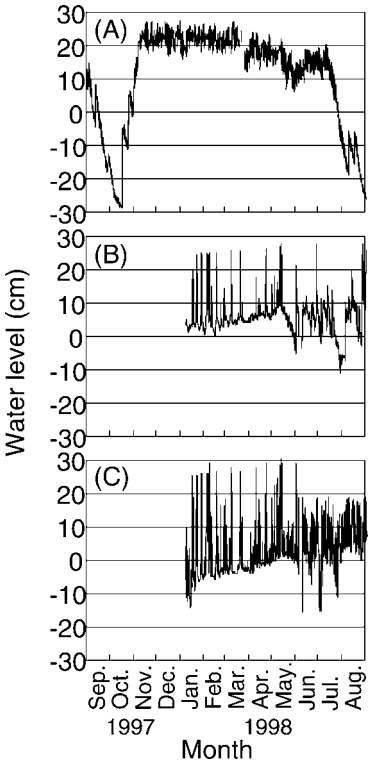

Fig. 2. Water fluctuations at the three flooded sites from September 1997 to August 1998. (A) FW site. (B) TU site. (C) TS site. For the TU and TS sites, measurements were started in January 1998. Water levels higher than 0 cm indicate flooding above the ground where the water-level meters were set. Data were recorded every hour at the FW site and every 4 hours at the TU and TS sites.

with maximum values occurring in the summer when watershed discharge is usually the lowest. Characteristics of *H. moscheutos* populations in Mill Swamp and the Muddy Creek estuary have been described in earlier studies (e.g., Spira, 1989; Kudoh and Whigham, 1997, 1998, 2001; Shimamura et al., 2005).

Seed bank sampling—To study changes in genetic structure following primary and secondary dispersal, we first conducted a control study to develop a sampling procedure and test our prediction that the genetic composition of the seed bank would not change in areas with no or minimal flooding. Because the control study necessitated sampling the seed bank in the autumn after primary seed dispersal and again the next spring after potential secondary seed dispersal, this part of the study was conducted over one year (1995–1996), and the second part of the study, described later, was conducted between autumn 1997 and spring 1998. In October 1995 we established the control site within the most upstream portion of Mill Swamp (Fig. 1A), where flooding did not regularly occur or flooding depth and the velocity of flooding water were very low. As indicated, we predicted that the genetic makeup of the seed bank would change little between the time the study was started (when there was no surface flow of water) and the following spring. Within the control site, nine sampling points were arranged on a 5-m grid (Fig. 1B). At each sampling point, a 1×1 m quadrat was established, and each was divided into four, 50×50 cm subquadrats. Soil, litter, and plant debris to a depth of 5 cm were sampled from two diagonal subquadrats at each sampling point in October, and the other two subquadrats were sampled 7 mo later (May 1996). In the laboratory, the samples were washed through a sieve that would retain *H. moscheutos* seeds. Seeds were handpicked (247 seeds in October and 200 in May) and analyzed individually for allozyme polymorphism (described in detail later). Based on the results of the control study (see Results), we used the same sampling procedure to sample additional sites in the Mill Swamp and Muddy Creek in 1997–1998.

Study sites—We selected three flooded sites, one in a fresh water wetland (FW) and two (TS and TU) in tidal wetland along Muddy Creek (Fig. 1A). The FW site was located in a portion of Mill Swamp characterized by flooding with standing water for long periods during late autumn, winter, spring, and early summer and by directional water flow (Fig. 1A). The FW site was dominated by *H. moscheutos*, and the density of *H. moscheutos* plants was higher at the FW site than at the other two sites. The TS site was located near the tidal stream and was regularly inundated at high tide (Fig. 1A). The TS site was within the low marsh portion (Jordan et al., 1984) of Muddy Creek, and it is characterized by mixed vegetation dominated by *H. moscheutos* and *Phragmites australis* (Cav.) Steud. The TU site was located near the upland–wetland boundaries and was tidally inundated less frequently than the TS site (Fig. 1A). The TU site was a typical high marsh habitat (Jordan et al., 1984) and was dominated by *Scirpus olneyi* A. Gray, *Spartina cynosuroides* (L.) Roth, and *Typha angustifolia* L. Density of *H. moscheutos* was lower in the TU site than in the TS site.

We monitored water level fluctuations at the TU and TS sites using automated water level meters (Remote Data Systems, Inc., Whiteville, North Carolina, USA). Water level data at the FW site were collected at an automated sampling weir (T. Jordan, SERC, personal communication). Water levels were measured from September 1997 to August 1998 in the FW site and from January to August 1998 in the TU and TS sites. The measurements were made at 4-h intervals at the TU and TS sites and at 1-h intervals at the FW site.

Seeds were collected from each site in October 1997 and May 1998 using the protocol described. Seven, six, and six sampling points were arranged on the grid lines at 3-m intervals in the FW, TU, and TS sites, respectively (Fig. 1B). The locations of the sampling points at each site were based on the presence of *H. moscheutos*. In total, 1289 and 1340 seeds were retrieved from the three sites (Appendix). We performed allozyme analyses on subsets of seeds and consequently obtained data on 424 seeds from the autumn sampling and 546 seeds from the spring sampling (Appendix).

Allozyme analysis—Allozyme polymorphism in *H. moscheutos* has been detected in three enzymes for the SERC population (Kudoh and Whigham, 1997). Kudoh and Whigham (1997) reported consistently clear and genetically

TABLE 1. Number of seeds collected from soil at all sampling points in autumn and the following spring. The Pearson's correlation coefficient (r) across sampling points is listed for each site. Asterisks indicate that r is significantly different from zero (***, $P < 0.001$), and *ns* indicates no statistical difference of r from zero ($P > 0.05$).

Site point	Autumn	Spring	Site point	Autumn	Spring	Site point	Autumn	Spring	Site point	Autumn	Spring
Control-1	26	22	FW-1	49	41	TU-1	52	116	TS-1	117	262
Control-2	14	5	FW-2	30	33	TU-2	107	149	TS-2	97	54
Control-3	8	6	FW-3	40	47	TU-3	46	43	TS-3	42	42
Control-4	9	8	FW-4	32	46	TU-4	37	53	TS-4	157	51
Control-5	37	18	FW-5	45	44	TU-5	150	48	TS-5	105	119
Control-6	38	33	FW-6	37	44	TU-6	55	71	TS-6	32	37
Control-7	23	15	FW-7	59	40						
Control-8	26	28									
Control-9	66	65									
Autumn–Spring correlation (r)	0.94***			0.046 *ns*			0.13 *ns*			0.37 *ns*	

TABLE 2. Absolute frequencies of alleles at the sampling points for the three study sites (FW, TU, and TS). Frequencies are listed for autumn (pre-flooding) and spring (post-flooding) seasons. The results for rare alleles were excluded (see Appendix for these alleles). The absolute differences between seasons, *D*, are also listed.

			Sampling point								
Site	Allele	Season	1	2	3	4	5	6	7	8	9
Control	*EST-a*	Autumn	18	9	6	7	35	33	12	32	53
		Spring	18	4	5	5	19	26	11	37	53
		D	0	5	1	2	16	7	1	5	0
	EST-b	Autumn	34	19	10	11	39	43	34	20	79
		Spring	26	6	7	11	17	40	19	19	77
		D	8	13	3	0	22	3	15	1	2
	PGI-a	Autumn	21	11	3	5	13	11	7	19	41
		Spring	16	2	2	4	6	18	4	20	45
		D	5	9	1	1	7	7	3	1	4
	PGI-c	Autumn	27	16	11	12	46	57	33	31	79
		Spring	27	6	6	8	28	39	22	32	80
		D	0	10	5	4	18	18	11	1	1
	PGM-a	Autumn	41	22	16	12	69	72	31	35	121
		Spring	35	5	10	10	32	63	21	39	124
		D	6	17	6	2	37	9	10	4	3
	PGM-b	Autumn	11	6	0	6	5	4	15	17	11
		Spring	9	5	2	6	4	3	9	17	6
		D	2	1	2	0	1	1	6	0	5
FW	*EST-a*	Autumn	34	13	33	26	37	30	57		
		Spring	25	14	34	39	35	36	31		
		D	9	1	1	13	2	6	26		
	EST-b	Autumn	64	47	47	38	53	44	61		
		Spring	57	52	60	53	53	52	49		
		D	7	5	13	15	0	8	12		
	PGI-a	Autumn	17	8	14	8	12	11	30		
		Spring	21	6	13	22	16	7	16		
		D	4	2	1	14	4	4	14		
	PGI-c	Autumn	81	50	62	54	74	54	84		
		Spring	55	50	78	68	56	74	58		
		D	26	0	16	14	18	20	26		
	PGM-a	Autumn	88	51	69	59	76	63	98		
		Spring	62	49	84	83	73	77	55		
		D	26	2	15	24	3	14	43		
	PGM-b	Autumn	10	9	11	5	14	11	20		
		Spring	20	17	10	9	15	11	25		
		D	10	8	1	4	1	0	5		
TU	*EST-a*	Autumn	86	132	36	38	110	56			
		Spring	158	195	39	53	30	25			
		D	72	63	3	15	80	31			
	EST-b	Autumn	18	82	56	36	190	54			
		Spring	74	103	47	53	66	117			
		D	56	21	9	17	124	63			
	PGI-a	Autumn	9	49	38	33	140	55			
		Spring	41	115	36	31	44	24			
		D	32	66	2	2	96	31			
	PGI-b	Autumn	36	21	14	10	24	9			
		Spring	41	40	6	15	5	42			
		D	5	19	8	5	19	33			
	PGI-c	Autumn	59	144	40	31	135	46			
		Spring	150	143	45	60	48	76			
		D	91	1	5	29	87	30			
	PGM-a	Autumn	99	206	82	65	281	97			
		Spring	226	275	79	97	69	137			
		D	127	69	3	32	212	40			
TS	*EST-b*	Autumn	229	179	63	314	200	34			
		Spring	472	99	72	89	217	55			
		D	243	80	9	225	17	21			
	PGI-a	Autumn	96	73	25	82	38	18			
		Spring	170	52	22	45	21	14			
		D	74	21	3	37	17	4			
	PGI-b	Autumn	11	5	13	96	29	8			
		Spring	26	5	17	23	43	15			
		D	15	0	4	73	14	7			
	PGI-c	Autumn	128	116	46	137	143	38			
		Spring	328	52	45	34	174	45			
		D	200	64	1	103	31	7			

TABLE 2. Continued.

Site	Allele	Season	Sampling point 1	2	3	4	5	6	7	8	9
	PGM-a	Autumn	229	184	71	307	200	55			
		Spring	472	106	77	96	187	62			
		D	243	78	6	211	13	7			

interpretable banding patterns in esterase (EST, E.C. 3.1.1), phosphoglucoisomerase (PGI, E.C. 5.3.1.9), and phosphoglucomutase (PGM, E.C. 2.7.5.1). Allozyme analyses were conducted to determine the genotypes of seeds for these three putative loci (*EST*, *PGI*, and *PGM*). Nongerminated seeds were submerged into concentrated sulfuric acid (H_2SO_4) for 30 min (Baskin and Baskin, 1998), resulting in no adverse effects on seed viability (R. Shimamura et al., unpublished data). The seeds germinated within 2 d at 25°C on wet filter paper in Petri dishes. Seedlings were transplanted into plastic pots with vermiculite and grown in a greenhouse until leaves or cotyledons were harvested for the enzyme extractions. We used either cotyledons or leaves for each individual seed in our analyses, and we preliminarily confirmed that leaves and cotyledons from the same individuals have identical banding patterns for the three allozyme loci. Freshly collected samples were kept at 4°C prior to protein extraction and electrophoresis.

Approximately 20 mg of leaf tissue were frozen with liquid nitrogen and ground in a 1.5-mL microtube. We used 0.5 mL of modified Shiraishi's (1988) extraction buffer (Kudoh and Whigham, 1997). Approximately 20 to 50 μL of the extracts were loaded on polyacrylamide vertical slab gels after refining by centrifugation (15 000 rpm for 45 min at 4°C). The electrophoresis was carried out at 4°C, 11 $mA \cdot cm^{-2}$ for 210 min with an electrophoresis chamber (NA-1116, NIHON EIDO, Tokyo, Japan). Three enzymes (EST, PGI, and PGM) were stained following Shiraishi (1988). The banding patterns were scored for all individuals following the interpretation of allelic variation reported by Kudoh and Whigham (1997).

Data analysis—At each sampling point for each sampling season, allele frequencies were calculated for each of the three polymorphic loci (Appendix). In the following analyses, we excluded rare alleles (<10% in relative frequencies) for each site (*PGI-b*; *PGI-b*; *PGM-b*; and *Est-a* and *PGM-b* for the control, FW, TU, and TS sites, respectively). By multiplying the number of seeds collected by the ratio of each of the alleles, we estimated the number of each allele at each sampling point (absolute frequency of alleles). We used absolute frequency rather than relative frequency of alleles in the statistical tests to include the effects of the difference in number of seeds at each sampling point; analyses using relative frequency are sensitive to the results of sampling points with few seeds.

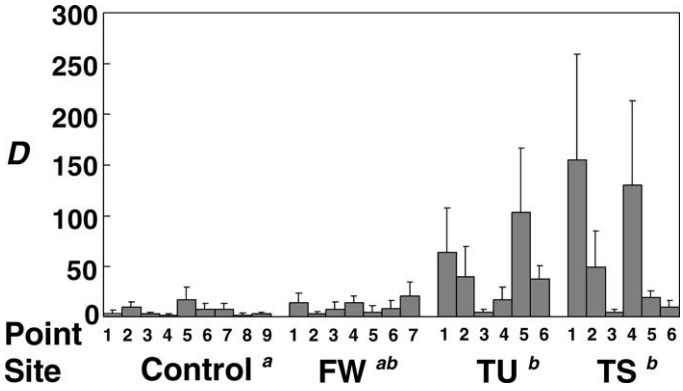

Fig. 3. Average *D* (difference in absolute allele number between autumn and spring samplings) for each sampling point of the four study sites. Bars indicate standard deviations. The letters next to the site show the results of the multiple comparisons between sites, and different letters indicate statistically significant differences in *D* between the sites ($P < 0.05$).

To quantify changes in absolute allele frequencies between seasons, for each sampling point and for each allele, we calculated difference (*D*) in absolute allele number between seasons, $D = |a - s|$, where *a* and *s* are absolute allele frequencies in autumn and spring, respectively. We conducted a nested one-way ANOVA on *D*, where site and point effects were tested. In the test, points were nested in sites, and site effect was tested against variance between points (Sokal and Rohlf, 1995). Following the significant site term in the nested ANOVA, we conducted multiple comparisons between sites by Scheffe's method (Sokal and Rohlf, 1995) to test the difference in *D* between sites with different hydrological regimes (i.e., Control, FW, TU and TS sites). The data were log-transformed in these analyses to ensure uniformity of variances. We used SuperANOVA ver.1.11 (Abacus Concept, Inc., Berkeley, CA, USA) for these statistical tests. For each site, we calculated between-season correlations (Pearson's correlation coefficients) in the number of seeds collected across sampling points. Furthermore, we calculated between-season correlations on the absolute frequency of alleles across all combinations of alleles and sampling points. The correlations measure overall similarities of allele distributions between seasons. If changes in allele distributions between seasons are absent or proportional (identical proportion across sampling points), we expect to observe correlation coefficients close to unity. Disproportional changes in allele distribution across sampling points are expected to reduce correlation coefficients. Correlation coefficients were statistically compared between sites for all combinations of site pairs with corrected probability levels using the sequential Bonferroni method (Rice, 1989).

RESULTS

Water fluctuation—The FW site was flooded from November to June, and the relative change in water level was small (Fig. 2A). The two sites in the brackish tidal wetland (TU and TS) were characterized by water level fluctuation of a greater magnitude (Fig. 2B, C). The TU site was flooded almost continuously (Fig. 2B) compared to the TS site, where water levels more typically varied from nonflooded to flooded (Fig. 2C).

Control site—Across the nine sampling points, the number of seeds collected per point had a high positive correlation between seasons ($r = 0.94$) in the control site (Table 1). Differences between pre- and postflooding seasons in absolute allele frequencies (*D*) were minimal, and overall average *D* for the control site was 6.0 (Table 2 and Fig. 3). We also found highly positive between-season correlations in the absolute frequency across all combinations of alleles and the nine sampling points ($r = 0.89$, Fig. 4A).

Comparisons among sites with different hydrologic regimes—Average *D* across alleles varied among sampling points, ranging from 1.5 to 16.8, 3.0 to 21.0, 5.0 to 103, and 4.5 to 155 for the control, FW, SU, and ST sites, respectively (Table 2 and Fig. 3). Variation among sites was also observed and overall averages of *D* were 6.0, 10.4, 44.4, and 60.9 for the control, FW, TU and TS sites (Fig. 3). In the nested ANOVA, both site and sampling-point effects were statistically significant (Table 3). Multiple comparisons detected statistical

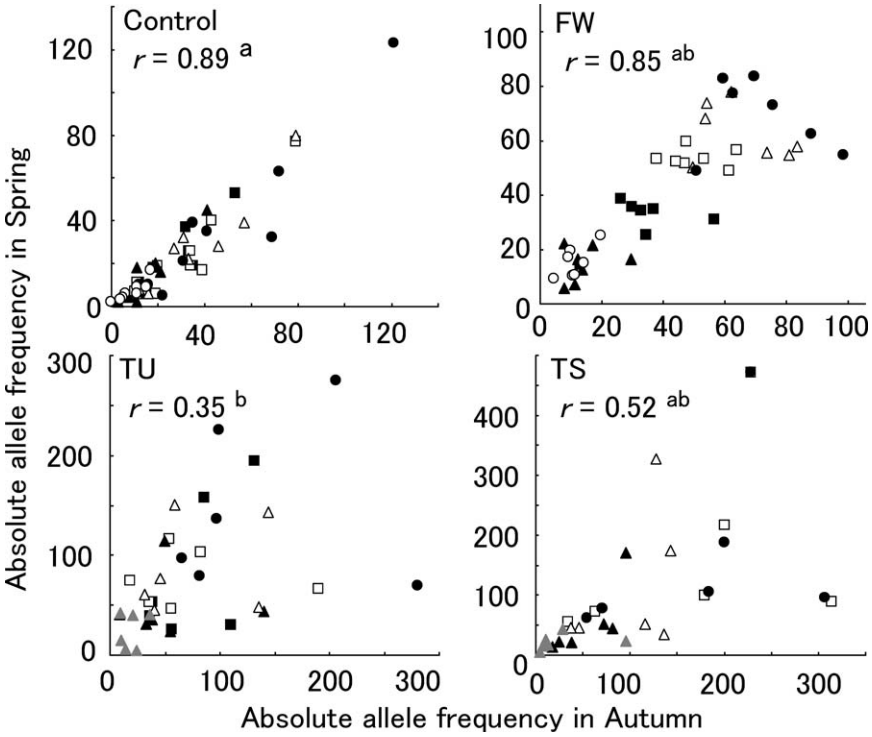

Fig. 4. Correlations between absolute frequencies of alleles detected in autumn and the following spring for the four study sites. Each data point represents a particular allele in each sampling point. Different alleles are shown by different symbols; closed and open squares, closed, gray and open triangles, and closed and open circles represent *Est-a* and *-b*, *Pgi-a*, *-b* and *-c*, and *Pgm-a* and *-b*, respectively. Correlation coefficients (r) are also listed. The letters next to r show the results of the multiple comparisons between sites, and different letters indicate statistically significant differences in r between the sites ($P < 0.05$).

differences between the control and the TU/TS sites ($P < 0.05$, Fig. 4). Between-season correlations for the number of seeds collected per sampling points were low for the FW, TU and TS sites, and none of them were significantly different from zero (Table 1). Correlations for absolute frequencies across combinations of alleles and sampling points between seasons were high in the control and FW sites and lowest in the TU site (Fig. 4). The correlation coefficient (r) was significantly lower in the TU site than in the control and FW sites ($P < 0.01$), and r was significantly lower in the TS site than in the control site ($P < 0.01$, Fig. 4).

DISCUSSION

Secondary seed dispersal has been quantified for relatively few species in wetland ecosystems, but where it has been examined, the seed banks in tidal and nontidal wetlands have been shown to be dynamic in space and time in natural (Parker and Leck, 1985; Leck and Simpson, 1995; Schneider and Sharitz, 1988; Huiskes et al., 1995; Hampe, 2004; Peterson and Baldwin, 2004a) and restored (Baldwin and DeRico, 1999; Baldwin, 2004; Leck and Leck, 2005) wetlands. The general spatial pattern for seeds in tidal wetlands appears to be the secondary dispersal of seeds toward higher elevation sites, particularly high marsh sites near the upland–wetland boundary (Leck and Graveline, 1979; Parker and Leck, 1985; Huiskes et al., 1995). Even when dispersal to higher elevation sites is not characteristic, secondary seed dispersal has been shown to be important. Griffith and Forseth (2002), for example, found that secondary seed dispersal likely occurs in the establishment of populations of a rare annual species (*Aeschynomene virginica*) in a freshwater tidal wetland, even though disturbance (vegetation removal) influenced population growth rates more than secondary seed dispersal (Griffith and Forseth, 2005). In nontidal wetlands, secondary seed dispersal has also been shown to be important in a variety of landscapes. In the semi-arid west, for example, Waser et al., (1982) found that secondary dispersal of seeds within individual drainage systems was important for the maintenance of metapopulations of *Mimulus guttatus*, a species with a high rate of local extinction. In seasonally flooded nontidal forested wetlands in the Southeast (Schneider and Sharitz, 1998) and Midwest (Middleton, 2000), seeds were widely scattered by water, especially accumulating near microtopographic features such as logs. Seeds may be dispersed as far as 600 m in floodplain

TABLE 3. Results of nested ANOVA on the absolute differences between seasons (D). Effects of site and sampling point (nested within sites) were tested. D was log-transformed in the analysis. Adjusted coefficient of determination of the model was 0.59. Asterisks indicate the term is significant; **, $P < 0.01$; ***, $P < 0.001$.

Source	df	SS	MS	F
Site	3	17.3	5.8	7.0**
Point (site)	24	19.6	0.82	5.6***
Residual	134	19.4	0.15	

forests that are seasonally flooded (Schneider and Sharitz, 1998).

The process of secondary seed dispersal and subsequent seedling establishment is clearly important for *H. moscheutos* in the Rhode River system, as demonstrated in our earlier studies with evidence for gene flow within established populations (Kudoh and Whigham, 1997, 2001). We examined genetic polymorphism in mature (i.e., flowering) plants in 10 populations of *H. moscheutos* in the same nontidal and tidal wetlands used in this study (Kudoh and Whigham, 1997). We found almost complete panmixia within the populations, and the genotypes were randomly distributed among the populations along the tidal stream. The results suggested that the observed genetic pattern most likely resulted from water dispersal of seeds. We further studied the genetic polymorphism of seeds that were floating in the water in the same study locations (Kudoh and Whigham, 2001). We found that seeds from the source populations were well mixed, but there were differences in the contributions of populations to the floating seed mixture based on the locations of the established populations. The exchange of seeds was greatest for populations close to the tidal creek, but seeds from populations that were well removed from the tidal stream also contributed to the genetic polymorphism of the floating seeds.

Our results clearly demonstrate that secondary dispersal can change the genetic composition of the seed bank, but the amount of change varies among habitats with differing hydrologic regimes. Results from the nontidal control study show that the genetic composition of the seed bank does not change significantly when there is little surface flooding of the wetland following primary seed dispersal. The results from other sites demonstrate that the amount of change in the genetic structure of the seed bank is influenced by habitat conditions, probably by hydrologic regimes during winter and spring flooding. In the FW site, which was in Mill Swamp, changes in the genetic composition of the seed bank were the smallest. The FW site is covered by standing water for a relatively longer period (Fig. 2A), but water flow is mostly in one direction. The FW site also has the highest densities of *H. moscheutos* (Kudoh and Whigham, 1997), and we often observed that floating seeds were trapped by standing and floating old stems (H. Kudoh et al., unpublished observations). The combination of one-way water flow and abundant floating obstacles presumably prevented the extensive movement of seeds in the FW site.

The greatest alterations in the genetic composition of the seed bank occurred in the tidal habitats (TU and TS sites) where flooding was more frequent. The highest average D was detected in the TS site (Fig. 3), and the lowest between-season correlation was observed in TU site (Fig. 4). These results suggested that more variation in the depth of flooding resulted in more frequent and more widespread dispersal of seeds. Even in the TU and TS site with relatively large seed movements, the results showed local variation in secondary seed dispersal within the sites. Variation patterns in D across sampling points (Fig. 3) suggested that all of sampling points were not necessarily modified equally, an indication of the localized movements of seeds even within the sites. In addition to hydrologic patterns, other factors, such as standing plant shoots, depth of litter layers, and variations in surface microtopography, may modify local patterns of secondary seed dispersal (Hart and Cox, 1995).

Overall, our results showed that changes in seed distribution by secondary dispersal modify the local genetic structure of the seed bank, at least at the scale of our sampling. Additional research, however, is still needed because the spatial mixing of seeds may reduce spatial aggregations of related genetic lineages. Relatedness among neighboring plants often determines the level of outcrossing of plant populations (Griffin and Eckert, 2003). Kudoh and Whigham (1997) reported that *H. moscheutos* populations in the study area were at Hardy–Weinberg equilibrium. This panmictic mating may be realized by the spatial shuffling effect by hydrochory (Kudoh et al., 2006). Allozyme markers used in this study are not sensitive enough to calculate genetic relatedness within and among seed samples. Further studies should evaluate to what extent hydrochory determines genetic relatedness between neighboring plants. Another point that should be addressed in future studies is whether secondary dispersal increases the probability that seeds will be lodged at safe sites, a pattern suggested for other species in nontidal wetlands (Schneider and Sharitz, 1988; Middleton, 2000). Dispersal of *H. moscheutos* seeds to safe sites is especially important because seeds are rarely found in the seed bank (Parker and Leck, 1985; Leck and Simpson, 1995) and few remain viable for more than a year (R. Shimamura and D. Whigham, unpublished observations). In tidal wetlands, dispersal of seeds to safe sites may be especially important because seeds that lodge in microsites that experience more frequent and deeper flooding appear to have a lower chance of becoming established (Baldwin et al., 2001; Peterson and Baldwin, 2004b). In other studies of *H. moscheutos*, H. Kudoh and D. Whigham (unpublished data) found that emerging seedlings of *H. moscheutos* died within a few days of being flooded, and seedling establishment appears to be limited to microsites (e.g., muskrat lodges, muskrat feeding stations, unattached blocks of peat that float up and down with the tide, litter wrack) that elevate the seedlings above the water during the critical establishment phase. It is not likely that primary dispersal (i.e., dispersal by gravity) would place seeds in very many safe sites, while tidal flooding, especially variations in water level, could move seeds to safer, higher sites.

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APPENDIX. Number of seeds, allele frequencies of three loci (*EST*, *PGI*, *PGM*) in autumn and the following spring for all the sampling points.

			Allele frequency							
			EST			*PGI*			*PGM*	
Site point	Season	Seeds analyzed	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*
Control-1	Autumn	26	0.35	0.65	0.00	0.40	0.08	0.52	0.79	0.21
	Spring	22	0.41	0.59	0.00	0.36	0.02	0.61	0.80	0.20
Control-2	Autumn	14	0.32	0.68	0.00	0.39	0.04	0.57	0.79	0.21
	Spring	5	0.40	0.60	0.00	0.20	0.20	0.60	0.50	0.50
Control-3	Autumn	8	0.38	0.63	0.00	0.19	0.13	0.69	1.00	0.00
	Spring	6	0.42	0.58	0.00	0.17	0.33	0.50	0.83	0.17
Control-4	Autumn	9	0.39	0.61	0.00	0.28	0.06	0.67	0.67	0.33
	Spring	8	0.31	0.69	0.00	0.25	0.25	0.50	0.63	0.38
Control-5	Autumn	37	0.47	0.53	0.00	0.18	0.20	0.62	0.93	0.07
	Spring	18	0.53	0.47	0.00	0.17	0.06	0.78	0.89	0.11
Control-6	Autumn	38	0.43	0.57	0.00	0.14	0.11	0.75	0.95	0.05
	Spring	33	0.39	0.61	0.00	0.27	0.14	0.59	0.95	0.05
Control-7	Autumn	23	0.26	0.74	0.00	0.15	0.13	0.72	0.67	0.33
	Spring	15	0.37	0.63	0.00	0.13	0.13	0.73	0.70	0.30
Control-8	Autumn	26	0.62	0.38	0.00	0.37	0.04	0.60	0.67	0.33
	Spring	28	0.66	0.34	0.00	0.36	0.07	0.57	0.70	0.30
Control-9	Autumn	66	0.40	0.60	0.00	0.31	0.09	0.60	0.92	0.08
	Spring	65	0.41	0.59	0.00	0.35	0.04	0.62	0.95	0.05
Control total	Autumn	247	0.41	0.59	0.00	0.27	0.10	0.63	0.85	0.15
	Spring	200	0.45	0.56	0.00	0.29	0.09	0.62	0.85	0.15
FW-1	Autumn	20	0.35	0.65	0.00	0.18	0.00	0.83	0.90	0.10
	Spring	21	0.31	0.69	0.00	0.26	0.07	0.67	0.76	0.24
FW-2	Autumn	23	0.22	0.78	0.00	0.13	0.04	0.83	0.85	0.15
	Spring	23	0.22	0.78	0.00	0.09	0.15	0.76	0.74	0.26
FW-3	Autumn	38	0.41	0.59	0.00	0.17	0.05	0.78	0.87	0.13
	Spring	41	0.37	0.63	0.00	0.13	0.04	0.83	0.89	0.11
FW-4	Autumn	28	0.41	0.59	0.00	0.13	0.04	0.84	0.93	0.07
	Spring	25	0.42	0.58	0.00	0.24	0.02	0.74	0.90	0.10
FW-5	Autumn	22	0.41	0.59	0.00	0.14	0.05	0.82	0.84	0.16
	Spring	38	0.39	0.61	0.00	0.18	0.18	0.63	0.83	0.17
FW-6	Autumn	26	0.40	0.60	0.00	0.15	0.12	0.73	0.85	0.15
	Spring	37	0.41	0.59	0.00	0.08	0.08	0.84	0.88	0.12
FW-7	Autumn	24	0.48	0.52	0.00	0.25	0.04	0.71	0.83	0.17
	Spring	27	0.39	0.61	0.00	0.20	0.07	0.72	0.69	0.31
FW total	Autumn	181	0.39	0.61	0.00	0.16	0.05	0.79	0.87	0.13
	Spring	212	0.37	0.63	0.00	0.16	0.09	0.75	0.82	0.18
TU-1	Autumn	23	0.83	0.17	0.00	0.09	0.35	0.57	0.96	0.04
	Spring	37[a]	0.68	0.32	0.00	0.18	0.18	0.65	0.97	0.03
TU-2	Autumn	26	0.62	0.38	0.00	0.23	0.10	0.67	0.96	0.04
	Spring	26	0.65	0.35	0.00	0.38	0.13	0.48	0.92	0.08
TU-3	Autumn	23	0.39	0.61	0.00	0.41	0.15	0.43	0.89	0.11
	Spring	23	0.46	0.54	0.00	0.41	0.07	0.52	0.91	0.09
TU-4	Autumn	26	0.52	0.48	0.00	0.44	0.13	0.42	0.88	0.12
	Spring	29	0.50	0.50	0.00	0.29	0.14	0.57	0.91	0.09
TU-5	Autumn	31[a]	0.37	0.63	0.00	0.47	0.08	0.45	0.94	0.06
	Spring	32	0.31	0.69	0.00	0.45	0.05	0.50	0.72	0.28
TU-6	Autumn	48[b]	0.51	0.49	0.00	0.50	0.08	0.42	0.88	0.12
	Spring	39	0.18	0.82	0.00	0.17	0.29	0.54	0.96	0.04
TU total	Autumn	117	0.53	0.47	0.00	0.38	0.14	0.48	0.91	0.09
	Spring	186	0.45	0.55	0.00	0.30	0.15	0.55	0.90	0.10
TS-1	Autumn	22	0.02	0.98	0.00	0.41	0.05	0.55	0.98	0.02
	Spring	20	0.10	0.90	0.00	0.33	0.05	0.63	0.90	0.10
TS-2	Autumn	20	0.03	0.93	0.05	0.38	0.03	0.60	0.95	0.05
	Spring	24	0.08	0.92	0.00	0.48	0.04	0.48	0.98	0.02
TS-3	Autumn	22	0.25	0.75	0.00	0.30	0.16	0.55	0.84	0.16
	Spring	25	0.14	0.86	0.00	0.26	0.20	0.54	0.92	0.08
TS-4	Autumn	23	0.00	1.00	0.00	0.26	0.30	0.43	0.98	0.02
	Spring	24	0.10	0.88	0.02	0.44	0.23	0.33	0.94	0.06
TS-5	Autumn	11	0.05	0.95	0.00	0.18	0.14	0.68	0.95	0.05
	Spring	28	0.05	0.91	0.04	0.09	0.18	0.73	0.79	0.21
TS-6	Autumn	28	0.46	0.54	0.00	0.29	0.13	0.59	0.86	0.14
	Spring	27	0.26	0.74	0.00	0.19	0.20	0.61	0.83	0.17
TS total	Autumn	126	0.11	0.88	0.01	0.29	0.15	0.55	0.94	0.06
	Spring	148	0.12	0.87	0.01	0.30	0.16	0.54	0.90	0.10

[a] *EST* genotype was not detected for one of the seeds.
[b] *PGM* genotype was not detected for one of the seeds.