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SPERM-MEDIATED GENE FLOW AND THE GENETIC STRUCTURE OF A POPULATION OF THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI*

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Abstract.—The genetic structure of populations of sessile and sedentary organisms is often characterized by microgeographic differentiation in gene frequencies and deviations from panmixia. In many terrestrial botanical systems, restricted gene flow via seed and pollen dispersal may have an important role in promoting such genetic patterns. Until recently, however, limited dispersal of the sexual propagules of benthic invertebrates has not been considered to play a comparable role in aquatic systems. Based on paternity analyses in the field using rare allozyme markers, it appears that concentrations of sibling sperm of the sessile, colonial ascidian *Botryllus schlosseri* decline rapidly within 50 cm of a source colony. In combination with spatially restricted dispersal of brooded larvae, limited dispersal of sperm should enhance the potential for genetic diversification and inbreeding. However, analysis of allelic and genotypic frequencies at three independent, polymorphic allozyme loci using *F*-statistics provides little evidence for microgeographic variation in gene frequencies. This lack of differentiation can be explained in terms of the absolute number (rather than concentration) of gametes and larvae dispersing from a point source, which—depending on diffusion and geometric assumptions—may actually increase with distance. In contrast to the absence of differentiation, levels of inbreeding are high, even within the confines of 25 × 25-cm quadrats. The absence of genetic diversification and presence of inbreeding caution against inferring levels and causes of gene flow from indirect analysis of genetic structure and, conversely, making predictions about genetic and breeding structure based solely on direct analysis of gene flow.

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Gene flow is the fulcrum upon which the processes regulating patterns of evolutionary diversification and breeding structure rest. A number of botanical studies suggest that limited dispersal of pollen, seeds, and spores restricts gene flow (Levin and Kerster, 1974; Werner, 1975; Handel, 1976; Jain, 1976; Paine, 1979; Clegg, 1980; Levin, 1981, 1984; Hamrick, 1982; Rai and Jain, 1982; Meagher and Thompson, 1987; Reed et al., 1988; but see Ellstrand and Marshall, 1985), promoting both the evolution of spatial genetic variation via drift and variable selection (Ehrlich and Raven, 1969; Endler, 1977; Wright, 1977; Levin, 1979, 1984; Turkington and Harper, 1979; Linhart et al., 1981; Slatkin, 1985; 1987; Golenberg, 1987; Waser, 1987), as well as inbreeding (Levin and Kerster, 1974; Allard, 1975; Levin, 1979, 1981, 1984; Shields, 1982; Bateson, 1983; Holsinger, 1986). In contrast, it has been generally assumed that the extensive dispersal potential of the planktonic gametes and larvae of sessile invertebrates leads to high levels of gene flow, thereby mitigating the evolution of diversification and inbreeding (Gooch, 1975; Crisp, 1978; Hedgecock, 1986). Nevertheless, the genetic structure of populations of sedentary and sessile aquatic invertebrates, like that of plants, is often characterized by microgeographic variation in gene frequencies (Ayre, 1983; Burton, 1983, 1986) and deviations from panmixia (Tracey et al., 1975; Pudovkin and Zhivotovskii, 1980; Ayre, 1983; Bucklin et al., 1984; Johnson and Black, 1984*a*; Hoffmann, 1986).

Until recently, limited dispersal of sexual propagules has not been strongly advocated as a mechanism enhancing the potential for microgeographic variation and deviations from panmixia in sessile, aquatic invertebrates (but see Gerrodette, 1981; Jackson, 1985, 1986; Grosberg, 1987; Keough and Chernoff, 1987; Stoddart, 1988; Davis and Butler, 1989; Knowlton and Jackson, 1990). Instead, these striking genetic patterns have been explained largely in terms of 1) strong, spatially varying post-settlement selection (e.g., Sabbadin and Graziani, 1967; Koehn, 1975; Koehn et al., 1976, 1984; Ayre, 1985; Hedgecock, 1986), 2) genetically based intraspecific variation in habitat preferences at the time of settlement (e.g., Doyle, 1976), 3) episodic settlement, with different cohorts of recruits experiencing different planktonic selection regimes (e.g., Johnson and Black, 1984*b*) or originating from dif-

ferent source populations (e.g., Johnson and Black, 1984*b*), 4) restricted dispersal of clonal propagules (e.g., Ayre, 1983; Hoffmann, 1986), 5) self-fertilization (Bucklin et al., 1984), and 6) intergenotypic aggression (Ayre, 1983).

Resolution of the causes of these genetic patterns requires that the contribution of gene flow be isolated from other processes regulating differentiation and breeding structure. Approaches to measuring levels of gene flow under natural conditions fall into two categories (reviewed in Handel, 1983; Slatkin, 1985, 1987). Indirect methods are based on mathematical inferences drawn from patterns of variation in the genetic structure of populations (Slatkin, 1985, 1987). One of the major theoretical advantages of indirect quantification of gene flow is that short-term and infrequent events of limited or extensive gene flow are likely to be temporally averaged into the genetic structure of the sampled population (Slatkin, 1985). However, one of the major disadvantages of indirect methods, particularly for taxa with limited dispersal, is that the combined effects of selection, drift, and gene flow are difficult to separate based simply on spatial patterns of allelic and genotypic frequencies (Rai and Jain, 1982; Hedgecock, 1986; Slatkin, 1987).

Direct measurements of gene flow involve the tracking of motile stages, dispersal vectors (e.g., pollinators, winds, and water currents), and genetic markers. Such direct measurements provide insight into the ecological processes leading to gene flow, but have several drawbacks that make it difficult to translate the data into estimates of gene flow. First, it is often difficult to know whether movement of progagules leads to gene flow. Second, because the magnitude of gene flow can vary temporally, actual levels of gene flow may be much higher or lower than measured during the short duration (in evolutionary terms) of most direct studies (Slatkin, 1985, 1987). Third, all potential components of gene flow must be considered together. For example, in plants, estimates of gene flow based on the movement of pollen compared to seeds can substantially differ (Smyth and Hamrick, 1987; Waser, 1987). Finally, there are enormous practical obstacles to following the movements of small, motile propagules over large distances in dilute media (i.e., air or water).

Given the theoretical and empirical complexities of measuring gene flow in aquatic environments, the isolated effects of dispersal of different classes of sexual propagule on genetic and breeding structure remain largely unquantified. In a previous paper (Grosberg, 1987), I measured dispersal distances of the larvae of the sessile, viviparous, colonial ascidian, *Botryllus schlosseri*. An in situ mark-recapture experiment showed that the majority of sibling larvae settle within 1 m of their birthplace. This pattern of limited larval dispersal was reflected in the results of histocompatibility assays: the probabilities of intergenotypic fusion in experimental grafts decline rapidly when geographic distances initially separating grafted colonies exceeds 50 cm (Grosberg, 1987). Laboratory studies of mating success as a function of distance between colonies in the field also suggest that populations of *B. schlosseri* may be genetically structured on fine spatial scales (Grosberg, 1987). However, I neither measured the distances over which sperm dispersed, nor did I examine the cumulative effects of limited dispersal on patterns of genetic differentiation and breeding structure. In this paper, I report the results of direct measurements of sperm dispersal in a natural population of *B. schlosseri*. I then compare the direct measurements of larval and sperm dispersal with an indirect analysis of genetic structure, using hierarchical fixation indices (Wright, 1951) based on allozyme data.

Materials and Methods

Study Site

I conducted this study in the Eel Pond at Woods Hole, Massachusetts. A detailed description of this tidal pond is given in Grosberg (1987, 1988). The Eel Pond is a small (~1 ha), shallow (maximum depth 5 m) embayment. Tidal currents are relatively slow (2–5 cm/sec, pers. obs.), and salinity range is broad (12–34 ppt, Grosberg, 1988). A narrow channel, which limits tidal exchange, connects the Eel Pond to the more oceanic waters of Vineyard Sound.

All samples were taken either directly from the wooden surfaces of the underside

of the floating dock adjacent to the supply house of the Marine Biological Laboratory (MBL supply dock), or from artificial substrata suspended beneath the dock. During June and July, such surfaces are heavily colonized by a number of epibenthic species (Grave 1933; Grosberg, 1981); colonies of *Botryllus schlosseri* are particularly abundant, often covering greater than 50%, and sometimes nearly 100%, of hard substrate beneath the MBL supply dock (Grave, 1933; Grosberg, 1981).

Reproductive Biology of Botryllus schlosseri

Colonies of *B. schlosseri* are composed of a number of morphologically identical zooids that are the asexual descendants of a sexually produced tadpole larva (reviewed in Milkman, 1967). These zooids are embedded in a cellulose-like tunic, and are interconnected by a common blood vascular system. Asexual fission of colonies is rare in *B. schlosseri* (Grosberg, 1987), although it occurs frequently in other clonal invertebrates (reviewed in Highsmith, 1982; Jackson, 1985, 1986). Approximately 5 to 10 weeks after settlement of the tadpole larvae from the plankton, colonies become sexually mature. Fully sexually mature colonies are hermaphrodites, and most zooids carry a lateral pair of testes and ovaries.

The sexual reproductive cycle is synchronized among zooids in a colony, such that all zooids ovulate or release sperm together. The sexual reproductive cycle of a colony—which is also synchronized with the asexual growth cycle—occurs on roughly a weekly period at 20°C (Sabbadin, 1955, 1958; Grosberg, 1988). On the first day of a cycle, mature zooids ovulate their ova into a brood chamber, where the ova are fertilized and develop into tadpole larvae. Meanwhile, several days after ovulation, the sperm are released into the plankton via the exhalent flow of water through the colony. Colonies are therefore cyclically protogynous, and this protogyny, perhaps in combination with gametic self-incompatibility (Oka, 1970; Scofield et al., 1982), minimizes the likelihood of self-fertilization in the field. On the last day of the sexual cycle, the mature larvae escape to the outside either through exhalent canals, or occasionally by rupturing the body wall of their parental zooid (pers. obs.).

Dispersal of Sperm

I used a mark/recapture technique, based on three rare electrophoretic markers, to estimate the distance over which the planktonic sperm from a known source colony fertilized the ova in other colonies. A previous electrophoretic survey of the Eel Pond population of *B. schlosseri* revealed the existence of five electromorphs at the phosphoglucose isomerase (*Pgi*) locus, two of which were quite rare (Grosberg, 1987). One of these electromorphs, designated *Pgi-4* (according to its relative anodal mobility from the origin), was found in 2 of 512 sampled colonies. The other rare *Pgi* electromorph, *Pgi-5*, occurred in 8 of the 512 sampled colonies. In this same electrophoretic survey, I found another rare electromorph at the malate dehydrogenase (*Mdh*) locus; this electromorph appeared in 10 of 512 colonies.

To breed homozygous lines carrying these rare markers, I mated pairs of colonies carrying identical rare electromorphs for all three markers, as described in Grosberg (1982, 1987). Clonal subsamples from the F_1 progeny of these matings were then electrophoretically assayed for the presence of *Pgi-4*, *Pgi-5*, and *Mdh-3* homozygotes using the methods of starch gel electrophoresis described in Grosberg (1987).

For each of the three classes of homozygote, a reproductively mature colony containing 600–650 zooids, each with large testes and ovaries, was selected as a source in each of three field experiments. Each source colony was allowed to attach to a separate circular glass plate, 2-mm thick and 10 cm in diameter (see Grosberg, 1982 for specific methods), that had been previously glued with thermal adhesive to a 10-cm diameter, 1-cm-thick piece of Styrofoam. This buoyant unit, with the source colony facing downward, was placed beneath the MBL supply dock into a dense stand of *B. schlosseri* colonies that extended over a flat horizontal area approximately 2.5 m by 8.0 m.

On 23 June 1984, I positioned beneath the MBL supply dock the *Pgi-4/4* source colony. I initiated identical experiments using the *Pgi-5/5* and *MDH-3/3* colonies on

7 July 1984 and 2 August 1984, respectively. Fifteen to seventeen days after each source colony was positioned beneath the dock, I set out four perpendicular transect lines that intersected at the source colony. Along each of these transects, at marked distances of 5 cm, 10 cm, 25 cm, 50 cm, and 1 m from the source colony, I removed with a metal spatula the reproductively mature *B. schlosseri* colony closest to the marked distance. I brought the 20 sampled colonies back to the lab, where I dissected out all of the sibling embryos that each colony contained. I then placed all of the embryos in each sibship into a 1.5-ml microcentrifuge tube along with 0.25 ml of 0.22 μ filtered seawater, and froze the tubes at −80°C.

In each of the first two experiments, I assayed 96 embryos from each sibship for the presence of either the *Pgi-4* or *Pgi-5* allele using horizontal cellulose acetate (CA) membrane electrophoresis. In the third experiment involving the *Mdh-3* marker, I assayed 48 embryos from each sibship. This procedure, by sampling newly fertilized embryos, should minimize the intrusion of post-fertilization selection on marker electromorph frequencies.

The CA membranes, as well as the loading bases, capillary applicators, and alignment bases were supplied by Helena Laboratories (Beaumont, Texas). The details of this technique for rapidly assaying small samples are given by Easteal and Boussy (1987). Individual embryos were removed from the micro-centrifuge tubes and transferred to the loading bases, one embryo to a well, where the embryos were macerated with a small plastic rod in 10 λ of 0.1 M Tris-HCl grinding buffer (recipe in Grosberg, 1987). Samples were then loaded onto the gels. The gels had previously been soaked in a pH 8.5 Tris-glycine buffer (14.4 g/liter glycine, 3.0 g/liter Tris). This buffer was also used as the electrode buffer. Gels were run at 150 V for 10–15 min, after which they were stained for *Pgi* or *Mdh* activity using the agar-overlay method of Easteal and Boussy (1987).

Genetic Analysis of Population Structure

I used allozyme frequencies at three loci—phosphoglucose isomerase (*Pgi*), malate dehydrogenase (*Mdh*), and leucine aminopeptidase (*Lap*)—to characterize allelic and genotypic frequencies over a variety of spatial scales in the Eel Pond population of *B. schlosseri*. I chose these loci because preliminary analyses using horizontal starch gel electrophoresis, along with breeding studies, showed that electromorphs at these loci segregated as Mendelian alleles and that the loci were unlinked (Sabbadin, 1978, 1982; pers. obs.).

I designed a set of sampling arrays on a spatial scale meant to reflect previously estimated maximum larval dispersal distances (Grosberg, 1982, 1987). On 17 June 1980, I suspended four square arrays (I–IV), each composed of four 25-cm × 25-cm asbestos-cement panels (A–D), 0.5 m below the MBL supply dock. Each of the panels was separated from the adjacent panel in the array by a 1-cm gap. The arrays themselves were positioned linearly as shown in Figure 1.

On 20 November 1980, I recovered the 16 panels from the Eel Pond and scraped all of the *B. schlosseri* colonies that had colonized each panel into a plastic bag. The panels carried between 77 and 126 *B. schlosseri* colonies. Upon returning to the lab, I blotted excess water from each of the colonies with paper towels and excised a sample of each colony containing 20–30 zooids. I then dissected out any embryos in these samples (to avoid sampling embryonic genotypes), and froze each sample separately at −80°C.

I electrophoretically analyzed 50 colonies from each of the panels, using the methods for horizontal starch gel electrophoresis described in Grosberg (1987). All three allozymes were assayed using the pH 8.0, Tris-citrate buffer system and staining recipes of Selander et al. (1971). Alleles at each locus are denoted numerically by their relative anodal mobilities.

Genotype frequencies and patterns of genetic differentiation within and among panels and arrays were analyzed at each locus using Wright's (1978) hierarchical fixation indices (*F*-statistics), calculated by the BIOSYS-1 algorithms (Swofford and Selander, 1981). Under the assumption of neutrality, F_{IT} reflects levels of inbreeding in the whole population, F_{IS} measures nonrandom mating within subpopulations, and F_{ST} reflects

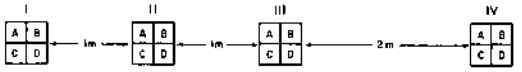

FIG. 1. The positions of experimental arrays (I–IV) and panels (A–D) within arrays used in the analysis of population genetic structure.

genetic differentiation among subpopulations (Slatkin, 1985).

There is some controversy about the best way to calculate F-statistics, particularly F_{ST}, so that finite samples yield relatively unbiased estimates of true parametric values (Weir and Cockerham, 1984; Slatkin, 1985). BIOSYS-1 calculates F_{IS} for each locus as the weighted average of $1 - H/2pqN$, where H is the observed number of heterozygotes, p and q are allelic frequencies, and N is the number of genotypes sampled (Kirby, 1975). F_{ST} is calculated at each locus as the weighted average across alleles of $\sigma_p^2/\bar{p}(1 - \bar{p})$, where σ_p^2 is the variance in allelic frequencies and $\bar{p}$ is the mean allelic frequency across subpopulations. F_{IT} is calculated from the expression

$$1 - F_{IT} = (1 - F_{ST})(1 - F_{IS}).$$

To evaluate the spatial scale at which the Eel Pond population might be subdivided or nonrandomly breeding, allelic and genotypic frequencies used in the calculation of the F-statistics were reckoned under several schemes of population subdivision. In some cases, individual panels were treated as subpopulations; in other cases, an entire array of subpanels was considered to be a subpopulation. Similarly, the total population used in the calculations varied from single arrays, to all arrays together.

RESULTS

Dispersal of Sperm

The plots in Figure 2 show that in all three sperm dispersal experiments, the frequency of embryos carrying the *Pgi-4*, *Pgi-5*, or *Mdh-3* allele in each sibship declines rapidly with distance from the homozygous source of marked sperm. Among the samples closest to the source colony, from 23–40% of the assayed embryos carried a marker that matched that of the relevant sperm source. Beyond 25 cm, the frequencies of the marker alleles in the samples declined to frequencies approaching those observed in the population as a whole.

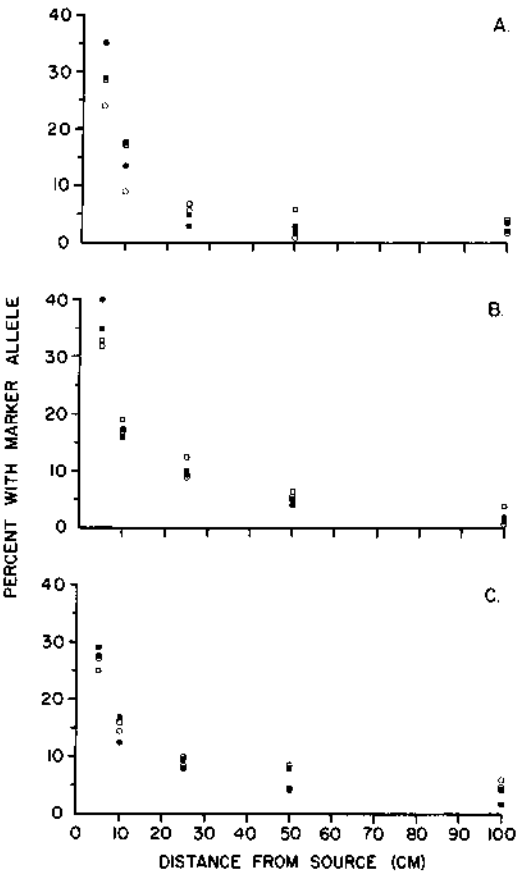

FIG. 2. The frequency of sibling embryos in a clutch carrying a given marker allele as a function of the distance from the source of labelled sperm. The data are taken from three experiments, using three different allozyme markers, conducted during different time intervals. (a) *Pgi-4*-carrying embryos; (b) *Pgi-5*-carrying embryos; (c) *Mdh-3*-carrying embryos. The different symbols represent frequencies along each of the four transects.

A one-way analysis of variance on arcsine transformed frequencies in each of the three experiments (with the four samples at each distance treated as replicates) confirms that frequencies of marker alleles in the samples depend strongly upon distance from the sperm source (*Pgi-4*: $F = 41.60$, $df = 4, 15$, $P < 0.001$; *Pgi-5*: $F = 122.36$, $df = 4, 15$, $P < 0.001$; *Mdh-3*: $F = 92.05$, df 4, 15, $P < 0.001$). The mean frequencies at each sampled distance, along with an a posteriori analysis of differences among means, are

TABLE 1. The mean frequencies and relative numbers (see text) of embryos carrying the relevant marker alleles in each of the three sperm dispersal experiments (i.e., the *Pgi-4* allele in the first experiment, the *Pgi-5* allele in the second experiment, and the *Mdh-3* allele in the third experiment) classified by distance from their respective source of marked sperm. Means with different letters differ at $P < 0.05$ (Student-Newman-Keuls test).

Distance	Frequency with marker	Grouping	Relative numbers (frequency × distance)
	Pgi-4		
5 cm	0.2725	A	1.375
10 cm	0.1350	B	1.350
25 cm	0.0550	C	1.375
50 cm	0.0325	C	1.625
100 cm	0.0325	C	3.250
	Pgi-5		
5 cm	0.3542	A	1.771
10 cm	0.1719	B	1.719
25 cm	0.1042	C	2.605
50 cm	0.0469	D	2.345
100 cm	0.0234	D	2.340
	Mdh-3		
5 cm	0.2708	A	1.354
10 cm	0.1510	B	1.510
25 cm	0.0937	C	2.342
50 cm	0.0625	D	3.125
100 cm	0.0469	D	4.690

shown in Table 1. In all three experiments, frequencies at distances ≤25 cm declined significantly; for the experiments involving *Pgi-5* and *Mdh-3* markers, frequencies continued to decline significantly through 50 cm. Although Figure 2 does not obviously show any consistent differences among transects in each experiment, there is some evidence for minor, but nevertheless statistically significant, differences among transects in the *Pgi-5* and *Mdh-3* experiments [Friedman's Method for Randomized Blocks (Siegel, 1956 pp. 166–172); *Pgi-4*: $\chi^2 = 7.38$, $df = 3$, $P > 0.05$; *Pgi-5*: $\chi^2 = 9.21$, $df = 3$, $P < 0.01$; *Mdh-3*: $\chi^2 = 23.50$, $df = 3$, $P < 0.001$].

Population Structure

Allelic frequencies for each of the 16 subpopulations in the four arrays are shown in Table 2. Only allelic and genotypic frequencies > 0.02 in all subpopulations were included in the statistical analyses. Frequencies did not vary significantly among

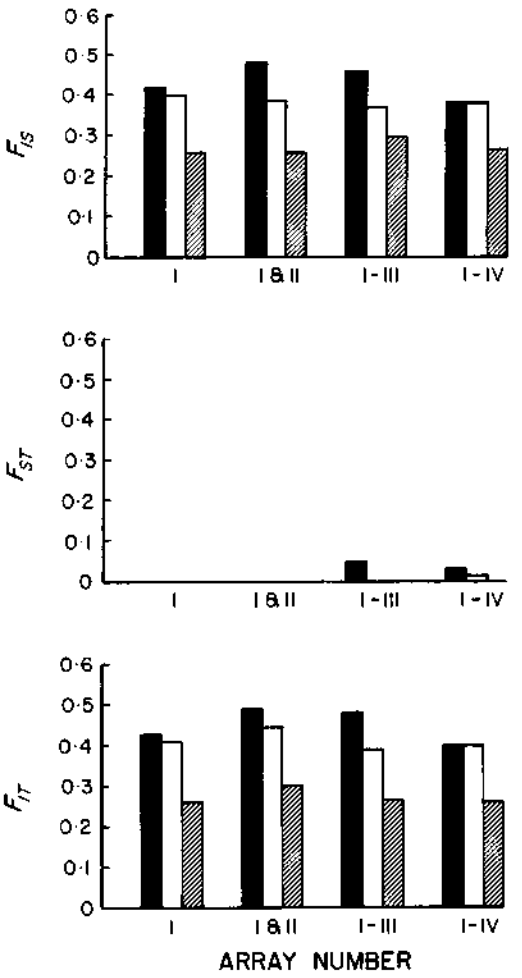

FIG. 3. Values of F_{IS}, F_{ST}, and F_{IT} for *Pgi* (solid bars), *Lap* (open bars), and *Mdh* (hatched bars), with increasingly distant arrays included in the calculations.

panels at *Mdh* ($G = 9.913$, $df = 15$, $P = 0.826$, $N = 1600$). In contrast, frequencies varied significantly at *Pgi* ($G = 57.734$, $df = 30$, $P = 0.002$, $N = 1600$) and were marginally significantly heterogeneous at *Lap* ($G = 25.757$, $df = 15$, $P = 0.041$, $N = 1600$).

Table 3 shows genotypic frequencies for the three loci on all panels. At all three loci, genotypic frequencies differed significantly from Hardy-Weinberg expectations, with consistent excesses in homozygote categories (*Pgi*: $G = 88.882$, $df = 5$, $P < 0.001$, $N = 800$; *Mdh*: $G = 45.815$, $df = 2$, $P < 0.001$, $N = 800$; *Lap*: $G = 112.235$, $df = 2$, $P < 0.001$, $N = 800$).

I calculated fixation indices based on three different schemes of the spatial scale of pop-

TABLE 2. Allelic frequencies in groups of colonists on each panel (= subpopulation) of the four arrays. Fifty colonies were assayed from each panel.

	Pgi					*Mdh*		*Lap*	
Panel	*1*	*2*	*3*	*4*	*5*	*1*	*2*	*1*	*2*
I-A	0.62	0.18	0.20	—	—	0.45	0.55	0.83	0.17
I-B	0.68	0.16	0.16	—	—	0.44	0.56	0.75	0.25
I-C	0.63	0.13	0.24	—	—	0.47	0.53	0.79	0.21
I-D	0.64	0.18	0.18	—	—	0.46	0.54	0.75	0.25
II-A	0.63	0.14	0.22	0.01	—	0.47	0.53	0.76	0.24
II-B	0.70	0.09	0.21	—	—	0.45	0.55	0.73	0.27
II-C	0.64	0.16	0.18	0.02	—	0.40	0.60	0.73	0.27
II-D	0.68	0.11	0.21	—	—	0.40	0.60	0.78	0.22
III-A	0.60	0.14	0.26	—	—	0.42	0.58	0.66	0.34
III-B	0.60	0.22	0.18	—	—	0.41	0.59	0.61	0.39
III-C	0.64	0.13	0.22	—	0.01	0.43	0.57	0.65	0.35
III-D	0.57	0.17	0.26	—	—	0.49	0.51	0.70	0.30
IV-A	0.52	0.12	0.36	—	—	0.55	0.45	0.68	0.32
IV-B	0.60	0.10	0.29	—	0.01	0.46	0.54	0.67	0.33
IV-C	0.51	0.06	0.43	—	—	0.49	0.51	0.75	0.25
IV-D	0.60	0.06	0.34	—	—	0.48	0.52	0.77	0.23

ulation subdivision. In the first scheme, I assumed that the population was subdivided at the scale of individual panels (i.e., subpopulations were individual panels); in the second, I assumed that the level of subdivision corresponded to each of the four arrays (each containing four panels). In these two cases, the total population included all 16 panels together. The results of these calculations are shown in Table 4. Under both of these schemes of population structure, values of F_{IS} and F_{IT} are large and roughly concordant across loci, as expected from the heterozygote deficiencies at *Pgi*, *Mdh*, and *Lap*. In contrast, values of F_{ST} are quite small, although the χ^2 test of Workman and Niswander (1970) indicates that values of F_{ST} at *Pgi* and *Lap* are significantly greater than 0. At all loci, the bulk of the contribution to F_{IT} can be attributed to inbreeding within subpopulations (i.e., F_{IS}) rather than differentiation among subpopulations (i.e., F_{ST}).

The third scheme explored the effects of spatial position and proximity of arrays of panels on the fixation indices. In the cal-

TABLE 3. Genotypic frequencies in groups of colonists on each panel (= subpopulation) of the four arrays. Fifty colonies were assayed from each panel. Genotypes with the rare alleles *Pgi-4* and *Pgi-5* are omitted.

	Pgi						*Mdh*			*Lap*		
Panel	*1/1*	*1/2*	*1/3*	*2/2*	*2/3*	*3/3*	*1/1*	*1/2*	*2/2*	*1/1*	*1/2*	*2/2*
I-A	0.46	0.18	0.14	0.06	0.06	0.10	0.26	0.38	0.36	0.74	0.18	0.08
I-B	0.56	0.12	0.12	0.08	0.04	0.08	0.28	0.32	0.40	0.66	0.18	0.16
I-C	0.56	0.10	0.12	0.06	0.04	0.12	0.28	0.38	0.34	0.68	0.22	0.10
I-D	0.52	0.16	0.08	0.08	0.04	0.12	0.28	0.36	0.36	0.64	0.22	0.14
II-A	0.52	0.12	0.10	0.06	0.06	0.14	0.30	0.36	0.34	0.66	0.20	0.14
II-B	0.54	0.14	0.18	0.01	0	0.12	0.28	0.34	0.38	0.60	0.26	0.14
II-C	0.52	0.14	0.10	0.10	0.10	0.12	0.22	0.36	0.42	0.58	0.30	0.12
II-D	0.54	0.10	0.18	0.04	0.04	0.10	0.18	0.44	0.38	0.70	0.18	0.12
III-A	0.48	0.10	0.14	0.06	0.06	0.16	0.24	0.34	0.42	0.52	0.28	0.20
III-B	0.48	0.12	0.12	0.12	0.02	0.12	0.18	0.44	0.38	0.42	0.38	0.20
III-C	0.50	0.12	0.16	0.04	0.06	0.12	0.24	0.38	0.38	0.48	0.34	0.18
III-D	0.40	0.16	0.18	0.06	0.06	0.14	0.30	0.38	0.32	0.62	0.18	0.20
IV-A	0.36	0.10	0.22	0.02	0.12	0.18	0.34	0.42	0.24	0.58	0.20	0.22
IV-B	0.48	0.04	0.22	0.04	0.06	0.18	0.28	0.36	0.36	0.58	0.30	0.12
IV-C	0.34	0.06	0.28	0.02	0.02	0.28	0.30	0.38	0.32	0.62	0.26	0.12
IV-D	0.44	0.06	0.26	0	0.06	0.18	0.28	0.40	0.32	0.68	0.18	0.14

culation of these F-statistics, individual panels were once again treated as subpopulations, with the aggregate of subpopulations 1–4 (i.e., array I), 1–8 (i.e., arrays I and II), etc., being sequentially included in the calculations. As Figure 3 shows, both F_{IS} and F_{IT} are consistently large at all loci (and even within a single array); neither F_{IS} nor F_{IT} increases as progressively more distant arrays are sequentially included in the calculation of the indices. F_{ST} for *Pgi* increases when subpopulations from array III are added to the sample. However, there appears to be no linear trend for values of F_{ST} to increase as the total distance among samples increases.

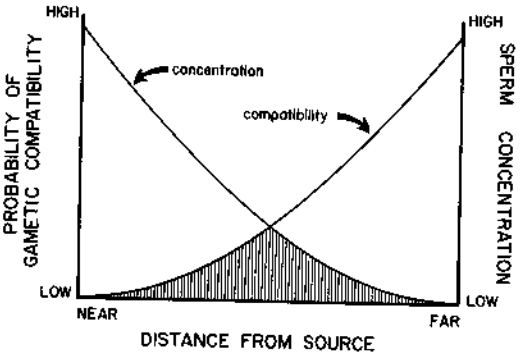

FIG. 4. The potential combined effects of sperm concentration, gametic compatibility, and distance from a source colony on male fertilization success (shaded area). The interaction between concentration and compatibility may yield a maximum of male fertilization success some intermediate distance from the source of sperm.

DISCUSSION

The effects of sperm or pollen movement on gene flow and breeding structure in sessile organisms depend on the probabilities that male gametes will move from their point of release and encounter receptive ova. If substantial turbulent mixing and advection primarily regulate levels of pollen and sperm transport, then recipient ova should receive a random sampling of genetically well-mixed male gametes (Clegg, 1980). If, on the other hand, diffusive processes predominate, then a concentration gradient of sibling sperm may form at steady state (Richardson, 1970; Crank, 1975; Denny, 1988; Okubo and Levin, 1989). Consequently (in the absence of gametic incompatibility systems and other complications), ova located close to a sperm or pollen source should have a disproportionately higher probability of being fertilized by male gametes from that source than would be expected under the assumptions of local panmixia. Indeed, despite the impacts of population structure (reviewed in Antonovics and Levin, 1980; Handel, 1983, 1985), pollinator behavior (e.g., Schaal, 1980), and environmental variables such as fluid velocity on gene flow (e.g., Bos et al., 1986; Pennington, 1985; Augspurger and Franson, 1987), numerous botanical studies demonstrate that sibling pollen grains are not spread uniformly across populations, but rather have a spatially limited sphere over which they are likely to fertilize ova (Bateman, 1947; Handel 1976; Schaal, 1980; Hamrick, 1982; Rai and Jain, 1982; Waser and Price, 1983; Tonsor, 1985; Bos et al., 1986; Meagher and Thompson, 1987; Okubo and Levin, 1989).

The patterns of fertilization frequencies reported in this paper, based on a paternity analysis of unborn, brooded embryos, show that in a dense, natural population of the colonial ascidian *Botryllus schlosseri*, the concentration of sibling sperm rapidly declines with increasing distance from a source of sperm. Within 50 cm of a source, the

TABLE 4. F-statistics for *Pgi*, *Lap*, and *Mdh* calculated under two different assumptions of underlying population structure. The first set of calculations assumes that panels within arrays are subpopulations; the second set assumes that arrays are subpopulations.

Locus	Panels as subpopulations			Arrays as subpopulations		
	F_{IS}	F_{ST}	F_{IT}	F_{IS}	F_{ST}	F_{IT}
Pgi	0.375	0.027*	0.398	0.392	0.012*	0.398
Lap	0.383	0.016*	0.393	0.387	0.011*	0.393
Mdh	0.258	0.006	0.262	0.236	0.003	0.238

* Denotes values of F_{ST} that exceed 0 at $P \geq 0.99$ [χ^2 test of Workman and Niswander (1970)].

form of this gradient approximates the simple inverse function expected from diffusive processes at steady state, without depletion (Crank, 1975). At distances greater than 50 cm, observed concentrations exceed those expected from a simple diffusion model. In some cases (e.g., *Pgi-5* and *Mdh-3*), subtraction of observed background allelic frequencies from fertilization frequencies reduces the descrepancy between theoretical expectations and observations at distances beyond 50 cm. However, because I measured background frequencies prior to when the sperm dispersal experiments were conducted, estimates of background frequencies (and corrections based on these estimates) should be viewed qualitatively.

The existence of a concentration gradient need not imply that the absolute number of progeny sired by a given individual (hence, levels of gene flow) declines with distance. In fact, exactly the opposite may be true. For example, assuming that fertilizable ova are spread uniformly (or randomly) on a planar surface, their relative numbers along concentric circles centered around a source of male gametes will scale in proportion to the perimeters of these circles. Thus, although the sampling design used here should estimate relative **frequencies** (hence concentrations) of sibling sperm according to distance from a source, estimation of the relative **numbers** of fertilized ova requires multiplication of the observed fertilization frequencies at each distance by the radius of the circle.

The last column of Table 1 shows the results of this normalization procedure based on the mean frequencies at each distance in each of the three experiments. The corrected results are like those predicted by the diffusion model: at distances less than or equal to 50 cm from the sperm source, these normalized values remain fairly constant under the assumptions of the geometric model. Beyond 50 cm, the normalized values suggest that numbers of ova fertilized by sperm from the source colonies may actually exceed values at closer distances. Once again, however, this inference should be viewed cautiously because of the inherent difficulties of measuring (rather than estimating by extrapolation from some theoretically or empirically based function—e.g., Werner, 1975) the shape of the tail of a concentration gradient using any but the rarest genetic markers. This is unfortunate because relatively few long-distance fertilizations can lead to extensive gene flow (Slatkin, 1985, 1987).

Taken together, the direct measurements of sperm-mediated gene flow in *Botryllus schlosseri* indicate that even when a concentration gradient exists of the sort described in this paper (and in many botanical studies) in which the **frequency** of embryos sired by a given sperm genotype declines with distance, the total **number** of sired embryos need not decrease (also see Okubo and Levin, 1989). Indeed, despite the coupling of a rapid decline in sperm concentration with both a comparable gradient of sibling larval dispersal (Grosberg, 1987) and preferential larval settlement near kin (Grosberg and Quinn, 1986), the homogeneity of allelic frequencies and consistently small values of F_{ST} imply that levels of gene flow were too high to permit microgeographic differentiation over the five months (encompassing approximately five generations [Grosberg, 1988]) that the sampled panels were in the Eel Pond.

Over larger spatial and longer temporal scales, this may not be the case. For example, in my own unpublished studies, allelic frequencies differ significantly at *Pgi* and *Mdh* among three Cape Cod tidal ponds that are separated by distances greater than several kilometers. Similarly, in a Venetian Lagoon population of *B. schlosseri*, there is evidence for microgeographic differentiation over scales of hundreds of meters for genetically specified color patterns (Sabbadin and Graziani, 1967) and allozyme frequencies (Sabbadin, 1978). These patterns are consistent with expectations based on limited gene flow, but over a larger spatial (and perhaps temporal) scale than that investigated here.

In this study, the primary genetic effect of the decline of sibling larval and sperm concentrations with distance from a source appears to be on the breeding system. The large values of F_{IS} and F_{IT}, along with heterozygote deficiencies at the three polymorphic loci, together indicate that within the 25-cm × 25-cm bounds of a subpopulation on the panels, levels of inbreeding are

high. This breeding system in the Eel Pond population of *B. schlosseri* contrasts with Sabbadin's (1978) report that genotypic frequencies at *Mdh* and *To* (tetrazolium oxidase) generally agreed with Hardy-Weinberg expectations in the Venetian Lagoon population of *B. schlosseri.* This difference may arise from different levels of turbulent mixing and advection in the two populations: the Venetian Lagoon population resides in tidal channels with high current speeds, whereas the Eel Pond population lies in very protected waters. If differences in levels of mixing (hence gene flow) underlie these differences in breeding system, then one would expect newly established populations in well-mixed waters to remain panmictic over many generations. In poorly mixed waters, however, after initial colonization by immigrants from elsewhere in the population, levels of inbreeding should increase through time as siblings co-settle, and the frequency of consanguineous matings increase. The temporal dynamics of breeding structure have yet to be examined in the Eel Pond, or elsewhere.

At first sight, gametic self-incompatibility—which evidently occurs in another population of *B. schlosseri* (Scofield et al., 1982) and a Japanese congener, *B. primigenus* (Oka, 1970)—could offset some of the inbreeding consequences of limited dispersal. Although the formal genetics of gametic incompatibility are not well-studied in *B. schlosseri,* gametic incompatibility in *B. primigenus* is controlled by a single, highly polymorphic, Mendelian locus whose formal genetics are similar to the S-locus, gametophytic incompatibility systems found in some angiosperms (Oka, 1970; de Nettancourt, 1977). If a sperm shares a compatibility allele with the diploid, maternally derived egg envelope, then the sperm is apparently incapable of fertilizing the egg within. Such an incompatibility system would eliminate self-fertilization, but could only reduce the frequency of successful full-sib matings by 50% (Grosberg, 1987). Unless the locus controlling gametic incompatibility were in linkage disequilibrium with all of the allozyme loci examined in this study (an impossibility given that the three loci segregate independently), the effects of a self-incompatibility system on genotypic frequencies at independent loci should ultimately be small.

Gametic incompatibility, however, could profoundly influence seasonal and spatial patterns of male fertilization success and sperm-mediated gene flow in *Botryllus schlosseri.* For instance, as the assemblage of *B. schlosseri* colonies seasonally develops, and levels of inbreeding intensify, matings between proximal colonies will be more likely to involve gametes that share incompatibility alleles (Fig. 4). Gametic incompatibility may therefore decrease local fertilization success, and oppose the effects of locally high sperm concentrations. Consequently, late-season levels of sperm-mediated gene flow would be greater than expected from the rapid decline in sperm concentration, with maximum male fertilization success at some intermediate distance from a source colony (Fig. 4). Such a local shadow of fertilization success is reminiscent of the seed shadows in *Stercula apetala* produced by granivorous insects (Janzen, 1971).

Whatever the effects of gametic incompatibility systems on breeding structure, the data reported in this paper imply that levels of gene flow in the Eel Pond population of *Botryllus schlosseri* are too high to promote fine-scale, short-term genetic diversification, but not so high as to preclude inbreeding at comparable spatial scales. These genetic patterns are consistent with the analyses of Pollak (1987, 1988), whose stochastic models predict that when rates of inbreeding (due to selfing or sib-matings) substantially exceed the reciprocal of population size, inbreeding will cause a much more rapid decline in heterozygosity than will genetic drift. In the case of *Botryllus schlosseri,* the calculations of Knowlton and Jackson (1990), based on the larval dispersal data in Grosberg (1987), indicate that Pollak's inbreeding criterion will be met when the frequency of parent-offspring (or full-sub) matings exceeds 0.005. It seems quite likely that in the Eel Pond population of *B. schlosseri,* close inbreeding occurs with a considerably higher frequency than 1/200 matings.

Undeniably, patterns of 1) pollen dispersal in wind-pollinated plants (Levin and Kerster, 1974; Waser and Price, 1983; Ton-

sor, 1985; Bos et al., 1986; but see Ellstrand and Marshall, 1985), 2) fertilization success in sea urchins (Pennington, 1985; D. R. Levitan, pers. comm.) and hydroids (Yund, 1990), and 3) behavior and development of sexually produced larvae of many clonal and some aclonal invertebrates (reviewed in Burton, 1983; Jackson, 1985, 1986; Grosberg, 1987) imply that restricted gene flow plays an important role in the evolution of microgeographic genetic differentiation in sessile and sedentary organisms. The data in this paper, however, caution against making predictions about genetic differentiation based on such dispersal patterns alone (also see Tonsor, 1985). In some cases, the primary microgeographic effects of limited dispersal may be on the structure of breeding systems and the evolution of social behavior (Breden and Wade, 1981; Wade and Breden, 1981, 1987; Michod, 1982; Uyenoyama, 1984; Pollak, 1987, 1988).

As it stands, this study represents one of the first that simultaneously compares direct and indirect measurements of gene flow and genetic structure in a sessile, aquatic invertebrate. Until comparable data are in hand for other taxa, it will be difficult to assess how the movement of dispersing propagules regulates the evolution of genetic diversification, breeding systems, and social interactions in sessile invertebrates.

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