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# LIMITED DISPERSAL AND PROXIMITY-DEPENDENT MATING SUCCESS IN THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI*

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*Abstract.*—Although the propagules of many sessile organisms have the capacity to disperse over large distances, dispersal is often spatially restricted. In this paper, I document, using a combination of mark/recapture techniques and histocompatibility assays, dispersal distance of the planktonic larvae of the sessile, colonial sea squirt *Botryllus schlosseri*. Both of these methods indicate that most larvae remain within a meter of their birthplace. Such limited dispersal should lead to increased matings among relatives, and the potential for inbreeding depression. However, the success of: 1) fertilization, 2) embryogenesis, and 3) larval metamorphosis all decrease as distance between mated colonies increases. The spatial scale over which this decrease in mating success occurs is concordant with the estimates of dispersal distance based on the larval mark/recapture data and histocompatibility assays. Taken together, these results imply that inbreeding depression is not a necessary consequence of limited dispersal and consanguineous matings in *B. schlosseri*.

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Dispersal is a critical, and often fatal, aspect of the life cycle of sessile or sedentary organisms. Ecologically, dispersal provides an opportunity to escape deteriorating local conditions, or to exploit favorable sites elsewhere (Southwood, 1977; Hamilton and May, 1977; Buss, 1979; Palmer and Strathmann, 1981; Olson, 1985; Strathmann, 1985). Evolutionarily, dispersal distance controls the potential for microgeographic differentiation due to selection or drift (Ehrlich and Raven, 1969; Wright, 1977; Levin, 1979, 1984; Slatkin, 1985), as well as the breeding and social structure of populations (Hamilton, 1964; Levin and Kerster, 1974; Levin, 1979, 1981, 1984; Shields, 1982; Bateson, 1983; Holsinger, 1986).

The propagules of many sessile organisms have the capacity to disperse extensively either actively or passively. However, in a number of plants (Paine, 1979; Hamrick, 1982; Waser and Price, 1983) and sessile, clonal marine invertebrates (reviewed in Knight-Jones, 1961; Ryland, 1981; Jackson, 1985, 1986), dispersal appears to be quite restricted. In a mating context, such philopatric dispersal leads to proximity of relatives, and the potential for inbreeding. The population genetic consequences of persistent inbreeding are well understood; inbreeding increases homozygosity (Jacquard, 1975; Wright, 1977) and stabilizes intragenomic allelic associations (Shields, 1982; Partridge, 1983). The fitness consequences of inbreeding are more ambiguous. Inbreeding depression has been documented in plant (Grant, 1975; Wright, 1977; Schoen, 1983) and animal (Bulmer, 1973; Hill, 1974; Wright, 1977; Shields, 1982; Levin, 1984) populations that normally outbreed. However, in plant populations in which dispersal of pollen and seed is limited (and inbreeding is presumably common), analysis of the costs and benefits of consanguineous matings reveals little consensus (Mitchell-Olds and Waller, 1985). Some studies show that mating success and progeny viability increase with distance between mates, presumably reflecting advantages to outcrossing (e.g., Coles and Fowler, 1976; Levin, 1984; Mitchell-Olds and Waller, 1985). Other data indicate that parental proximity has no clear effect on seed-set or viability (Grant, 1954; Williams, 1960; Wilkens, 1982). Alternatively, in populations of *Delphinium nelsonii* and *Ipomopsis aggregata*, seed-set and viability of progeny are highest in matings between parents separated by distances similar to the dispersal distances of pollen and seeds (Price and Waser, 1979; Waser and Price, 1983).

In contrast to the growing body of data that documents dispersal, gene flow, and the fitness consequences of inbreeding in populations of plants with limited dispersal, there are few comparable data for naturally inbred populations of sessile or sedentary animals (Strathmann et al., 1984). In this paper, I characterize the dispersal pattern of planktonic larvae of the sessile, colonial

ascidian *Botryllus schlosseri*. The combined results of larval mark/recapture experiments and histocompatibility assays suggest that most larvae disperse less than a few meters from their birthplace. Limited dispersal of sibling larvae should lead to high frequencies of matings among kin. However, mating success (measured by relative fertility, normal embryogenesis, and metamorphosis) decreases according to distance between parental colonies. In turn, the region of maximal crossing success corresponds to the range of dispersal of the majority of sibling larvae.

## Materials and Methods

### *Biology of* Botryllus schlosseri

*B. schlosseri* is a seasonally abundant colonial sea squirt that colonizes hard substrata in the low intertidal, and the submerged surfaces of floating docks, throughout the temperate zones of the northern hemisphere (van Name, 1945; Berrill, 1950; Grosberg, 1982). Colonies are founded by a sexually produced tadpole larva, which, upon locating a suitable substratum, attaches and metamorphoses into a primary zooid (Milkman, 1967). Like the larvae of many colonial ascidians (e.g., Kott, 1974; van Duyl et al., 1981; Olson, 1985), *B. schlosseri* larvae can metamorphose upon their release into the plankton (Grave and Woodbridge, 1924; Grosberg and Quinn, 1986).

A colony of *B. schlosseri* is composed of morphologically and genetically identical units termed zooids, which are imbedded in a gelatinous tunic. Each zooid can feed and sexually reproduce independently of the others; however, zooids usually remain physiologically connected by a ramifying and anastomosing blood vascular system (Sabbadin, 1971, 1978). The blood vascular system terminates around the periphery of the colony in crowns of finger-like projections that are termed ampullae. Colonial growth involves the development of asexual buds produced by the adult zooids. The development of buds is cyclic and synchronized among all zooids composing a colony. Thus, all buds are of the same developmental stage at any given time. An asexual cycle occurs every five to seven days (according to temperature); the feeding adults in a colony are resorbed, and a developing generation of buds synchronously replaces the adults in a process called takeover.

Once a colony reaches sexual maturity, the sexual cycle is locked in phase to the asexual cycle. Just after asexual takeover, the ova contained within the newly formed adult zooids are synchronously (i.e., within a few hours) ovulated (Milkman, 1967; Sabbadin, 1971). Fertilization follows shortly after ovulation, and the embryos are brooded until they complete development into tadpole larvae. The release of a clutch of larvae coincides with the regression of the brooding adult zooids during takeover. As ovulation and development are synchronized among zooids within a colony, and brooded embryos may be counted through a dissecting microscope, the determination of larval production is straightforward. Finally, it is important to note that although colonies are hermaphroditic the testes within zooids do not mature until several days after ovulation; therefore, self-fertilization rarely occurs in nature (Milkman, 1967; Sabbadin, 1971).

### *Study Site*

All of the field studies were performed on a floating dock (the Marine Biological Laboratory [MBL] Supply Dock) in the Eel Pond at Woods Hole, Massachusetts. The Eel Pond is a small (~0.05 hectares), shallow (maximum depth at high water is ~5 m) tidal pond on Cape Cod. Although the complete hydrodynamics of the Eel Pond are unknown, tidally generated currents probably do not exceed 2–3 cm/sec beneath the MBL Supply Dock (Keen, pers. comm.).

From June through October, the sides and undersurfaces of the MBL Supply Dock are heavily colonized by a number of solitary and colonial marine invertebrates (Grave, 1933; Grosberg, 1981). During June and July, the sessile epibenthos is dominated by colonies of *B. schlosseri* which may cover nearly all hard surfaces on the MBL Supply Dock (Grave, 1933; Grosberg, 1981, 1982).

### *Estimates of Dispersal Distance*

I used two methods to estimate the dispersal distance of sibling larvae of *B. schlosseri*. One method involved a larval mark/recapture technique in which larvae carried

a rare electrophoretic marker. With this technique, I mapped the recruitment sites of colonies founded by sibling larvae that were released from a point source. I also used a histocompatibility assay to assess the dispersion of related colonies on the MBL Supply Dock.

*Larval Mark/Recapture Study.*—To find a genetic marker that would allow precise identification of larvae derived from a source colony, I conducted an electrophoretic survey of 512 randomly selected colonies living along a 25 m transect on the side of the MBL Supply Dock. The general procedures I used for horizontal starch gel electrophoresis are contained in Selander et al. (1971). Modifications for studies of *Botryllus* involved sample preparation. *B. schlosseri* broods its embryos, hence, they must be removed prior to electrophoretic analysis. Four or five living or frozen (at −80°C) zooids from each colony were homogenized in 0.5 ml of buffer consisting of 0.1 M Tris-HCl, 0.001 M EDTA, and 0.0005 M NADP adjusted to pH 7.0. The resulting slurry was centrifuged in capillary tubes, and the supernatant was used to wet 4 mm filter paper wicks.

Gels run according to the pH 8.0, Tris-citrate system of Selander et al. (1971), and stained to detect phosphoglucose isomerase (PGI) activity, revealed five distinct electromorphs. One of these, designated PGI-fast for its extreme cathodal position, was identified in only two of the 512 colonies. No PGI-fast homozygotes were found. These two colonies were then mated (see Grosberg [1982] for detailed methods), and clonal subsamples of the progeny were surveyed for PGI-fast homozygotes. One such homozygous $F_1$ colony was used as a source colony for larvae carrying the rare PGI marker. This colony was attached to a circular glass plate, 10 cm in diameter. In turn, this plate was fixed with a stainless steel screw to the center of a 1 m diameter circular asbestos-cement panel. The panel, with the source colony attached to the lower surface, was suspended horizontally 0.5 m below the MBL Supply Dock.

To ensure that all progeny derived from the source colony were homozygous for the PGI-fast marker, just prior to the synchronized ovulation of eggs in the source colony, the plate carrying it was detached from the panel, and brought into the lab. There, the plate and colony were washed three times in 0.22 $\mu$ filtered seawater (fsw), and placed in a 10 liter aquarium, filled with fsw. A sibling PGI-fast homozygous colony with ripe testes was then placed in the aquarium. After 24 hours, several embryos were removed from the source colony to ensure that cleavage had begun. The remaining embryos were censused, and the source colony was then returned to the MBL Supply Dock and reattached to the settlement plate. I repeatedly crossed the source colony and its mate over four sexual cycles. All told, these four clutches produced 5,856 larvae that commenced development.

Two days after the fourth clutch of larvae was released, I removed the 1 m settlement panel from the Eel Pond, and mapped on an acetate overlay the position of every *B. schlosseri* recruit. I assigned each colony a number, then removed the colony and froze it at −80°C. I then electrophoretically assayed each colony to determine if it was homozygous for the PGI-fast allele. I considered homozygous colonies to be siblings derived from the source colony. In the 23 days that the panel was in place, no recruits reached sexual maturity; thus, any recruited colonies that were not PGI-fast homozygotes must have immigrated.

As colonies may grow asymmetrically from their point of settlement, there is no a posteriori method to determine actual settlement position. Therefore, I assigned by eye a position that corresponded roughly to the settlement site of each colony. My criterion for assigning this position was that the position be as near as possible to the center of the largest area of symmetrical growth. Whatever bias the technique may have introduced was uniform. I corroborated my technique by determining the actual centroids of colonies with a digitizing tablet. My estimates of settlement position did not deviate significantly from those suggested by the analysis of centroids (paired comparisons $t$-test, $N = 50$, $P > 0.2$). Settlement positions of marked recruits were recorded as x, y coordinates, which were converted to polar coordinates for distance analyses.

*Histocompatibility Assays.*—In *Botryllus schlosseri,* self/nonself recognition, or his-

tocompatibility, assays provide a useful technique to quantify the dispersion of related individuals for several reasons. First, the formal genetics of histocompatibility are described (Oka and Watanabe, 1957; Sabbadin, 1962; Scofield et al., 1982). A single Mendelian locus governs fusion between allogeneic colonies such that contiguous colonies sharing one, or both, alleles at this locus will fuse by their blood vascular systems. If colonies do not share an allele, rejection (accompanied by tissue necrosis) follows contact. Second, in all populations studied, the locus is highly polymorphic (Karakashian and Milkman, 1967; Mukai and Watanabe, 1975; Scofield et al., 1982), with upwards of 100 alleles estimated in the Eel Pond (Grosberg and Quinn, 1986). Given this level of polymorphism, the likelihood that any two allogeneic colonies will share a histocompatibility allele should be determined by their pedigree relatedness. Finally, fusions between allogeneic individuals are unlikely to be confounded by fusions between clonemates because fragmentation of colonies producing physiologically discrete clones is rare (Grosberg et al., 1985; Stoddart et al., 1985). (Over a three-year period, of nearly 5,000 colonies followed from settlement to death, only 10 asexually fragmented [Grosberg, 1982].) These considerations indicate that histocompatibility assays provide a reliable means to assess the dispersion of relatives in the Eel Pond population of *B. schlosseri*. If relatives are spatially associated, then fusion frequencies should decline as distance between juxtaposed colonies increases.

To determine the relationship between fusion frequency and the distance between colonies, I established a 3 m line transect along the side of the MBL Supply Dock, and removed all *B. schlosseri* within 10 cm of both sides of the transect. I noted the position of each colony along the transect, assigned it a reference number, and then placed each colony in a perforated 250 ml plastic container set in a seawater table. I set up 100 fusion assays in each of seven distance categories (i.e., 0–5 cm, 6–10 cm, 11–20 cm, 21–50 cm, 51–100 cm, 101–200 cm, and 201–300 cm) by attaching pairs of colonies, with their growing edges separated by ~1 mm, to glass plates (methods in Grosberg, 1982). After the colonies were fixed to the plates, they were placed in seawater tables. One to three days later, I scored the pairs of colonies according to whether their blood vascular systems had fused at the site of juxtaposition.

I used two sampling regimes to provide a standard estimate of fusion frequency unbiased by distance. In one estimate, I chose randomly from the transect 200 colonies, and initiated 100 assays as above. Additionally, I gathered ~1,000 colonies from docks throughout Eel Pond, and paired these colonies randomly with respect to location.

### *Progeny Viability and Interparental Distance*

To examine the relationship between proximity of mated colonies and the success of 1) fertilization, 2) embryonic development through hatching into a swimming larva, and 3) metamorphosis into the sessile feeding stage, I mated two sets of colonies from different sites to other colonies from varying distances. On the side of the MBL Supply Dock, 10 cm below the water line, I set out a 4.5 m transect running landward to seaward. At 0.5 m intervals, I centered a 15 cm × 15 cm quadrat, and removed all *B. schlosseri* within the bounds of each of the 10 quadrats. From the landward-most and seaward-most quadrats, I chose the five largest colonies carrying ova. I attached these colonies to glass plates, and maintained the colonies in running seawater. These colonies, designated F(1–5) and F(6–10), provided the eggs used in the experiment. I refer to these colonies as egg-parents.

From each of the 10 quadrats, I chose one colony with ripe testes, labeled M(1–10), to mate to the egg-parents. Each of the 10 egg-parents was mated to each of the 10 sperm-parents. Although every egg-parent was mated to the same 10 sperm-parents, the distance between a given pair of mates depended on whether the egg-parent was from the landward or seaward end of the transect; for example, M(1) was adjacent to F(1–6), but 4.5 m from F(6–10).

As asexual takeover began in the egg-parents, I carefully washed each colony in filtered seawater, then transferred it to a large glass fingerbowl containing 1 liter of filtered seawater. Shortly after ovulation, when all

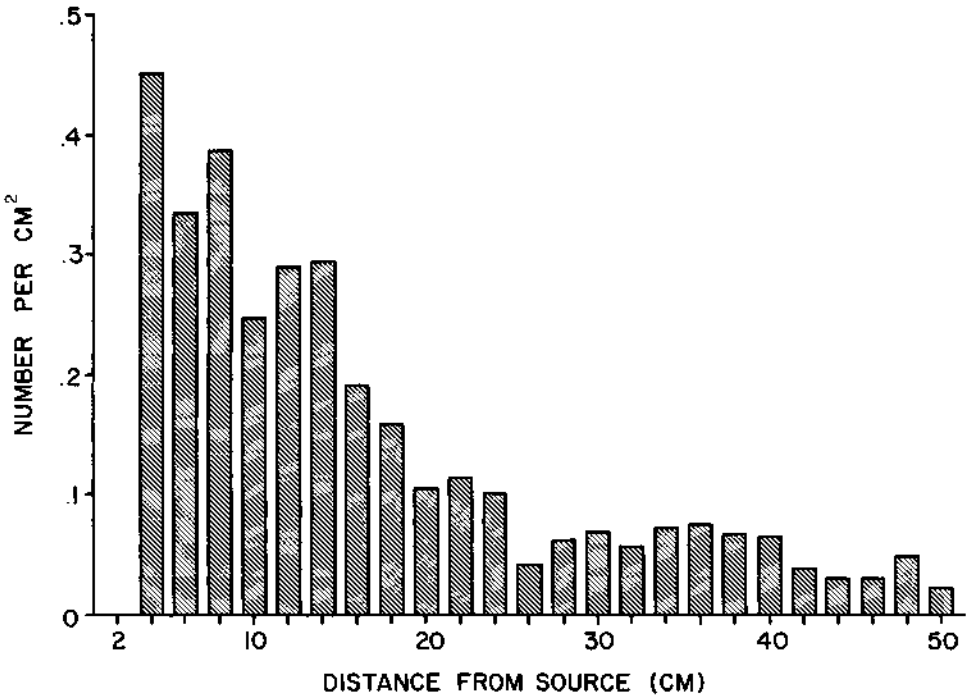


FIG. 1. The density of *Botryllus schlosseri* colonies carrying the rare electrophoretic marker PGI-fast as a function of distance from the source colony. Of 5,856 larvae released from the source colony, 645 were recovered after four weeks as recruits carrying PGI-fast.

germinal vesicles had disappeared in the ova (indicating competency to be fertilized), I removed 1,000 ova from each egg-parent. Each clutch of 1,000 eggs was split into 10 groups of 100 that were transferred into 50 ml of 0.22 $\mu$ filtered seawater (fsw). To each of the 10 tubes derived from a single clutch, I added sperm from one of the sperm-parents. I obtained the sperm by dissecting 15–20 ripe testes from each of the 10 sperm-parents. I then washed each group of testes three times in fsw. The testes were stored intact in 25 ml of fsw at 3°C until sperm were needed for fertilizations. The sperm suspensions used for fertilizations were created by gently macerating one ripe testis in 3 ml fsw. Sperm were never stored for more than 48 hours. I have previously shown that storage of sperm for this period has no discernible effect on fertilization, embryonic development, or larval metamorphosis (Grosberg, 1982).

After the sperm were added to the tubes containing the eggs, I agitated the tubes briefly and incubated them for 2 hours at 18°C. I then pipetted each batch of ova from the culture tubes, washed the ova three times in filtered seawater, and transferred the 100 ova to 150 mm × 30 mm plastic petri dishes filled with 150 ml filtered seawater.

After 8 hours at 18°C, I scored the total number of eggs that had undergone at least one cleavage. I used this number divided by the total number of ovulated eggs to estimate fertilization success. After 24 hours, I removed all uncleaved ova. Every 24 hours thereafter, until the embryos hatched into swimming tadpole larvae, I monitored the cultures. The proportion of embryos that had cleaved but not hatched 7 days post-fertilization were scored as fertilized, but unhatched. On day 8, I counted the total number of hatched larvae that had successfully metamorphosed from swimming larvae into the sessile primary zooid (the oozooid) from which the colony develops.

## RESULTS

### *Estimates of Dispersal Distance*

*Larval Mark/Recapture Study.*—After four weeks, 1,036 colonies of *B. schlosseri* had recruited to the panel suspended be-

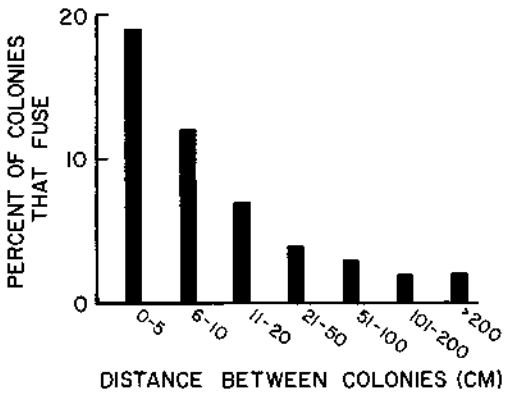


FIG. 2. The percent of juxtaposed colonies that fused according to the distance between colonies. One hundred pairs of field-collected colonies were established in each distance category.

neath the MBL Supply Dock. Of these colonies, 645 (62.3%) were homozygous for the PGI-fast marker. Given that the source colony produced 5,856 larvae, this represents a recapture rate of recruits of 11.0%. Juvenile mortality of colonies during the first week after settlement approaches 90% (Brunetti, 1974; Grosberg, 1982). Thus, the actual number of sibling larvae derived from the source colony that settled on the panel was probably much higher than the 11% recovery would otherwise indicate.

The density of sibling recruits as a function of distance from the source colony is shown in Figure 1. This histogram reflects a strongly leptokurtic distribution of recruits (mean distance = 24.47 cm, SD = 13.23 cm, $N = 645$), with greater than 80% of the marked recruits located within 25 cm of the source colony.

*Histocompatibility Assays.*—Figure 2 demonstrates that the probability of fusion between pairs of colonies strongly depends on distance. Nearly 20% of colonies separated by 0–5 cm fused, whereas fewer than 5% of colonies separated by 21–50 cm fused. At distances greater than 2 m, fusion frequencies dropped to 2%. As the highest density of sibling recruits in the mark/recapture experiments was within 20 cm of the source, this pattern of attenuating fusion probability agrees well with the pattern of larval dispersal.

To provide a baseline fusion frequency unbiased by distance effects, I sampled 100 pairs of colonies that were randomly drawn from the population along the transect sampled above. Of these 100 pairs, six fused. In a more extensive sampling of 500 random pairings of colonies collected haphazardly from floating docks and pilings throughout the Eel Pond, 22 pairs (4.4%) fused. The agreement between the fusion frequencies calculated from the 100 pairs along the MBL Supply Dock transect, and from the more extensive sample in the Eel Pond, implies that the limited sample from the transect reflects the allelic diversity at the histocompatibility locus in the Eel Pond. Thus, colonies taken within 5 cm of each other are 4–5 times more likely to fuse than if they had been drawn at random from the population.

### *Progeny Viability and Interparental Distance*

I determined the influence of distance between mates on three dependent variables related to the viability of progeny: 1) the percent of ova fertilized; 2) the percent of fertilized ova that hatched and formed motile, normal larvae; and 3) the percent of larvae that successfully settled and metamorphosed into feeding zooids. A mixed model hierarchical ANOVA (SAS Institute Inc., 1985) of the arcsine-transformed data showed that neither the location of the egg-parents (i.e., landward or seaward end of the transect), nor the identity of the egg-parent itself, significantly affected any of the three dependent variables (Table 1). The distance between the sperm-parents and egg-parents did have a signficant effect on all three variables (Table 1). For metamorphic success, there is a significant location × distance interaction; however, the contribution of this interaction to the overall sum of squares is less than one-fiftieth that of the effect due to distance between mates. Accordingly, for the regression analyses of the effects of distance on the three dependent variables, I pooled the data across locations and egg-parents.

The graphical results of the effects of distance between mates on the three dependent variables are shown in Figure 3. Although the strength of the relationships between mate proximity and the three components

TABLE 1. Analysis of variance on the effects of egg-parent, location of egg-parent, and distance between mates on three variables related to progeny viability (see text). *F*-tests for effects of location use egg-parent (location) term as the error mean square. All other *F*-tests use distance egg-parent (location) term as error mean square.

| | Dependent variable: Fertilization success | | | |
|---|---|---|---|---|
| Source of variation | SS | *d.f.* | *F* | Significance level (*P*) |
| Location of egg-parent | 0.002 | 1 | 0.65 | = 0.44 |
| Egg-parent (location) | 0.020 | 8 | 0.43 | = 0.90 |
| Distance between mates | 0.170 | 9 | 3.30 | < 0.002 |
| Location × distance | 0.022 | 9 | 0.44 | = 0.91 |
| Distance × egg-parent (location) | 0.409 | 72 | | |

| | Dependent variable: Hatching success | | | |
|---|---|---|---|---|
| Source of variation | SS | *d.f.* | *F* | Significance level (*P*) |
| Location of egg-parent | 0.003 | 1 | 1.05 | = 0.33 |
| Egg-parent (location) | 0.026 | 8 | 1.19 | = 0.31 |
| Distance between mates | 2.152 | 9 | 86.23 | < 0.001 |
| Location × distance | 0.045 | 9 | 1.81 | = 0.08 |
| Distance × egg-parent (location) | 0.199 | 72 | | |

| | Dependent variable: Metamorphic success | | | |
|---|---|---|---|---|
| Source of variation | SS | *d.f.* | *F* | Significance level (*P*) |
| Location of egg-parent | 0.001 | 1 | 1.02 | = 0.34 |
| Egg-parent (location) | 0.012 | 8 | 0.59 | = 0.78 |
| Distance between mates | 2.773 | 9 | 124.36 | < 0.001 |
| Location × distance | 0.054 | 9 | 2.43 | = 0.018 |
| Distance × egg-parent (location) | 0.199 | 72 | | |

of fitness is variable, the graphs show success rates ≥95% in matings between colonies separated by 1 m, or less. Between 1 m and 1.5 m, all characters decline concordantly.

Across all distances, the percent of ova fertilized ranged between 90% and 95%. Fertilization was most successful in crosses between individuals separated by ≤1 m, and declined slightly in matings beyond this distance. This decline produced a small, but significant negative relationship between distance and percentage of ova fertilized ($R^2 = 0.19$, $P < 0.001$).

The effect of interparental distance on the percent of fertilized ova that hatched into normal larvae is greater than its effect on fertilization success. Where interparental distances were ≤1 m, hatching success remained above 95%. Beyond 1 m, hatching success declined dramatically, with 80% success in matings between colonies separated by 3 m, or more. This results in a large and significant negative relationship between distance and hatching success ($R^2 = 0.69$, $P < 0.001$).

The pattern of declining settlement and metamorphic success, although somewhat less striking than that of hatching success, shows a similar drop where mates are separated by ≥1 m. Distance explains over 50% of the variance in metamorphic success ($R^2 = 0.54$, $P < 0.005$).

## DISCUSSION

The enormous difficulty of monitoring the movements of large numbers of small larvae has hindered an understanding of how dispersal affects the genetic structure of sessile marine invertebrate populations (Hedgecock, 1982). Even when direct observations of individual larvae provide estimates of minimum dispersal distance (e.g., Olson, 1985), documentation of gene flow remains an elusive goal. Because I was unable to sample large areas for the presence of sibling larvae, and since gene flow depends disproportionately on relatively few

long distance movements, my results do not permit quantification of gene flow or neighborhood size. Nevertheless, the indirect measurements of dispersal distance based on the larval mark/recapture experiment, and the decline of fusion frequencies with increasing distance between paired colonies, together suggest that 1) many larvae of *Botryllus schlosseri* recruit within a meter of their birthplace, and 2) the distance between colonies and their relatedness will be negatively correlated. In fact, the concordance between estimates of dispersal distance, and the decrease in fertilization, hatching, and settlement success according to distance between mates, implies that gene flow does not overwhelm genetic substructuring of the Eel Pond population of *B. schlosseri.* Other reports of spatial association of similar *B. schlosseri* genotypes over scales of less than a few meters support the conclusion that limited dispersal produces associations of related individuals in other populations (Sabbadin and Graziani, 1967; Sabbadin, 1978).

In a mating context, associations of kin potentially, but not inevitably, lead to inbreeding. For example, flowers of wind-pollinated plants may receive a random subsample of pollen from the entire population, rather than receive pollen disproportionately from neighbors (Schaal, 1975; Linhart et al., 1981). Among insect-pollinated plants, pollen carryover may increase gene flow, hence increasing levels of outbreeding, over that predicted on the basis of pollinator movements alone (Schaal, 1980; Levin, 1981). The situation in many brooding, viviparous marine invertebrates is potentially much the same. In *Botryllus,* fertilized ova are held until the larvae are fully developed; however, the sperm are released into the plankton. If sperm travel across the population and remain viable during their journey, and if dilution effects are small, then gene flow due to sperm dispersal may counter the effects of limited larval dispersal.

In the absence of synthetic populations with labeled sperm sources, precise estimates of the component of gene flow due to sperm dispersal are not available for any sessile marine invertebrate. Two lines of circumstantial evidence indicate that sperm dispersal may not greatly disrupt the effects of limited larval dispersal. First, *B. schlosseri* sperm, once mixed with seawater, remain active for less than five minutes; subsequently, they appear unable to fertilize ova (pers. observ.). Second, once sperm leave the boundary layer and enter the plankton, dilution may limit gene flow. Even in some wind-pollinated plants, the distribution of pollen grains from a source is leptokurtic (Levin and Kerster, 1974; Waser and Price, 1983). Pennington's (1985) in situ study of fertilization in the sea urchin *Strongylocentrotus droebachiensis* revealed a similar pattern: sperm dilution much beyond 0.5 m downstream rendered fertilizations unlikely. In *Botryllus,* the importance of dilution effects remains difficult to quantify; but in combination with the comparatively short lifespan of the sperm, gene flow due to sperm dipersal may be quite limited.

Two other mechanisms may counteract the effects of limited larval dispersal on breeding structure. First, some congeners of *Botryllus* possess gametic compatibility systems like the gametophytic incompatibility systems that control fertilization in plants (Oka, 1970; Burnet, 1971). Oka (1970) reported that *B. primigenus* sperm sharing a histocompatibility allele with the diploid, maternally derived egg envelope, cannot fertilize that egg; thus, ova derived from a colony carrying an *A* and *B* allele at the histocompatibility locus cannot be fertilized by sperm carrying either an *A* or *B* allele. Scofield et al. (1982) provide evidence for a similar block to fertilization in a Monterey Bay, California population of *B. schlosseri.* However, both Sabbadin (1982, pers. comm.) and I, in our studies of *B. schlosseri* populations from the Venetian Lagoon and the Eel Pond, have been able to cross-fertilize colonies carrying the same set of histocompatibility alleles. More recently, I have shown in laboratory sperm competition experiments that when an *A/B* colony is fertilized by an *A/C* colony, the *A* and *C* paternal alleles appear in the $F_1$'s with equal frequency (Grosberg, unpubl.).

Even if the block were perfectly efficient, at least 50% of full-sib sperm/egg interactions would be compatible. Consider the case where two colonies heterozygous for different histocompatibility alleles (e.g., $A/B \times C/D$) are crossed. Four compatibility ge-

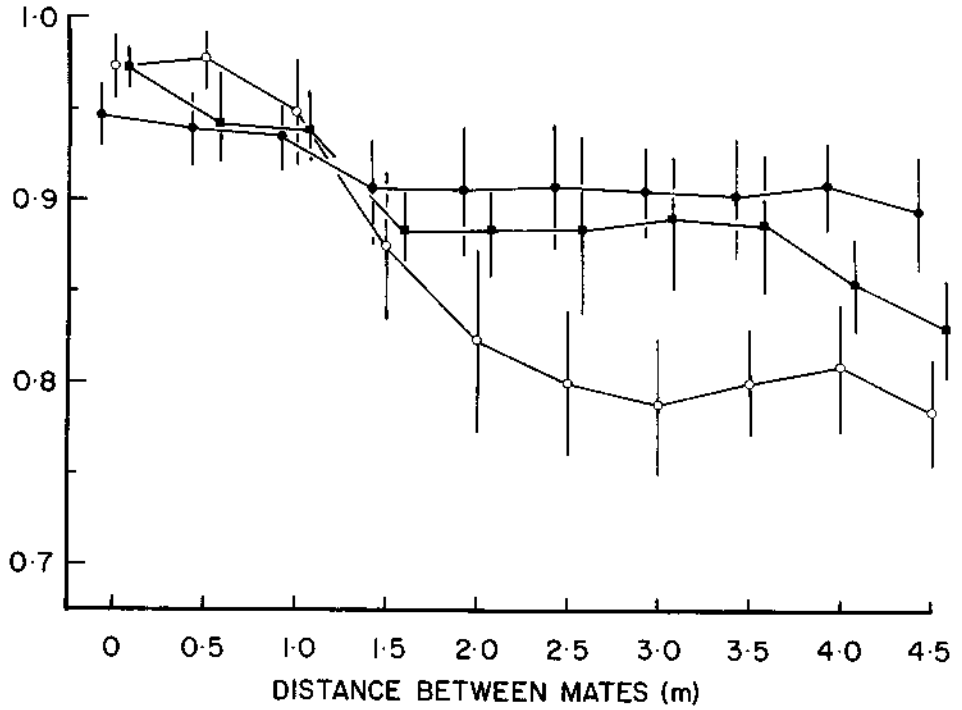


FIG. 3. The proportion of successful fertilizations (solid circles), larval hatching (open circles), and larval metamorphosis (squares) according to distance between mated colonies. The plots show the means of data taken from 10 matings (each initiated with 100 ova) in each distance category. The error bars show ± one standard deviation.

notypes of $F_1$'s would be produced in equal frequencies: *A/C, A/D, B/C,* and *B/D.* In matings between full-sib $F_1$'s, all *A/C* × *A/C* fertilizations would entirely fail; however, *A/C* × *A/D* or *B/C* fertilizations would be 50% successful, and *A/C* × *B/D* fertilizations would be 100% successful. The same probabilities would hold for all classes of matings among a full sibship.

A second mechanism that can reduce inbreeding in *B. schlosseri* results from the synchronization of asexual (hence, reproductive) cycles that occurs when two colonies fuse (Watanabe, 1953; Sabbadin, 1978). In a given sexual cycle, ovulation precedes by several days the maturation of the testes; thus, self-fertilizations are unusual (Milkman, 1967). When two desynchronized colonies fuse, their reproductive cycles rapidly become synchronized, and protogyny prevents cross-fertilization (Sabbadin, 1978). Although effective in preventing matings between fused relatives, this mechanism would not prevent matings among neighboring, but unfused, kin.

Despite the randomizing effects of fertilization barriers and planktonic dispersal of sperm, the results of the proximity-dependent mating experiments indicate that 1) breeding structure is not panmictic and 2) outbreeding is costly at least through metamorphosis. The magnitude of outbreeding depression can be roughly calculated using the cumulative mean stage-specific survivorships through metamorphosis. For crosses between proximate individuals, survivorship through metamorphosis is 89%. At a crossing distance of 1.5 m, survivorship declines to 70%. At 4.5 m, survivorship declines still further to 58%. The cumulative decrease in survival leads to a 35% cost, relative to crosses between proximate individuals, to outcrossing at distances ≥2.5 m. Thus, the fitness costs of outbreeding documented here are substantial, even if they were offset by increased vigor of outbred progeny later in the life cycle.

There are at least two genetic processes that can account for outbreeding depression in *Botryllus*: 1) the loss, or dilution, of alleles

that confer high location-specific fitness in environments where selection varies spatially (Maynard Smith, 1966; Antonovics, 1968; Jain, 1976; Ament, 1979; Turkington and Harper, 1979), and 2) the disruption by recombination of favorable epistatic interactions (Wright, 1960; Hedrick et al., 1978; Ohta, 1980; Partridge, 1983). As the favorability of epistatic interactions is often environmentally dependent (Spiess, 1977), these two mechanisms are not mutually exclusive. In the absence of reciprocal transplant experiments, the role of local adaptation is difficult to discern. However, because outbreeding proves costly to *Botryllus schlosseri* in the uniform and relatively benign environment of the laboratory, it is probable that outbreeding depression is partially exacted by the disruption of favorable epistatic interactions.

The establishment of epistatic interactions is promoted by a combination of temporally stable and spatially heterogeneous environments, as well as continuous inbreeding (Fisher, 1958; Wallace, 1968; Balkau and Feldman, 1973; Endler, 1977; Hedrick et al., 1978; Gillespie, 1981; Partridge, 1983). In contrast, extensive dispersal and outbreeding, through gene flow and recombination, inhibit the formation of epistatic interactions. Thus, in a spatially uniform environment, the benefits of maintaining favorable epistatic interactions can explain the persistence of philopatry and inbreeding, but can not provide the impetus for their evolution (Fisher, 1958). This implies that epistatically based outbreeding depression likely followed, rather than caused, the evolution of philopatry.

The evolution of philopatry may be affected by factors initially outside the context of population breeding structure. For example, if dispersal is hazardous, then the costs of inbreeding depression (or sib-competition) may be outweighed by the ecological benefits of short-distance dispersal. In the case of the soft coral *Alcyonium siderium,* proximity of juvenile recruits to their parental colony increases juvenile survival (Sebens, 1983). In sedentary organisms, philopatric dispersal can promote and maintain local adaptation (Balkau and Feldman, 1973; Feldman and Krakauer, 1976; Gillespie, 1981; Slatkin, 1985), and prevent propagules from being transported away from favorable sites (e.g., Levin, 1984; Olson, 1985). Although the maintenance of local adaptation may play a role in the evolution of philopatry in the Eel Pond, there appear to be no obvious physical or biotic differences along the transects on the MBL Supply Dock. Alternatively, if, as the data suggest, larvae suffer high mortality, then selection may have favored the reduction of time in the plankton, hence reducing dispersal distance. In fact, among nonfeeding larval forms such as those of many clonal marine invertebrates (including *Botryllus*), there is little apparent benefit to prolonging the planktonic phase beyond the time necessary to locate favorable habitats (Strathmann, 1985).

Once a persistent pattern of inbreeding is established, it should rapidly purge a deme of deleterious recessives (Bengsston, 1978; Shields, 1982). Thus, matings between individuals drawn from populations which outbreed, or which have brief histories of inbreeding, should show higher levels of inbreeding depression due to the unmasking of deleterious recessives (Shields, 1982). Historical differences in breeding structure could explain the discrepancy between my data, and Sabbadin's (1971) observation that self-fertilized lines derived from Venetian Lagoon populations show high levels of inbreeding depression.

There is another important ecological consequence of limited dispersal in colonial animals: philopatry promotes the cosettlement of potentially histocompatible, hence fusible, genotypes (Grosberg and Quinn, 1986; Jackson, 1985). If dispersal were extensive and settlement random, then colony fusion would be an unusual event. As it is, fusion frequencies can exceed 20% on fine spatial scales (also see Scofield et al., 1982). If colony fusion is beneficial to one or both genotypes composing a chimera (reviewed in Buss, 1982), then philopatry may be favored because it indirectly enhances the probability of colony fusion (Jackson, 1986).

Philopatric dispersal among sessile organisms is not limited to *Botryllus schlosseri.* Indeed, direct observations on the behavior of pollinators (Schaal, 1980; Waser and Price, 1983; Handel, 1983), the dispersal of pollen (Schaal, 1980; Handel, 1983;

Waser and Price, 1983) and seeds (Schaal, 1980), and the movement of invertebrate larvae and gametes (e.g., Ayling, 1980; Gerrodette, 1981; Kojis and Quinn, 1982; van Duyl et al., 1981; Keough, 1984; Olson, 1985; Heyward and Babcock, 1986; Jackson, 1985) indicate that relatives are likely to be spatially associated in a broad array of sessile taxa. In addition, preferential larval settlement near siblings, now known for arborescent bryozoans (Keough, 1984) and *B. schlosseri* (Grosberg and Quinn, 1986), should further enhance kin associations.

The intensity of selection favoring the evolution of philopatry will depend on a complex balance between the costs of 1) locally increased crowding and habitat deterioration, 2) inbreeding depression, and 3) moving to other sites; and the benefits of 1) lower mortality during dispersal, 2) remaining near favorable sites, 3) maintaining epistasis and local adapation, and 4) cooperation and nepotism among kin (Bengsston, 1978; Greenwood, 1984). Among organisms in which genetically distinct conspecifics are known to fuse—including colonial marine invertebrates (Buss, 1982), fungi (Esser and Blaich, 1973), myxomycetes (Carlile, 1973), slime molds (Buss, 1982), red algae (Tveter and Mathieson, 1976), and social insect colonies (Thorne, 1984)—the costs and benefits of fusion must also be factored into the analysis. Until there are more data documenting these costs and benefits, understanding the widespread evolution of philopatry, and its striking association with colony fusion in marine invertebrates, will remain an elusive goal.

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