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KIN INTERACTIONS IN A COLONIAL HYDROZOAN (*HYDRACTINIA SYMBIOLONGICARPUS*): POPULATION STRUCTURE ON A MOBILE LANDSCAPE

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Abstract.—Many sessile colonial organisms intensively compete with conspecifics for growing space. This competition can result in either cooperative fusion or aggressive rejection between colonies, and some species have evolved highly polymorphic genetic systems that mediate the outcome of these interactions. Here we demonstrate the potential for interactions among close kin as the basis for the evolutionary maintenance of a genetically polymorphic allorecognition system in the colonial hydroid *Hydractinia symbiolongicarpus*, which lives on gastropod shells occupied by hermit crabs. Fusion between hydroids in the laboratory is restricted mainly to encounters between full siblings, whereas other encounters result in aggressive rejection. Natural selection acting on the costs or benefits of fusion between colonies could be responsible for the present maintenance of such a highly specific behavioral response, but only if encounters between fusible colonies still occur in contemporary populations. The large size of these hydroid populations and the mobility of the crabs should limit the potential for interactions among closely related hydroids on the same shell. However, RAPD polymorphisms among a large sample of hydroids from a population off the coast of Massachusetts indicate that genetically similar colonies are often found together on the same shell. Some genetic distances between colonies on the same shell were low relative to genetic distances between colonies on different shells or genetic distances between known full siblings from laboratory matings. We conservatively estimate that 2–18% of co-occurring colonies may be full sibling pairs. These observations suggest that encounters between genetically similar hydroids are common, despite the mobile nature of their habitat, and these encounters may provide frequent opportunities for natural selection to influence the evolution of cooperative and agonistic behaviors and their polymorphic genetic basis.

Key words.—Allorecognition, genetic polymorphism, *Hydractinia symbiolongicarpus*, Hydrozoa, population structure, RAPDs.

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Many plants, fungi, and animals have self-nonself recognition systems that mediate the nature and outcomes of reproductive and somatic interactions between conspecifics (reviewed in Buss 1987; Bonner 1988; Grosberg 1988). These allorecognition systems typically exhibit exceptional specificity and presumably corresponding high levels of genetic polymorphism (i.e., allotypic diversity), sometimes exceeding by an order of magnitude levels of variation found at almost all other polymorphic loci (Wright 1939; Grosberg 1988; Potts and Wakeland 1990; Charlesworth 1995; Parham and Ohta 1996). In the absence of exceptionally high mutation rates, the evolution and persistence of extensive allotypic diversity likely involves some form of balancing selection (Grosberg 1988; Potts and Wakeland 1993), resulting in: (1) unusually high levels of sequence divergence among alleles (Potts and Wakeland 1993); (2) high ratios of nonsynonymous to synonymous substitutions (Clark and Kao 1991); and (3) transpecific coalescence of allelic genealogies (Haring et al. 1990). These patterns are most likely the outcome of some combination of either overdominant (Hughes and Nei 1988, 1992) or frequency-dependent (Slade and McCallum 1992; Potts and Wakeland 1993) selection acting on allorecognition phenotypes (Hedrick 1994; Apanius et al. 1997).

In the context of conspecific interactions, what functions of allotypic diversity could lead to selection favoring extreme genetic polymorphism? In nearly 30 families of flowering plants, allorecognition takes the form of homomorphic self-incompatibility (Charlesworth 1995). These gametophytic and sporophytic self-incompatibility (SI) systems govern interactions between prospective mates or their gametes, limiting mating or fertilization to pairs with different compatibility types. Allotypic polymorphism eliminates the potential for self-fertilization and reduces the likelihood of inbreeding, particularly when relatives live in close proximity. If inbreeding is costly (Thornhill 1993) or populations are small (Vekemans et al. 1998), negative frequency-dependent selection should favor rare SI alleles and the accumulation of allotypic diversity (Charlesworth 1995). Allorecognition systems may also influence mating patterns in animals, including some colonial ascidians (Oka 1970), echinoderms (Metz and Palumbi 1996), and mammals (Lenington et al. 1994; Potts and Wakeland 1993). As in plants, these mate-recognition systems may reduce inbreeding load and promote the accumulation of allotypic diversity by disassortative mating (reviewed in Bodmer 1972; Potts and Wakeland 1993; Hedrick 1994).

In many fungi (Raper 1966; Metzenberg 1990; Nauta and Hoekstra 1994) and colonial animals (reviewed in Buss 1987, 1990; Grosberg 1988), allorecognition systems govern whether somatic tissue contacts between allogeneic (i.e., conspecific nonself) individuals lead to fusion or rejection. Fusion is a form of parabiosis, usually restricted to allotypically similar or identical individuals, that results in the coalescence of two or more genotypes in one soma. Allotypically dis-

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parate individuals usually reject one another, and in at least some cnidarians rejection is accompanied by inducible aggressive behavior, involving the deployment of specialized tentacles or stolons armed with stinging cells (reviewed in Ayre and Grosberg 1996).

The strength of natural selection favoring the evolution of polymorphism in an allorecognition system that regulates somatic interactions mainly depends upon two factors: (1) the relative costs and benefits of fusion versus rejection; and (2) the frequency of encounters between fusible and rejecting allotypes (Grosberg and Quinn 1988; Ratnieks 1991). Many of the costs of fusion arise from the mixing of genetically distinct cell lineages, each of which retains the capacity to differentiate into either gametes or somatic tissue (Buss 1987, 1990). Under these conditions, one member of a chimera could parasitize the other, possibly by limiting the differentiation of the other's multipotent cells into gametes, by using the somatic tissues of the other member of the chimera for provisioning gametes and brooding embryos, or by unidirectional translocation of nutrients (Rinkevich and Loya 1983). Partial or complete displacement of one cell lineage by another appears to occur in chimeras of fungi, myxomycetes, sponges, and colonial ascidians (Buss 1982, 1990; Grosberg 1988; Pancer et al. 1995; Stoner and Weissman 1996; Matapurkar and Watve 1997). The most obvious benefits of fusion arise from the immediate increase in size of one or both members of a chimera (Buss 1982, 1990). In many organisms, a small increase in size often yields a disproportionately large gain in survivorship or decrease in age at first reproduction. In addition, a chimeric soma houses more genetic diversity than a nonchimeric one, and such diversity may confer a selective advantage on the chimera in a heterogeneous environment (De Boer 1995).

Like fusion, rejection potentially entails substantial costs, especially when it is associated with agonistic behavior. In this context the most significant costs likely arise from the risk of injury by an opponent and the production of highly specialized tissues (Buss et al. 1984). However, rejection accompanied by agonistic responses may allow some genotypes to dominate others in competition for space (Francis 1973; Bigger 1980; Buss et al. 1984; Ayre and Grosberg 1995, 1996).

The other key component of selection on a genetically polymorphic allorecognition system is the dispersion of allotypes on the spatial scale of interactions between individuals. In contrast to our growing understanding of the costs and benefits of the regulation of intraspecific somatic interactions, there is considerably less known about the frequency of encounters between similar and dissimilar allotypes (but see Grosberg 1987; Potts and Wakeland 1993). The degree of allotypic similarity or disparity between interacting conspecifics depends on standing levels of allotypic diversity in a population and, for sedentary organisms, patterns of dispersal of sexually and asexually produced propagules. Specifically, if allotypic diversity is high and dispersal of propagules is extensive, then allotypes will be well mixed at the spatial scale of interactions between genotypes. Consequently, encounters between similar allotypes will be rare and opportunities for somatic fusion will also be unusual. Under these circumstances, ongoing selection acting on allorecognition polymorphism (via the costs and benefits of fusion) should be weak.

In principle, the simplest way to estimate the potential for selection to act on the costs and benefits of somatic fusion is to count the frequency of chimeras in natural populations. In practice, chimeras may be difficult to identify on morphological grounds (but see, e.g., Craig 1994), and counts of surviving chimeras may underestimate the potential for interactions between fusible genotypes. Alternatively, the spatial distribution of allorecognition alleles could be characterized directly if the genes and gene products that confer allotypic specificity were known. It is not yet possible to do so, except in a few vertebrates and angiosperms. However, in highly polymorphic populations with many rare allorecognition alleles, sharing of allotypic markers between individuals is correlated with relatedness because rare alleles are more likely to be shared by descent (Getz 1981; Lacy and Sherman 1983; Crozier 1987; Grafen 1990). Estimates of relatedness can thus be used as a proxy for the distribution of similar allotypes. To the extent that some outcomes of allogeneic contact such as fusion occur only between pairs of individuals that share a large fraction of allotypic markers (Grosberg et al. 1996b), the formation of chimeras will be limited to close kin. Thus, we can estimate the potential for fusion in nature by characterizing the relatedness of individuals involved in allogeneic contacts.

The life cycles of many sessile colonial invertebrates—including sponges, cnidarians, bryozoans, and ascidians—feature a suite of traits that should promote intraspecific competition for space and frequent allogeneic interactions among kin (Jackson 1985, 1986; Buss 1990). These life-history traits include indeterminate growth, asexual fragmentation, dense larval settlement, and limited dispersal of sexually produced propagules (reviewed in Knowlton and Jackson 1993; Grosberg et al. 1996b). Such life cycles should, in turn, favor the evolution of allorecognition systems that allow individuals to distinguish conspecific self from nonself and close relatives from more distantly related individuals (Jackson 1985, 1986; Grosberg 1988, 1992; Feldgarden and Yund 1992; Grosberg et al. 1996b).

Like many other sessile marine invertebrates, the athecate colonial hydrozoan *Hydractinia symbiolongicarpus* possesses a highly polymorphic allorecognition system that limits allogeneic fusion to interactions between close relatives (Hauenschild 1954, 1956; Ivker 1972; Grosberg et al. 1996b; Mokady and Buss 1996). However, the life cycle of *H. symbiolongicarpus* differs in one critical respect from that of many other sedentary, colonial animals: the sessile adult colonies live attached to small and mobile patches of habitat, specifically gastropod shells occupied by the hermit crab *Pagurus longicarpus* (Buss and Yund 1989). The hermit crabs inhabit the muddy and sandy benthos of shallow coastal bays, which lack the hard substratum required by *Hydractinia*. Previous studies indicate that about one-third of shells colonized by hydroids carry two or more individuals (Yund et al. 1987; Yund and Parker 1989). As these colonies grow and encounter one another, one of three outcomes ensues: (1) fusion, involving the anastomosis of the stolons of the two allogeneic colonies and the exchange of their nutrients and other gastrovascular contents; (2) aggressive rejection, accompanied

by the induction of specialized organs of aggression, the hyperplastic stolons (Ivker 1972; Buss et al. 1984; Lange et al. 1989); or (3) transitory fusion, in which initial fusion is followed by rejection (Hauenschild 1954; Shenk and Buss 1991). Laboratory matings indicate that fusion occurs in roughly 40% of encounters between full siblings. Contacts between half siblings or more distant relatives nearly invariably lead to aggressive rejection (Grosberg et al. 1996b).

At first sight, the life cycle of *Hydractinia symbiolongicarpus* suggests that encounters between kin should be rare. Although full or half siblings from the same clutch could potentially recruit to the same shell on a passing hermit crab, the crabs are abundant (often > 200 per square meter; R. Grosberg, unpubl. data) and highly mobile. This should favor the distribution of relatives among different shells. It should also severely limit the potential for interactions between kin of lesser relatedness (e.g., cousins) because such interactions depend on associations between individuals removed by more than one generation (e.g., sisters on different shells spawning in the same place at the same time). In addition, the proximity in spawning aggregations of numerous hermit crabs bearing male hydroid colonies should increase the proportion of (nonfusible) maternal half siblings among members of a clutch relative to (fusible) full siblings. For these reasons, we did not expect to find either general evidence of genetic structure on the spatial scale of single gastropod shells or specific evidence of co-occurring full siblings. Under such an assumption, it is unclear how natural selection could maintain the observed allotypic specificity in this and other ecologically similar populations. In this study, we test this assumption by analyzing patterns of RAPD band-sharing between *H. symbiolongicarpus* colonies on the same shell in a natural population.

Materials and Methods

Sampling

Frequency of Multiply Colonized Shells.—We sampled hydroids from a large population living in Barnstable Harbor, Massachusetts, on the south side of Cape Cod Bay (approximately 41°43′N, 70°17′W). During low tides on 5 June, 15 August, and 13 October 1995 (dates spanning the growing season of these hydroids) one of us (RKG) collected hydroids along a line parallel to the shore and about 100 m offshore from the high tide mark. We collected a single *Hydractinia*-occupied shell (the shell closest to the toe of RKG's right boot) about every 1 m, for a total of 200 shells. We returned the shells to the laboratory and counted the number of colonies on each.

Genetic Similarity among Co-occurring Hydroids.—During low tides in late June 1996 we collected from the same population 106 gastropod shells inhabited by the hermit crab *Pagurus longicarpus* and that carried at least two *Hydractinia symbiolongicarpus* colonies. The shells were collected over two tide cycles across an intertidal area approximately 500 m long and 100 m wide. The exterior surface of most of these shells was extensively covered by *H. symbiolongicarpus.*

We characterized the RAPD banding patterns of 198 hydroid colonies from 93 of these shells. Two of the shells carried four hydroid colonies, eight shells carried three colonies, and 83 shells carried two colonies, yielding a total of 119 pairwise comparisons among co-occurring genotypes. We generated RAPD markers using five Operon RAPD primers (F-06, F-07, M-02, M-04, S-01) in a protocol described elsewhere (Levitan and Grosberg 1993; Grosberg et al. 1996a). Almost all RAPD bands in this species are inherited as dominant Mendelian markers, and RAPD band sharing is an effective measure of genetic differentiation among siblings and family groups of *Hydractinia* (Levitan and Grosberg 1993; Grosberg et al. 1996a).

Analysis

Our general goal was to determine the association between familial relatedness and co-occurrence on shells by comparing measures of inferred genetic distance (based on degree of sharing of polymorphic RAPD bands) between colonies found on the same shell to colonies on different shells. Our more specific goal was to identify cases in which putative full siblings recruited to the same shell.

Genetic Distances.—We used several different methods to characterize the correlation between co-occurrence on shells and genetic distance among colonies from Barnstable Harbor. For each method we performed one-tailed tests of the a priori hypothesis that genetic distances are greater between hydroids on different shells than between hydroids on the same shell.

First, we used the RAPDistance program of Armstrong et al. (1994) to calculate all pairwise genetic distances among colonies. Many of the available distance metrics are linear functions of each other and so are highly correlated for real datasets. Therefore, we followed the advice of Armstrong et al. (1994) and compared four metrics that use different information from the pattern of RAPD band sharing and that are usually poorly correlated with each other: $d = 1 - (n_{11}/n)$ (Russell and Rao 1940); $d = 1 - [(n_{11} + n_{00})/n]$ (Apostol et al. 1993); $d = 1 - [2n_{11}/(2n_{11} + n_{01} + n_{10})]$ (Nei and Li 1979); $d = 1 - [(n_{11}n_{00} - n_{10}n_{01})/(n_{11}n_{00} + n_{10}n_{01})]$ (Yule and Kendall 1950); where n is the total number of polymorphic bands in the population, n_{11} is the number of bands present in both colony i and colony j, n_{10} is the number of bands present in colony i but absent in colony j, n_{01} is the number of bands absent in colony i but present in colony j, and n_{00} is the number of bands absent in both colonies. From these four possibilities, we chose a single d metric that was most suitable for subsequent statistical analysis (having a normal distribution around an intermediate mean).

Second, we compared d between pairs of colonies on the same shell (total of 119 pairs) to mean d between pairs of colonies on different shells (total of 19,384 pairs). However, a simple comparison of these two means will be biased by nonindependence among observations (Danforth and Freeman-Gallant 1996). We therefore used the methods below to overcome this nonindependence.

First, we used the R package of programs by Legendre and Vaudor (1991) to implement Mantel's (1967) test of correlation between the structure of two matrices. The first matrix consisted of d values between all pairs of 198 colonies. The second matrix represented the dispersion of colonies among shells: entries for pairs of colonies on the same shell were

coded 0, and all other entries (for colonies on different shells) were coded 1. Mantel's r-statistic and the associated t-test indicate the matrixwide correlation between genetic distance and co-occurrence on shells.

Second, we resampled the observed matrix of d values among colonies using a nonparametric resampling routine written by D. Cutler (University of California, Davis). We first resampled the original 198 hydroid genotypes with replacement (bootstrapping) to produce a pseudoreplicate matrix of genetic distances. In each pseudoreplicate, we assigned genotypes to shells at random (83 shells with two colonies, eight shells with three colonies, two shells with four colonies), then we calculated the mean d (n = 119) between colonies assigned to the same shell. We repeated the procedure 10,000 times and counted the frequency of pseudoreplicates in which the mean d between colonies assigned to the same shell at random was less than the mean d between colonies actually collected from the same shell in Barnstable Harbor. We then resampled the observed d values among colonies on the same shell (n = 119) and among colonies on different shells (n = 19,384) without replacement (jackknifing) to produce a pair of pseudoreplicates of equal size (n = 93 for both classes of genetic distance). In each pseudoreplicate, no hydroid genotype was represented more than once. This comparison is analogous to the subsampling procedure devised by Danforth and Freeman-Gallant (1996). We resampled 10,000 times and counted the frequency of pseudoreplicate pairs in which the mean d between colonies on the same shell was significantly less than the mean d between colonies on different shells by a two-sample t-test. Both of these resampling methods test the average degree of genetic difference among colonies that occur on the same shell.

Third, we compared each d between a pair of colonies on a shell (e.g., colonies 1A and 1B on shell 1) to the mean d between one of those colonies and all other colonies on other shells (e.g., between colony 1A and colonies 2A through 106B) by a one-sample t-test. Each such test included only d values that are biased by the same single observation (e.g., RAPD bands observed in colony 1A). We noted all cases in which d between colonies on the same shell was significantly less than the mean d between shells at the 0.05 level of significance. We used a Bonferroni adjustment for multiple t-tests (Rice 1989) to assess the experiment-wide significance of each comparison. This one-sample method allowed us to identify specific cases of close genetic similarity between colonies on the same shell.

Identifying Full Siblings.—The estimation of familial relatedness (r) from genetic fingerprints (such as dominant RAPD polymorphisms) is notoriously difficult because the estimate of r for any pair of individual genotypes is biased and has a large variance (Lynch 1988; Lynch and Milligan 1994), even for large numbers of unlinked markers. Thus, we did not attempt to calculate r for pairs of co-occurring hydroid genotypes as a way to identify possible full sibling pairs (r = 0.5). Instead, we measured the degree of RAPD band sharing for known full sibling pairs (in which r is known to be 0.5) and used this observed RAPD band sharing as an empirical standard to identify specific cases in which co-occurring genotypes might be full siblings (e.g., Apostol et al. 1993).

First, we scored the same RAPD polymorphisms using the same five Operon primers for nine to 17 full siblings from each of five different laboratory matings (designated X, V, β, γ, δ) between parental colonies haphazardly collected from Barnstable Harbor. We used the within-family mean d to characterize the degree of measured genetic difference between individuals of known relatedness (r = 0.5). We then chose the largest of these five means (from the β family) as the most inclusive standard by which to judge genetic differences between colonies of unknown relatedness that were found on the same shell in the Barnstable Harbor population. We noted all cases in which d between two colonies on the same shell was less than the mean d among β full siblings. We discuss below the ability of this standard to distinguish correctly full-sibling pairs from unrelated pairs (Type I error) and the probability of misidentifying unrelated colonies as full siblings (Type II error).

Second, we performed two types of clustering analysis based on RAPD band-sharing to identify cases in which colonies found on the same shell formed pairs more closely related to each other than to all other colonies in the sample. In the first analysis, we used SYSTAT (Wilkinson 1989) to implement Ward's minimum variance method for clustering based on values of Nei and Li's d (Levitan and Grosberg 1993).

In the second analysis, we coded each polymorphic RAPD band as a two-state character (scored 1 for present or 0 for absent) for each hydroid genotype. We analyzed this character state matrix by parsimony using PAUP 3.1 (Swofford 1993). We could not perform complete heuristic searches of tree space for 198 OTUs because the search quickly found very large numbers of trees of equivalent length, so we resorted to a series of abbreviated analyses. We performed an initial series of 20 heuristic searches of tree topologies, using TBR branch swapping, in which genotypes were added at random and a maximum of 100 trees of equivalent length were retained at each step. We then calculated the 50%-majority-rule consensus of the 100 shortest trees produced by these 20 random addition sequences. Finally, we used this consensus as the starting tree in a more extensive heuristic search in which a maximum of 1000 trees were retained at each step. We then calculated the 50%-majority-rule consensus from the resulting 1000 shortest trees, and counted the cases in which colonies on the same shell were grouped together as sister genotypes more closely related to each other than to any of the other 196 colonies.

Analysis of Rare RAPD Markers.—Dominant markers from DNA fingerprints tend to underestimate familial relatedness when unrelated individuals share many common markers (Reeve et al. 1992). We repeated both of the analyses for identification of co-occurring full siblings (comparison to laboratory full siblings and Ward's minimum variance cluster analysis) using only relatively rare RAPD markers found in less than 40% of the 198 sampled colonies. Such rare markers may be more effective in identifying and distinguishing members of full sibling families (Apostol et al. 1993; Blouin et al. 1996).

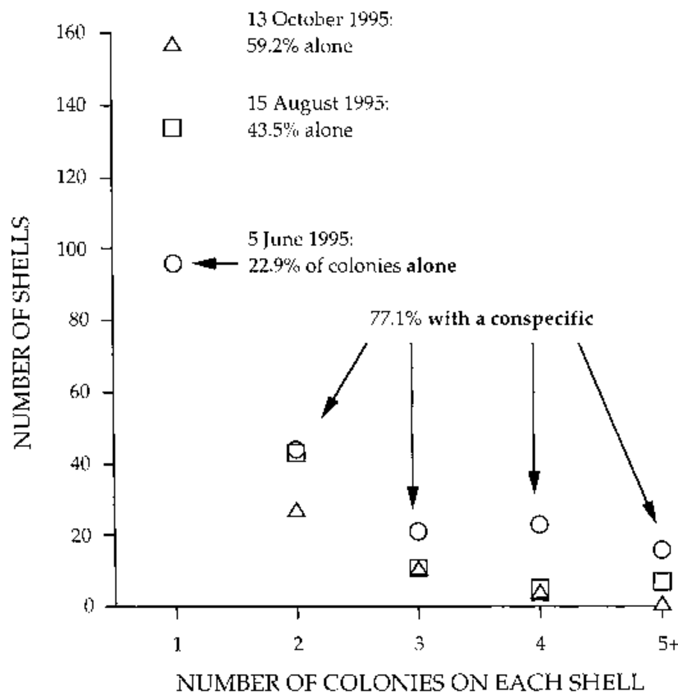

FIG. 1. Numbers of hermit crab–inhabited gastropod shells from Barnstable Harbor, Massachusetts, occupied by one or more than one *Hydractinia symbiolongicarpus* colonies in samples of 200 shells collected on 5 June (circles), 15 August (squares), and 13 October (triangles) 1995.

RESULTS

Frequency of Multiply Colonized Shells

The frequency of shells occupied by more than one hydroid colony declined through the summer of 1995 from about 52% in June to about 21% in October (Fig. 1). However, because many shells had more than two colonies, the proportion of hydroid colonies that occurred with one or more conspecifics was high throughout the summer: about 77% in June, declining to about 40% in October.

Scoring and Distance Measures

We scored 101 polymorphic RAPD bands among 198 *H. symbiolongicarpus* colonies. The number of scored bands per primer varied from 15 (F-06) to 27 (M-02). The mean frequency of these bands among colonies was 0.28 ± 0.24 (SD; range 0.01–0.93). Mean number of bands scored per colony was 28.37 ± 5.08 (range 15–43). These counts and frequencies are typical of other RAPD studies of *Hydractinia* species (Levitan and Grosberg 1993; Grosberg et al. 1996a; D. Levitan and R. Grosberg, unpubl. data)

We evaluated four distance metrics that used information ranging from simple matching based on shared present bands alone (Russell and Rao 1940) to complex matching based on shared present and shared absent bands discounted by bands found in one genotype but not the other (Yule and Kendall 1950). All of these distance metrics range from zero (complete mismatching) to one (complete identity). Only the Nei and Li d appeared normally distributed with a relatively large coefficient of variation (Fig. 2). The Nei and Li d has the additional advantage that it does not incorporate information from the shared absence of bands in two colonies (n_{00}). Shared absence (null alleles) of dominant markers like RAPDs may include multiple allele classes whose sharing does not reflect common ancestry (Levitan and Grosberg 1993; Grosberg et al. 1996a). The distributions of other distance metrics had a pronounced right-hand tail (Yule and Kendall 1950; Apostol et al. 1993), were clustered near one extreme (Russell and Rao 1940; Yule and Kendall 1950), or had a small coefficient of variation < 0.05 (Russell and Rao 1940). For these reasons we used the Nei and Li d in all of the analyses described below.

Genetic Distance and Co-occurrence of Colonies

Mean d between colonies on the same shell (0.460 ± 0.087, range 0.294–0.647) was less than mean d between colonies on different shells (0.506 ± 0.087, range 0.207–0.864). The difference between these means was small, and the distribution of genetic distances between colonies on different shells completely overlapped the distribution of d-values between colonies on the same shell (Fig. 3). Moreover, the smallest genetic distances that we observed were found between colonies on different shells in Barnstable Harbor. These broadly overlapping distributions give the impression that colonies on the same shell are not likely to be close relatives. However, all three of our statistical analyses suggest that this difference is in fact highly significant and that similar genotypes tend to occur together on the same shell.

Matrix Method.—The matrix correlation between d and co-occurrence on shells was strong: Mantel's $r = 2.452$, $t = 7.169$, $P \ll 0.001$ (Table 1). This result indicates that, on average, colonies on the same shell share more RAPD bands than colonies on different shells. Thus, low genetic distances occur more commonly between colonies on the same shell than would be expected if genotypes were randomly distributed among shells with respect to relatedness.

Resampling Methods.—Both resampling methods also indicated greater genetic similarity between colonies on the same shell compared to colonies on different shells.

Among 10,000 bootstrap pseudoreplicates in which colonies were randomly assigned to shells, we found no cases in which the mean d among colonies randomly assigned to the same shell was as small as the mean d among colonies actually found on the same shell in Barnstable Harbor. This strongly suggests that similar genotypes occur together on shells much more often than expected by chance alone.

Among 10,000 jackknife pseudoreplicates in which genetic distances (93) were selected from observed values of d at random without replacement, we found in all cases that the mean d between colonies on the same shell was significantly ($P < 0.05$) less than the mean d between colonies on different shells. This difference was significant at the $P = 0.01$ level in 99.9% of pseudoreplicates and at the $P = 0.001$ level in 96.2% of pseudoreplicates.

One-Sample Tests.—We found 22 cases of 119 (18.4%) in which the single d between a pair of colonies on the same shell was significantly ($P < 0.05$) less than the mean d between one of those colonies and all colonies on other shells. However, the Bonferroni test (Rice 1989) has a critical value

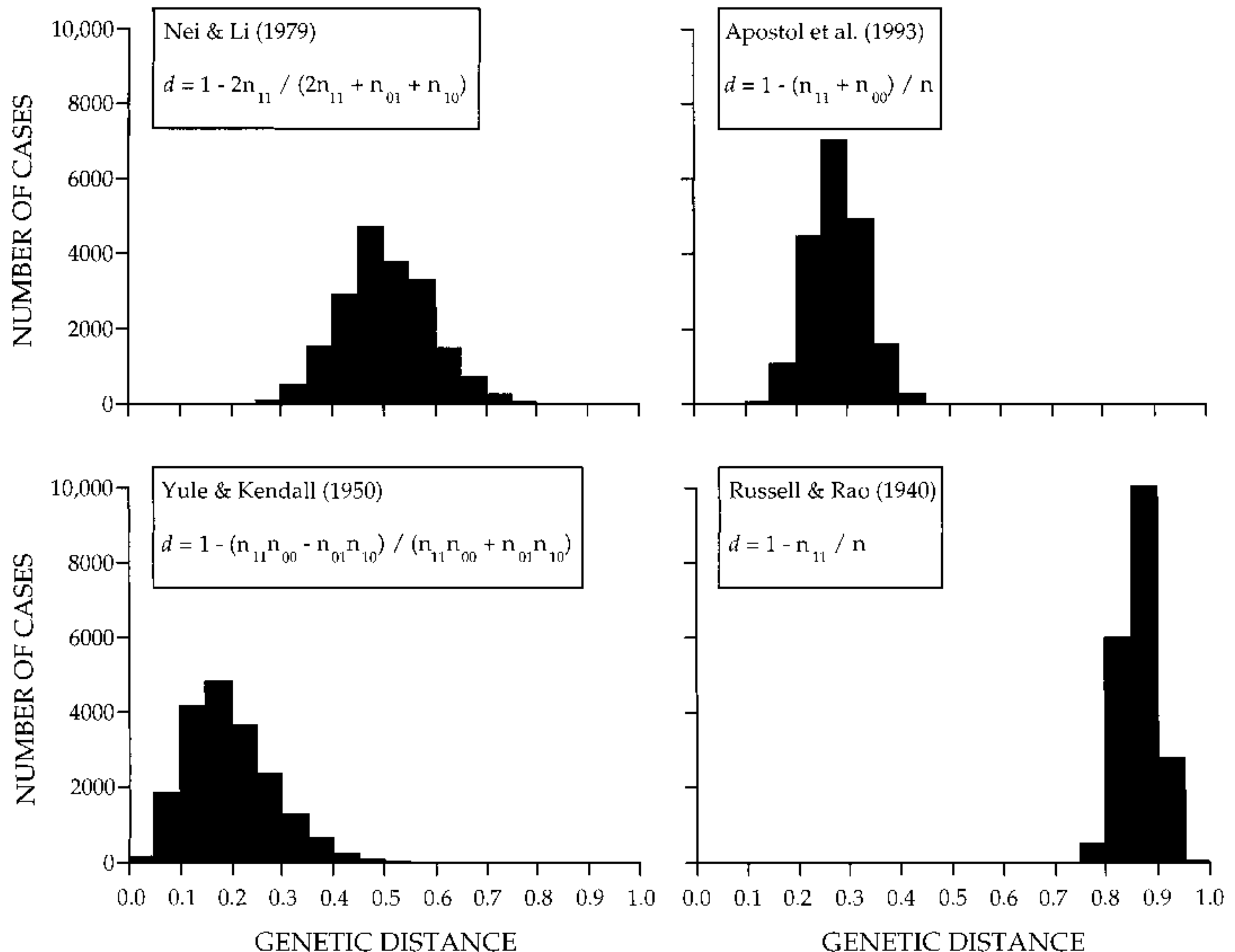

FIG. 2. Frequency distributions of four measures of pairwise genetic distance (d) among 198 *Hydractinia symbiolongicarpus* colonies from Barnstable Harbor, Massachusetts, based on RAPD markers present in each genotype (n_{11}), absent in each genotype (n_{00}), or present in one genotype but not the other (n_{01}, n_{10}).

of $P = 0.05/119 = 0.00042$, and all of the individual P-values associated with these tests were relatively high ($P \geq 0.002$), therefore none of the individual comparisons were judged to be statistically significant. Nevertheless, the matrix test and resampling tests all suggest that there must be some cases of genetically similar colonies on the same shell. Five percent of these 119 comparisons should be significant at the $P = 0.05$ level by chance alone; thus, approximately six of the significant results may be spurious and about 16 (13.4%) are real.

Identifying Full Siblings on the Same Shell

Comparison with Laboratory Full Siblings.—Mean d between known full siblings varied among families from 0.325 ± 0.066 (β family, $n = 105$ pairwise distances) to 0.288 ± 0.067 (γ family, $n = 105$). The mean d for all known full siblings pooled across all five families was 0.306 ± 0.075 ($n = 437$) (Fig. 4). We noted that the grand mean d among all known *nonsibling* pairs (i.e., d between members of different families) in laboratory matings was 0.490 ± 0.084 ($n = 1771$), only slightly less than the mean d observed among colonies on different shells in Barnstable Harbor (0.506 ± 0.087). The standard deviations around these means were all small and similar to each other.

We used the mean d among β full siblings (0.325) as an indicator of the largest amount of genetic difference that might be found among known full siblings. We used this standard for identifying putative full siblings among genetically similar colonies of unknown relatedness in the sample from Barnstable Harbor. By this standard, eight of 119 (6.7%) pairs of colonies on the same shell were judged to be full siblings ($d < 0.325$).

Use of the largest mean d among full siblings (from the β family) could be criticized as too permissive because many full-sibling pairs (from all five families) were genetically more similar to each other ($d < 0.325$) than the average for

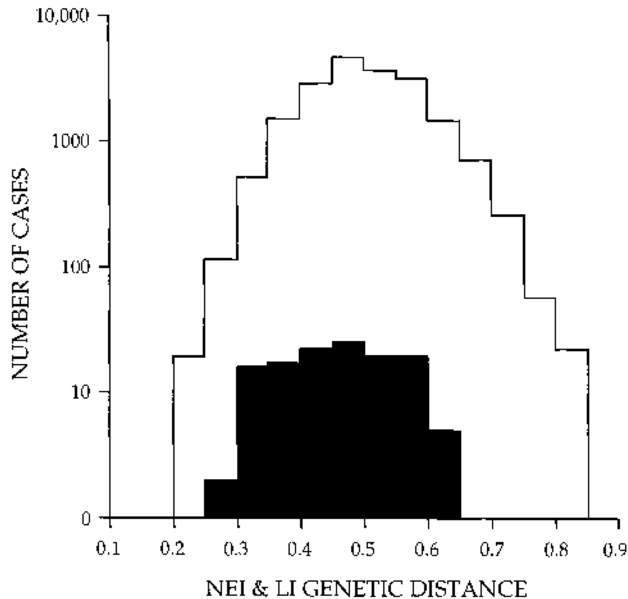

FIG. 3. Frequency distributions of Nei and Li's (1979) d between *Hydractinia symbiolongicarpus* colonies on the same shell (shaded histogram; $n = 119$) or between colonies on different shells (open histogram; $n = 19{,}384$).

TABLE 1. A sample of Mantel's (1967) test of matrix correlation between Nei and Li's (1979) pairwise genetic distances and shell residency of *Hydractinia symbiolongicarpus*. The sample matrices include four shells (labeled 1–4) each with two hydroid colonies (labeled A and B); entries for co-occurring colonies are shown in bold face. Entries in the shell residency matrix are scored 0 for colonies on the same shell or 1 for colonies of different shells.

	Sample Genetic Distance Matrix Colony Identification						
	1A	1B	2A	2B	3A	3B	4A
1B	**0.429**						
2A	0.574	0.569					
2B	0.511	0.469	**0.500**				
3A	0.500	0.400	0.725	0.551			
3B	0.396	0.298	0.500	0.478	**0.404**		
4A	0.547	0.263	0.583	0.652	0.439	0.407	
4B	0.571	0.433	0.725	0.633	0.333	0.404	**0.368**
	Sample Shell Residency Matrix						
1B	**0**						
2A	1	1					
2B	1	1	**0**				
3A	1	1	1	1			
3B	1	1	1	1	**0**		
4A	1	1	1	1	1	1	
4B	1	1	1	1	1	1	**0**

Correlation between the Two Matrices	Mantel's r	t	P
In this example:	0.223	1.593	.056
For all 198 colonies:	2.452	7.169	**≪.001**

pairs of β siblings. The use of an excessively permissive standard could lead to a high rate of Type II error and the misidentification of co-occurring colonies as full siblings when they are not closely related to each other. In fact, the β-family standard is extremely conservative and unlikely to misclassify unrelated pairs of colonies on the same shell in Barnstable Harbor as full siblings. First, this standard actually misclassified 169 of 437 (38.6%) distances between pairs of known laboratory full siblings as nonsiblings (Fig. 4). Second, this standard misclassified only 37 of 1771 (2.1%) known nonsibling pairs as full siblings. Third, this standard misclassified only 314 of 19,384 (1.6%) pairwise distances among colonies on different shells in Barnstable Harbor as full siblings. Such pairs must have been misclassified because we were very unlikely to collect full siblings on different shells by chance alone from a population of thousands of hydroids. Thus, our identification of multiple full-sibling pairs is conservative and may substantially underestimate the frequency of encounters between full siblings in Barnstable Harbor. It is likely that we have misclassified some pairs of full siblings on the same shells as nonsiblings (i.e., the power of our test is low and the Type I error rate is about 38%), and there is only a very small probability that any of the eight cases classified above as full siblings on the same shell are actually misclassified and not close relatives (i.e., the Type II error rate is only about 1–2%).

Cluster Analyses.—Clustering by genetic distance using Ward's minimum variance method produced the branching diagram shown in Figure 5. We found three cases (on shells 57, 87, and 94) in which the clustering analysis indicated that two colonies on the same shell were sister genotypes. All three of these were cases in which d between the sister genotypes was significantly less than mean d between one of them and all other genotypes (see One-Sample Tests above).

The parsimony analysis produced 1000 equally parsimonious trees of 2423 steps. In the consensus of these trees, we found two cases (on shells 87 and 94) in which two colonies on the same shell were sister genotypes.

The clustering analyses indicate close relationships between a few pairs of co-occurring genotypes, but these results were not completely congruent with the analysis of genetic distances among full siblings. We judged only one of the three pairs of sister genotypes (on shell 94) to be a full-sibling pair based on comparison of the genetic distance between genotypes 94A and 94B ($d = 0.316$) to distances among β-family full siblings ($d = 0.325$). The case with the lowest P-value associated with any of the one-sample t-tests (for genotypes 87A and 87B) was not judged to be a pair of full siblings because d between these genotypes was relatively high but much less than most distances between genotype 87A and all other genotypes that we collected from Barnstable Harbor.

Analysis of Rare RAPD Markers

We found 72 RAPD markers that occurred in less than 40% of colonies collected from Barnstable Harbor. Analysis of genetic distances and clustering based on these relatively rare markers produced results concordant with the results above based on all 101 polymorphic bands.

Nei and Li genetic distances were generally larger because pairs of colonies tended to share fewer rare RAPD bands. The mean d between colonies on the same shell was 0.727 ± 0.153; mean d between colonies on different shells was

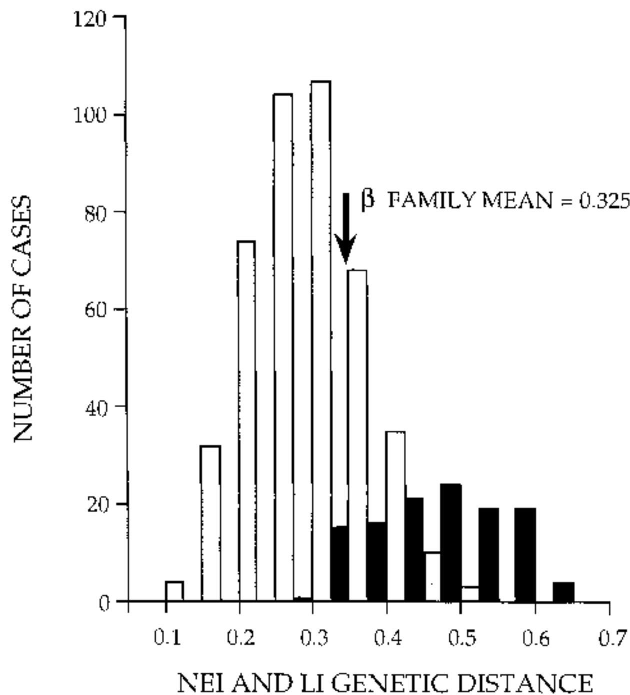

FIG. 4. Frequency distributions of Nei and Li's (1979) d between *Hydractinia symbiolongicarpus* colonies on the same shell in Barnstable Harbor, Massachusetts (shaded bars; $n = 119$) and between full siblings from five laboratory matings of unrelated parents collected from the same population (stippled bars; $n = 437$). The arrow indicates the largest within-family mean d (from the β family).

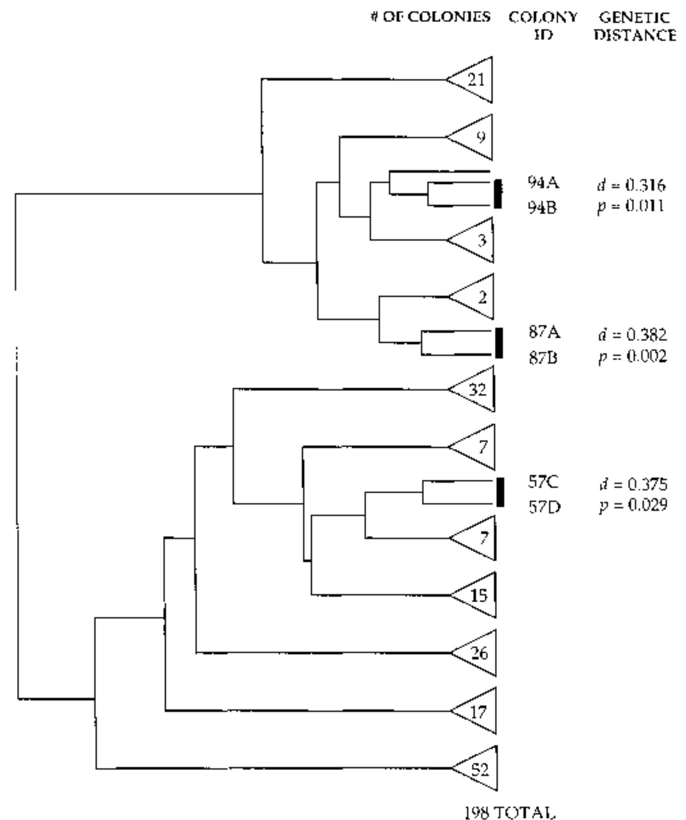

FIG. 5. Clustering of Nei and Li's (1979) d among 198 *Hydractinia symbiolongicarpus* colonies based on Ward's minimum variance method (Wilkinson 1989). Three pairs of colonies found on shells numbered 57, 87, and 94 (marked by vertical bars) cluster together as sister genotypes. Genetic distance (d) between the members of each pair was significantly less than mean d between one of the pair and all other colonies (indicated by the P value from a one-sample t-test).

0.800 ± 0.122. The mean d between full siblings from laboratory families was 0.473 ± 0.148; mean d between nonsiblings was 0.768 ± 0.118. The largest within-family mean d (from the V family) was 0.562. By this standard, we inferred that 18 co-occurring pairs of colonies (15.1%) were full siblings (compared to 6.7% using all RAPD markers). The V-family mean d misclassified many known full siblings as nonsiblings (119 of 437 pairs, or 27.3%), but misclassified few known nonsiblings as full siblings (58 of 1774 pairs, or 3.2%). These Type I and Type II error rates were similar to frequencies based on all RAPD markers (38.6% for known full siblings, 2.1% for known nonsiblings).

Ward's cluster analysis of genetic distances grouped five pairs of co-occurring colonies as sister genotypes (compared to three pairs using all RAPD markers). Three of these five pairs were cases in which d between the sister genotypes was significantly less than mean d between one of them and all other genotypes.

DISCUSSION

Extraordinary genetic polymorphism characterizes the self-nonself recognition systems of many plants and animals. In the vertebrate major histocompatibility complex (MHC), pathogen and parasite defense appear to drive the evolution of allelic diversity through some combination of frequency-dependent selection and overdominance. However, alternative pathogenic and social mechanisms of selection (via the regulation of autoimmunity or mate choice) may also contribute to the evolution and maintenance of genetic diversity at MHC loci (reviewed by Brown and Eklund 1994; Apanius et al. 1997). Similarly, there is strong evidence that selection against inbreeding maintains many rare self-incompatibility (SI) alleles in populations of flowering plants, but this mating function may have been co-opted from an original role of SI markers in disease resistance (Dickinson 1994; Charlesworth 1995). Thus, in both MHC and SI systems, multiple selective processes may have contributed to the evolution of allelic diversity.

In the somatic fusion-rejection systems of colonial marine animals, it is less clear how or if selection currently maintains allotypic diversity (Feldgarden and Yund 1992; Grosberg 1992). In large part, this is because the molecular and genetic basis of allotypic specificity remains unknown; thus, it is difficult to measure the effects of selection on the distribution of allelic frequencies and impossible to infer the phylogenetic relationships among alleles. For example, transpecific genealogies of SI alleles in plants and MHC alleles in animals suggest that many alleles diverged before more recent speciation events (Klein et al. 1993; Ono et al. 1993; Richman and Kohn 1996; Richman et al. 1996) and that similar alleles have been maintained in reproductively isolated species,

probably by selection on allelic function. Comparable evidence for selective maintenance of allotypic diversity is still lacking for colonial marine animals. However, recent studies documenting the potential costs of fusion among colonies (e.g., Stoner and Weissman 1996) and our data indicating that encounters between fusible colonies are more common than expected highlight the need for detailed studies of the molecular genetic basis of allotypic variation.

Close Encounters with Kin

Unlike other sedentary colonial animals with highly specific allorecognition behavior, the hydroid *H. symbiolongicarpus* lives on mobile patches of habitat that should minimize both the frequency of allogeneic contacts between closely related (and therefore somatically fusible) genotypes and the strength of selection favoring rare allorecognition alleles. Instead, we found that close relatives tend to occur on the same gastropod shells. The strongest and most general result is the matrix comparison of genetic distances versus spatial dispersion of genotypes among shells (Table 1), which shows unequivocally that similar genotypes are not randomly distributed among different shells, but instead tend to occur together on the same shell. Moreover, the hitchhiking life cycle of these hydroids should prevent the recruitment of kin other than full- or half-sibling pairs onto the same shell. Thus, genetically similar colonies on the same gastropod shell are actually expected to be siblings.

How many of these instances are potentially fusible full-sibling pairs? Minimally, the results of our clustering analysis suggest that about 2.5% (3/119) of co-occurring pairs are more closely related to each other than to any other genotypes in our sample (Fig. 5). Comparison to genetic distances among known full siblings from laboratory matings suggests that about 6.7% (8/119) of these pairs are full siblings. Maximally, the one-sample comparisons suggest that as many as 18.4% (22/119) of colonies that co-occur with a conspecific are genetically more similar to the co-occurring colony than they are to other genotypes.

Several ecological and behavioral processes before, at, and after the time of settlement could separately or together produce this pattern. First, full-sibling larvae from the same clutch may remain together as they develop, and these siblings may recruit to the shell of the same passing hermit crab. Such philopatry is well known in other colonial and solitary marine invertebrates (reviewed in Knowlton and Jackson 1993). Second, larvae may selectively attach to shells already occupied by close relatives. Other studies of recruitment behavior in tunicates (Grosberg and Quinn 1986) and bryozoans (Keough 1984) show that some larvae preferentially settle near kin, but *H. symbiolongicarpus* larvae apparently do not (Yund et al. 1987). Third, the intensity of postsettlement agonistic interactions between young colonies may vary as a function of the relatedness between contestants (Reeve 1989), as in social insects (Bourke 1997) or vertebrates (Pfennig et al. 1993; Emlen 1997). In sessile organisms, this should promote the co-occurrence of kin and reduce the frequency of associations between more distantly related genotypes.

Did We Underestimate Sibling Interactions?

We estimate that 2–18% of co-occurring pairs of *H. symbiolongicarpus* colonies are full siblings. Three potential biases suggest that we may have understimated the real frequency of full-sibling encounters. First, we measured an empirically high Type I error rate (about 38%) for classifying genetic distances as full-sibling pairs; thus, we may have misclassified up to one-third of co-occurring full-sibling pairs from Barnstable Harbor as nonsiblings. Second, the use of many relatively common RAPD bands for calculating genetic distances may have underestimated the genetic distances among unrelated pairs (on different shells) because such pairs will more often share these common RAPD markers rather than rare markers. We therefore may have overestimated the relative genetic distances between full-sibling colonies on the same shell. Third, some colonies that we treated as single genotypes may have been chimeras themselves. We sampled colonies from Barnstable Harbor in late June, in the middle of the growing season for these hydroids (Yund et al. 1987). Full-sibling juveniles that colonized the same shell early in the settlement season may have initially fused. Such chimeric hydroid colonies are difficult to identify by morphological features, and dominant markers such as RAPDs cannot distinguish these chimeras from single genotypes. However, we expect that a survey of genotypes of single colonies from the same population using codominant markers (such as microsatellite polymorphisms) will reveal the common occurrence of genetically chimeric colonies with more than two alleles at some loci.

At the same time, we have not tried to distinguish possible full-sibling pairs from half siblings. The distinction is potentially significant because fewer half-sibling interactions result in initial fusion, and most of these are transitory fusions that eventually lead to agonistic rejection within a few weeks (Grosberg et al. 1996b). If many of the closely related, co-occurring pairs that we identified in Barnstable Harbor are actually half siblings, then the strength of natural selection acting on the costs and benefits of fusion will depend on when these costs and benefits are exacted during the life cycle. If, in turn, the consequences of fusion between colonies are manifest early in the life of a chimeric colony, then transitory fusions (and consequently half-sibling interactions) are potentially as important as full-sibling chimeras. However, if the consequences of fusion are manifest only late in the life of a chimeric colony, then the costs and benefits of fusion are relevant only to interactions between full siblings because half-sibling fusions typically last less than one to three weeks. Unfortunately, the time course of costs and benefits of chimera formation are currently unknown for *Hydractinia* or any other colonial invertebrate.

Selection Past or Present?

The high levels of allotypic diversity characteristic of every population of clonal invertebrate so far studied could reflect both the influence of natural selection in contemporary populations as well as evolutionary forces (including selection) that acted on populations in the past. Fusion between colonies should be common when levels of allorecognition polymorphisms are low (Parham and Ota 1996), and the as-

sociated costs of fusion should be exacted frequently. Consequently, to the extent that fusion is costly, selection will favor allorecognition specificity and the accumulation of rare allotypes that are incompatible with the majority of the population (Grosberg and Quinn 1988; Yund and Feldgarden 1992). As polymorphism accumulates, the incidence of fusion will decline and the strength of selection favoring rare allotypes will also decline (Apanius et al. 1997). Moreover, as levels of allotypic diversity increase, the likelihood increases that fusible individuals (sharing many allorecognition alleles) will also be close relatives. Therefore, the costs of fusion will be discounted by the relatedness between the fused pair, and fusions will become restricted to rare interactions between clonemates or close relatives (Buss and Green 1985; Grosberg and Quinn 1986; Grafen 1990).

Barring any ecological or behavioral processes (such as philopatry or kin recognition) that promote the spatial association of kin, the ongoing maintenance of high standing levels of allorecognition polymorphism in a well-mixed population is probably not caused by selection acting on the costs and benefits of such rare fusion events. Such polymorphism must instead be explained by: (1) selection acting in the past; (2) nonselective processes such as mutation and migration; (3) the inheritance of ancestral allorecognition polymorphisms; or (4) the pleiotropic effects of selection acting on phenotypic traits other than somatic interactions among colonies.

This scenario presupposes that populations are well mixed and that the probability of interactions between compatible allotypes depends mainly on their frequencies in the population. However, if populations exhibit genetic structure at the same spatial scale at which individuals interact, then fusion frequencies may be much higher than predicted under a random-encounters model (Grosberg and Quinn 1986; Ratnieks 1991; Grosberg et al. 1996b). *Hydractinia symbiolongicarpus* exhibits kin structure at the level of colonies occupying the same small habitat patches on which fusions and rejections occur. This unexpected correspondence between dispersion and genetic similarity makes fusion (and its consequences) more common than expected given the life history of this hydroid. Thus, the scope for selection to act on the evolution and maintenance of somatic allorecognition polymorphism is potentially large.

Alternatively, allotypic polymorphism underlying specificity in somatic interactions may instead be a pleiotropic effect of negative frequency-dependent selection acting on gametic interactions between mates as a barrier to inbreeding (Oka 1970; Fuke 1983; Lenington et al. 1994). However, we have looked for and found no evidence of gametic incompatibility as a function of somatic compatibility in brother-sister matings (R. Grosberg, unpubl. data). Thus, consanguineous matings are possible, but F_{IS}-values (based on isozyme allele frequencies) give no evidence of inbreeding in the Barnstable Harbor population (R. Grosberg and B. Cameron, unpubl. data) and, given the size and mixing of the adult hydroid populations, opportunities for such matings must be rare. For these reasons, we doubt that this pleiotropic effect is strong enough to be measured or to influence the evolution and accumulation of rare allorecognition alleles in *H. symbiolongicarpus*.

Consequences for the Evolution of Allorecognition Behavior

The observation that closely related individuals frequently interact and that such interactions involve potentially costly behaviors such as fusion and fighting suggests that selection acting directly on the specificity of these behaviors plays an important role in the maintenance of allotypic diversity. What are the opportunities for this selection to act in nature?

Gastropod shells colonized by *H. symbiolongicarpus* colonies frequently bear more than one conspecific. We found more than half of occupied shells with two or more *H. symbiolongicarpus* colonies early in 1995, which declined to about 20% of occupied shells late in the year (Fig. 1). Because many shells had more than two colonies, a large proportion of hydroid colonies in these samples occurred with a conspecific on the same shell: about 77% of colonies in the early sample, declining to about 40% of colonies in the late sample. These estimates are similar to previous counts of shell use by *Hydractinia* (Yund et al. 1987).

These counts of shell use show that a large proportion of hydroid colonies in natural populations have the opportunity to interact with a conspecific during their lives. Of these encounters, we conservatively estimate that 2–18% involve full-sibling pairs. The proportion of these encounters that result in permanent fusion between full siblings varies among families from 15% to 50% (Grosberg et al. 1996b; D. Levitan and R. Grosberg, unpubl. data). The product of these proportions gives some indication of the frequency of fusions in natural populations. For example, if only 40% of all individuals encounter a conspecific on the same shell, the frequency of full-sibling encounters is in the lowest range of our estimates (2%), and those families consist of few somatically compatible genotypes (15%), then only about 0.12% of individuals in this population may experience the costs or benefits of life as a chimeric colony.

Alternatively, if many (77%) colonies encounter conspecifics, and if the true frequency of full-sibling encounters (18%) and compatible genotypes among those full siblings (50%) are in the highest range of our current estimates, then as many as 6.9% of individuals may form long-lasting chimeras. These estimates ignore the potential importance of short-lived chimeras (transitory fusions) in which full or half siblings fuse for a few days or weeks and then reject each other. These two estimates of the frequency of chimera formation (0.12–6.9%) encompass an earlier estimate for another *Hydractinia* species based on counts of fused colonies of opposite gender (Yund and Parker 1989).

Direct identification of chimeras in nature, using codominant markers, could be used to test these predicted frequencies. Our observation of genetic structure in this population on the scale of individual habitat patches suggests that the true frequency of chimeras will be nearer the larger of these two numbers (6–7%). Whether this frequency of chimera formation is sufficient to promote the evolutionary maintenance of multiple rare allorecognition alleles will depend on the selection differential between single-genotype and multiple-genotype colonies.

Although several *Hydractinia* species colonize hermit crab-occupied shells, other species (reviewed in Buss and Yund 1989), such as the eastern Pacific *H. milleri*, live in extensive

aggregations on rock faces in the lowest intertidal zone (Fraser 1937). These aggregations appear to consist of large numbers of closely related genotypes (D. Levitan and R. Grosberg, unpubl. data). Such aggregations may develop by the philopatric recruitment of larvae in the neighborhood of the parents. The potential for interactions between closely related and perhaps somatically compatible colonies must be considerably higher in *Hydractinia* species with this life history. Such abundant interactions could potentially favor the evolution of extreme allorecognition polymorphism, even more diverse than the approximately five loci and five to seven alleles per locus that confer allorecognition specificity in *H. symbiolongicarpus* (Grosberg et al. 1996b). A comparative analysis of habitat use and allorecognition behavior, based on a phylogeny for *Hydractinia* species, could be used to reconstruct the evolutionary history of self-nonself recognition in these species and to understand the interaction between evolutionary changes in habitat use and the specificity and genetic basis of allorecognition behavior.

A full understanding of allorecognition polymorphism in colonial invertebrates ultimately depends on the cloning and characterization of allorecognition loci and their allelic variants (as in the well-known MHC and SI systems). Specific knowledge of allelic diversity will lead to at least three important improvements. First, fusion-rejection behavior in somatic interactions can be related to the allotypes of the contestants, and this relation should reveal any possible correlation between sequence divergence and competitive success. Second, competing hypotheses about selective processes, especially somatic fusion-rejection behavior versus gametic compatibility and inbreeding, can be evaluated by comparing these pleiotropic effects in competitive or reproductive interactions between known genotypes. Third, the genealogies of allorecognition alleles will help to reveal the potential significance of different selective processes in the maintenance of allelic diversity. Transpecific genealogies imply either selective maintenance of functionally divergent alleles in different species or the evolution of allelic diversity via past processes independent of current selection (Parham and Ota 1996), whereas monospecific genealogies would suggest that selection acts in contemporary populations mainly on allelic frequencies (rather than differences in allelic function).

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