

Kin or self-recognition? Colonial fusibility of the bryozoan *Celleporella hyalina*

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SUMMARY We estimated fusion frequency with respect to coancestry in the bryozoan *Celleporella hyalina*, whose briefly planktonic sexually produced larvae settle on algal substrata and proceed to form encrusting colonies by iterative budding. Frequency of fusion between paired colonies growing on an artificial substratum was positively correlated with coefficient of relatedness, with allorecognition ability increasing during the early stages of colonial growth after larval settlement. Parents repressed the growth of F₁ progeny with which they

had fused. The results are concordant with the Feldgarden-Yund model of selection for self-recognition, which regards fusion with kin as an inevitable source of error whose cost diminishes with increasing relatedness. Contrary to fusion compatibility, gametic compatibility is negatively correlated with coancestry, indicating a need for further research on the possibility of common or linked genetic control that has opposite effect at somatic and gametic levels.

INTRODUCTION

Life histories of several major aquatic invertebrate taxa, notably Porifera, Cnidaria, Bryozoa, and Tunicata, are characterized by the sexual production of motile larvae that settle, metamorphose, and initiate asexual reproduction by iterative budding. In many species, the asexual offspring remain interconnected, forming a colony of modules (polyps or zooids) genetically identical to each other and to the larva from which they were ultimately derived. The colony therefore represents a genetic individual, or genet sensu Harper (1977), whose genome is replicated among the component modules and may become further replicated if the colony itself divides through natural or artificial processes.

Modular colonies typify many marine and some freshwater benthic communities, where they often compete for space (Buss 1990). Close neighbors may engage in competitive overgrowth (Jackson 1977) or tissue destruction (Lang 1973), both processes leading to the demise of the loser or at least to a stand-off. In addition to overt competition between colonies, the insidious process of cell-lineage parasitism, in which the somatic and/or germ cell lineage of one partner replaces that

of the other, may ensue if colonies should fuse into a chimera (Buss 1982, 1987; Sabbadin 1982; Stoner et al. 1999). Colonial fusion, tissue destruction, and perhaps even competitive overgrowth are based on allorecognition, which therefore assumes importance in protecting the interests of the genet. Allorecognition systems are consequently to be expected in all benthic colonial invertebrates (Grosberg 1988). In some species (Neigel and Avise 1983a,b; Wulff 1986) fusion is apparently restricted to tissues of the same genet (complete matching), occurring when edges of the same colony grow into juxtaposition or when edges of a previously fragmented colony grow together again. In other species, when larvae settle close together, fusion also occurs between different genets if they share kinship (partial matching) but with decreasing frequency as the coefficient of relatedness decreases (Sabbadin 1982; Grosberg et al. 1996; Mokady and Buss 1996).

Partial matching could be selectively advantageous if the ratio of benefit gained from increased colony size (Hughes and Jackson 1985; Wulff 1986) to cost through risk of cell-lineage parasitism (Buss 1982, 1987) remains high for close sexual relatives, falling progressively faster for more distantly related colonies (Grosberg 1988). The selection model of par-

tial matching depends on frequent interaction within families of genets, arising in benthic invertebrates from philopatric larval settlement (Jackson 1986), possibly combined with kin recognition, allowing siblings to preferentially settle close to each other. Although aggregation of kin occurs in some species (Keough 1984; Grosberg and Quinn 1986; Hart and Grosberg 1999), others settle randomly (Hoare and Hughes 2001). Thus, it might be predicted that partial matching will be found among species typified by aggregated kin settlement and complete matching among those typified by random settlement with respect to kin. On the other hand, the high allelic polymorphism characteristic of invertebrate allorecognition systems may have evolved in response to selection for fusion with self rather than kin (Feldgarden and Yund 1992). By restricting fusion to self, allorecognition eliminates the risk of cell-lineage parasitism while reaping the benefits of increased size and colonial integrity when growing fragments regain contact and more secure attachment when colonial edges meet as they wrap around three-dimensional substrata (Feldgarden and Yund 1992).

Strong evidence for partial matching has been adduced for species whose life history and/or larval behavior promote kin recognition (Grosberg and Quinn 1986; Grosberg et al. 1996; Hart and Grosberg 1999); however, comparable studies have been lacking for species unlikely to experience kin recognition. Partial matching in such latter species would support the Feldgarden-Yund model of error-prone self-recognition.

We conducted fusibility tests on pedigree families of the cheilostome bryozoan *Celleporella hyalina*, known to settle randomly with respect to kin (Hoare and Hughes 2001) and to frequently experience colonial fragmentation due to flexion of algal substratum (Cancino 1983; Manríquez 1999). Herein, it will be convenient to define coancestry and family structure solely in terms of genets. The term “family” therefore will refer to a set of colonies, ignoring modules (zooids) within those colonies. Each family is comprised of a maternal genet (egg provider), one or more paternal genets (sperm providers), and their F_1 progeny of sibling or half-sibling genets derived from sexually produced larvae.

METHODS

Experiment 1: fusion of mature colonies

Colonies of *C. hyalina* were collected from the Menai Strait, Anglesey (53°16'S, 4°5'W) and induced to release larvae by using the dark–light reaction (Ryland 1960). Acetate sheet that had been steeped in seawater for 4 weeks to develop a biofilm was provided for larval settlement. Postmetamorphic colonies were fed on the microflagellate *Rhinomonas reticulata* and after growing to a suitable size were propagated by taking cuttings (detailed in Manríquez et al. 2001). Each genet therefore was represented by a set of replicate colonies. Genets A, D, E, J, M, and Q were established from larvae released by colonies collected in April and genets N and S

from those collected in October 1996. All the above genets proved to be incapable of producing viable offspring by self-fertilization but were sexually compatible with each other (Manríquez 1999; Manríquez et al. 2001). Sibling and half-sibling colonies were established as follows. Colonies of *C. hyalina* contain both male and female zooids and normally function as self-sterile simultaneous hermaphrodites (Hughes et al. 2002). Virgin colonies from genet A were exposed to allosperm obtained by light-induced release from colonies of genet E and presented at a minimum concentration of $10 \mu\text{l}^{-1}$ (Manríquez et al. 2001). Larvae subsequently released by the mated colonies were settled on acetate and the postmetamorphic colonies propagated as above to generate a set of n sibling genets AE1–AE n , where A and E refer to the maternal and paternal genets, respectively. Other colonies from genet A were exposed to allosperm from genet M to produce the sibling set AM1–AM n . The procedure was repeated for maternal genet Q mated with paternal clones D and J to yield the sibling sets QD1–QD n and QJ1–QJ n . Each family therefore was comprised of a maternal genet, two paternal genets, and two sets of sibling genets, which were half-sibs of each other. Genets N and S were selected as being unrelated to families 1 and 2.

To stage fusion trials, cuttings 30–40 autozooids in size were taken from the above genets and glued in pairs onto conditioned 4.5×7.5 -cm acetate sheets. Each member of a pair was orientated with its meristem facing toward and 500–600 μm distant from that of the other. Each sheet was pinned by a 2.4×7.5 -cm glass microscope slide into the slots of a plastic histology rack. Histology racks, each carrying 12 sheets, were housed in 2-l plastic bottles containing aerated, 0.2- μm filtered, UV-irradiated seawater (FSW). *Rhinomonas reticulata* was added daily to maintain a food concentration of about 100 cells μl^{-1} , and the water was changed every 2 days. Temperature was maintained at 15–16°C, with a photoperiod of 12-h dark:12-h light.

Once having grown into contact, paired colonies were examined weekly under a binocular microscope at 40 \times magnification. Colonial fusion was recognized by the appearance of coalescent zooids (sensu Craig 1994) along the contact zone (Fig. 1). Each coalescent

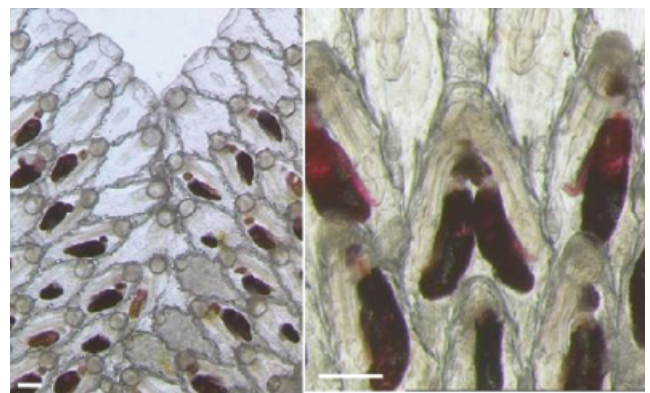


Fig. 1. (Left) Zone of contact between adjacent colonies of identical genotype. (Right) A coalescent zooid formed along the contact zone, showing two stomachs linked to a common orifice. Dark red coloration within stomachs is due to ingested *Rhinomonas reticulata* (Cryptophyceae: Cryptomonadida). Scale bars, 100 μm .

zooid is formed by the fusion of a pair of budding autozooids or of basal male zooids within the contiguous meristems of adjacent colonies, producing a bifid zooecium with two proximal ends, budded separately in the adjoining colonies, which converge into one distal end with a single orifice. Presence of coalescent zooids is a conservative criterion for fusion because contiguously parallel zooids might become integrated by funicular connections through the lateral pore plates (Bobin 1977) without visible modification of the zooecium (Craig 1994). Intercolonial pore plates, however, may differ morphologically from intracolony plates (Ishii and Saito 1995; Shapiro 1992). Moreover, although intercolonial pore plates facilitate neurological integration of colonies of *Membranipora membranacea*, they appear not to be involved in metabolic translocation (Shapiro 1996). The presence of coalescent zooids, on the other hand, shows that full colony integration must have occurred. Fusion trials were conducted on the coancestry classes self: self (coefficient of relatedness [c.r.] = 1), mother: F₁ offspring (c.r. = 0.5), father: F₁ offspring (c.r. = 0.5), sib: sib (c.r. = 0.5), half-sib: half-sib (c.r. = 0.25), and unrelated pairs (c.r. < 0.25), using genets listed in Table 1. The experiment was terminated after 17 weeks, when the number of basal zooids was counted in each pair of colonies and in isolated control colonies of each genet. The size ratio of the smaller to the larger colony was computed to reveal any interactive effect on postcontact growth, distinction between fusion partners remaining visible at the suture zone. As controls, genets

were also represented by colonies grown simultaneously in isolation and the ratio of fused to isolated colonial size calculated.

Experiment 2: fusion of young colonies

Four sets of sibling genets and two of half-siblings were generated by mating A × E, A × M, Q × D, and Q × J, where the second genet in each pair was the sperm donor. Unrelated genets were obtained by mating genets N × S. Having developed late-stage embryos, mated genets were subjected to dark–light treatment to induce larval release. Individual larvae were pipetted into approximately 500-μl settlement chambers, each constructed from an Eppendorf microtube lid fitted with a small disc of conditioned acetate and glued to a transparent plastic tray. Once a larva had settled, its position on the acetate disc was mapped and another larva of selected parentage was pipetted into the chamber and allowed to settle. Settlement units were then placed in a vessel containing 6 l FSW, to which *R. reticulata* was added daily. After 1 week, acetate discs bearing pairs of healthy postmetamorphic colonies were removed from the settlement chambers and glued individually to 4.5 × 7.5-cm conditioned acetate sheets. The sheets were placed 12 per histology rack and the colonies cultured as in experiment 1. Fusion trials were conducted on the coancestry classes sib: sib, half-sib: half-sib, and unrelated pairs. Pairing of

Table 1. Experiment 1: genet pairings used in fusion trials

	Self Self	Mother Offspring	Father Offspring	Sib Sib	Half-Sib Half-Sib	Unrelated
Family 1	AM1:AM1- AM6:AM6 AE1:AE1- AE3:AE3 A:A E:E M:M	A:AM1- A:AM6 A:AE1- A:AE3	E:AE1- E:AE3 M:AM1- M:AM6	AM1:AM2- AM1:AM6 AM2:AM3- AM2:AM6 AM3:AM4- AM3:AM6 AM4:AM5- AM4:AM6 AM5:AM6	AM1:AE1- AM1:AE3 AM2:AE1- AM2:AE3 AM3:AE1- AM3:AE3 AM4:AE1- AM4:AE3 AM5:AE1- AM5:AE3 AM6:AE1- AM6:AE3	N:AM1- N:AM6 N:AE1- N:AE3 S:AM1- S:AM6 S:AE1- S:AE3
Family 2	QD1:QD1- QD4:QD4 QJ1:QJ1- QJ3:QJ3 D:D Q:Q J:J	Q:QD1- Q:QD4 Q:QJ1- Q:QJ3	D:QD1- D:QD4 J:QJ1- J:QJ3	QD1:QD2- QD1:QD4 QD2:QD3- QD2:QD4 QD3:QD4 QJ1:QJ2- QJ1:QJ3 QJ2:QJ3	QJ1:QD1- QJ1:QD4 QJ2:QD1- QJ2:QD4 QJ3:QD1- QJ3:QD4	N:QD1- N:QD4 N:QJ1- N:QJ3 S:QD1- S:QD4 S:QJ1- S:QJ3

A pair is denoted by letters separated by a colon; a series of pairs is denoted by hyphenation. Each genet, propagated into the required number of colonies, occurs in a number of different pairings. Family 1 is comprised of maternal genet A, paternal genets M and E, six sibling genets (AM1–AM6) from mating between A and M, and three sibling genets (AE1–AE3) from mating between A and E; AM1–AM6 are half-siblings of AE1–AE3. Equivalent labeling defines family 2, derived from mating between maternal genet Q and paternal genets D and J. Unrelated genets N and S were used for pairings with members of families 1 and 2.

coancestry classes requiring colonial propagation was precluded by the need to grow colonies directly from metamorphosed larvae.

Two factors known to influence the growth characteristics of contiguous bryozoan meristems are the relative sizes of colliding colonies (Buss 1980, 1982; Winston and Jackson 1984; Harvell and Padilla 1990; Stocker 1991; Nandakumar and Tanaka 1997) and the angle of contact between zooids (Jackson 1979; Karande and Swami 1988; Turner and Todd 1994). Both factors can be discounted in the present experiments because all colonies were similar in size to their partners at initial contact, whereas fusion and rejection were assessed only once opposing zooids lay in parallel. The recorded frequencies of fusion and rejection therefore are a true reflection of treatment effects.

Field samples

Fronds of *Laminaria saccharina* bearing colonies of *C. hyalina* were collected in May and June 1999 from the Menai Strait, Anglesey, UK (53°16'S, 4°5'W). Sections of frond, in total bearing 36 pairs of contiguous colonies, were excised by scalpel. Each section was placed in a Petri dish containing 5 ml of FSW that was dosed with *R. reticulata* and replaced daily. Algal tissue was removed as it decayed, and by the end of the third week the colonies had become detached from their substrata. The contact area was examined microscopically for evidence of fusion, as above.

RESULTS

Experiment 1: fusion of mature colonies

Perimeters of paired colonies met within 4–6 weeks, when the budding zooids in the short tangential zone were brought into distal contact. The opposing zooids grew away from the acetate, using each other's undersurface as substratum to form a low ridge. As the zone of contact lengthened, budding zooids met at a progressively shallower angle, eventually touching in parallel along their lateral walls. At this stage, 6–8 weeks after initial contact, three responses were apparent. First, ridge formation continued along the entire contact zone, indicating allogeneic rejection. Second, ridge formation ceased once buds lay in parallel, obliterating any physical

distinction between colonies along the contact zone but without the formation of coalescent zooids. This outcome was unassignable to fusion or rejection and therefore was recorded as indeterminate. Third, coalescent zooids appeared, indicating colonial fusion. Frequencies of rejection, indeterminacy, and fusion were not significantly different between families (family \times response, $\chi^2_2 = 1.838$, $P = 0.399$; family \times coancestry, $\chi^2_4 = 0.729$, $P = 0.948$) and were identical for mother–offspring and father–offspring pairings. Based on pooled families, percentage frequencies of fusion, indeterminacy, and rejection, respectively, were as follows: self:self, 95.7%, 4.3%, 0% ($n = 23$); parent:F₁ offspring, 93.7%, 6.3%, 0% ($n = 32$); sib:sib, 91.7%, 8.3%, 0% ($n = 24$); half-sib:half-sib, 30.0%, 20.0%, 50.0% ($n = 30$); unrelated, 0%, 3.2%, 96.8% ($n = 31$).

All colonies grew at similar rates except where F₁ offspring had fused with their parents, in which case the parental colonies grew normally whereas growth of the offspring was impaired. There was no significant difference in final size ratio between mother:F₁ and father:F₁ pairings ($t_{30} = 0.341$, $P = 0.735$). Size ratio of experimental colonies versus isolated controls (Table 2) was significantly smaller in offspring than in parental colonies but was not significantly different between maternal and paternal colonies (one-way analysis of variance for data pooled across families, $F_{2,61} = 13.560$, $P < 0.001$; planned contrasts: maternal vs. F₁ offspring $P < 0.001$, paternal vs. F₁ offspring $P < 0.001$, maternal vs. paternal $P = 0.784$). Parental colonies therefore reduced the postfusion growth of their F₁ offspring.

Experiment 2: fusion of young colonies

Paired colonies made contact after about 2 weeks. Frequencies of rejection, indeterminacy, and fusion were not significantly different between families (family \times response, $\chi^2_2 = 2.452$, $P = 0.294$; family \times coancestry, $\chi^2_2 = 0.014$, $P = 0.993$).

Pooled across families, frequencies of fusion, indeterminacy, and rejection were, respectively, as follows: sib:sib, 89.4%, 8.5%, 2.1% ($n = 47$); half-sib:half-sib, 77.1%, 8.3%, 14.6% ($n = 48$); unrelated, 6.3%, 25.0%, 68.7% ($n = 48$).

Table 2. Experiment 1: postfusion size ratio in parent–offspring pairings

	Fused Colonies	Parental Control	Offspring Control
Family 1			
Mother–offspring	0.5789 (0.1014), 9	0.9294 (0.0143), 9	0.5674 (0.1004), 9
Father–offspring	0.5022 (0.0922), 9	0.8893 (0.0292), 9	0.4511 (0.0901), 9
Family 2			
Mother–offspring	0.5671 (0.1219), 7	0.8503 (0.0476), 7	0.6117 (0.1371), 7
Father–offspring	0.7514 (0.1270), 7	0.8502 (0.0330), 7	0.7346 (0.1225), 7

Fused Colonies are calculated as the number of basal zooids in the smaller colony relative to that in the larger. Parental Control is the postfusion size of the maternal or paternal colony relative to another colony from the same genet grown in isolation. Offspring Control is the postfusion size of the offspring relative to another colony from the same genet grown in isolation. Families are defined in Table 1. Values are means, with standard errors in parentheses and numbers of replicates listed after the comma.

Comparison of experiments 1 and 2

Response frequencies of mature and young colonies were similar for sibs (experiment \times response, $\chi^2_2 = 0.521$, $P = 0.771$), whereas for half-sibs fusion was significantly more frequent among young colonies ($\chi^2_2 = 17.110$, $P < 0.001$). For unrelated genets, indeterminacy was significantly more frequent among young colonies ($\chi^2_2 = 9.219$, $P = 0.010$).

Field samples

Separate colonies could be recognized by their distinct centers of origin (an ancestrula surrounded by a characteristic pattern of early daughter zooids, from which radial growth is established). All pairs of contiguous colonies remained interlocked after their algal substratum had disintegrated. The ridged border between colonies and absence of coalescent zooids, however, indicated allogeneic rejection in all cases. Coalescent zooids occurred within all colonies, indicating isogeneic fusion where meristems had grown into contact.

DISCUSSION

Observed differences in response frequency between young and mature colonies (see comparison of experiments 1 and 2, above) suggest that at 2 weeks after metamorphosis, when most colonial contacts were made in experiment 2, the development of immunocompetence was still incomplete. Maturation of immunocompetence is delayed for several weeks after metamorphosis in the hydroid *Hydractinia symbiolongicarpus* (Fuchs et al. 2002) and in the scleractinian coral *Pocillopora damicornis* (Frank et al. 1997). A shift in allogeneic discrimination during colonial development can be predicted theoretically in terms of a decreasing ratio of benefit to cost (Reeve 1989). This prediction may apply, for example, to several species of alcyonarian coral whose larvae aggregate at settlement and proceed to form chimeras (Barki et al. 2002). Random larval settlement in *C. hyalina*, however, greatly reduces opportunity for allogeneic fusion during the phase of incomplete immunocompetence, which itself may simply reflect developmental constraint.

Parental repression of the postfusion growth of F_1 allotypes (experiment 1) cannot readily be explained in terms of coancestry because the coefficient of relatedness is the same as for sibling fusion, in which no postfusion effect on growth was observed. To our knowledge, this phenomenon has not been reported for any other organism yet was a consistent experimental result.

The observed decrease in fusion frequency between sibs through half-sibs to unrelated colonies of *C. hyalina* is qualitatively predictable from the Mendelian inheritance of recognition alleles (Scofield et al. 1982; Grosberg 1988; Grosberg et al. 1996) and the operation of a partial matching rule.

Assuming that histocompatibility requires at least one shared allele at a polymorphic recognition locus, as occurs in *Botryllus schlosseri* (Oka and Watanabe 1957; Sabbadin 1962, 1982), that sexual progeny results from the mating of heterozygous parents in outcrossing populations and that fusion and rejection are the only possible allogeneic reactions, trials in experiment 1 would be expected to yield fusion/rejection frequencies of parent–offspring 100/0%, sibling 75/25%, and half-sibling 50/50%. Rejection frequencies were indeed as expected for parent–offspring and half-sib pairings but were higher than expected for sib pairings. Contrary to the above assumption of binary response, it remains possible that indeterminacy was a valid category representing allogeneic reaction intermediate between fusion and rejection, as occurs in *H. symbiolongicarpus* (Grosberg et al. 1996). Also contrary to the *Botryllus* model, a multilocus allorecognition system may operate in bryozoans, again as has been proposed for *H. symbiolongicarpus* (Grosberg et al. 1996).

Larval settlement of *C. hyalina* shows no sign of kin recognition (Hoare et al. 1999), and at least in the population studied, larval dispersal promotes outcrossing (Hoare et al. 1999; Goldson et al. 2001), whereas experimentally enforced inbreeding results in severe loss of fitness (Hoare and Hughes 2001). Fusion among siblings therefore is probably so rare as to have little evolutionary significance in the experimental population. In contrast, isogeneic fusion probably is almost ubiquitous in *C. hyalina* because it enables colonies to complete transition from the fan shape determined by early patterns of budding (Cancino and Hughes 1988) to the circular form of later stages. Isogeneic fusion could also serve to reunite colonial fragments after flexion fracture and is probably a general feature of cheilostome bryozoans, potentially occurring whenever meristems of the same genet grow into contact (Hastings 1979).

Histocompatibility data for other bryozoans are less robust. All studies report isogeneic fusion (Moyano 1967; Stebbing 1973; Humphries 1979; Chaney 1983; López Gappa 1989; Craig 1994; Ishii and Saito 1995; Shapiro 1996). Allogeneic fusion, however, was previously known only for two species. Chaney (1983) reported fusion among the F_1 progeny of one colony of *Thalamoporella californica* (i.e., sibs and/or half-sibs) but found rejection among unrelated colonies. In *Fenestrulina* sp., Craig (1994) recorded fusion in 13 of 27 pairs settled on artificial substratum in the field and concluded that either colonial fusion is not determined genetically in *Fenestrulina* sp., that larvae settle next to kin, or that little genetic variation was present in the sampled population.

The above considerations suggest that the adaptive function of allorecognition in *C. hyalina* is to discriminate self from nonself rather than different levels of kinship. Partial matching perhaps originally evolved in the context of gametic compatibility, serving as a mechanism for avoiding inbreeding and then was co-opted, with reversed effect, to govern fusi-

bility. Evidence for common genetic control of fusion and gametic compatibility, however, is equivocal: rejection frequency increases from 0% among sibs to 50% among half-sibs; gametic compatibility, expressed as embryo production, increases from 28% among sibs to 38% among half-sibs (Hoare and Hughes 2001); allosperm received from sibs has no effect on intracolony sex ratio, whereas allosperm from half-sibs increases femaleness just as strongly as that from unrelated partners (Hughes et al. 2002). Moreover, Grosberg and Hart (2000) showed that fusion and gametic compatibility in *H. symbiolongicarpus* are unlikely to share the same genetic system. Alternatively, allogeneic fusion among a proportion of kin could simply reflect errors incurred by an intrinsically constrained allorecognition system (Feldgarden and Yund 1992; Grosberg et al. 1996). We are aware of no mechanism whereby fusion might prevent inbred mating between cosettled relatives in *C. hyalina*, as has been described in some populations of the ascidian *Botryllus schlosseri* (Sabbadin 1979). In any case, cosettlement of relatives probably is too rare to be of evolutionary significance in outcrossing populations of *C. hyalina*.

We conclude that partial matching is likely to be a general characteristic of fusion compatibility in modular colonial invertebrates, driven primarily by selection operating on an error-prone genetic system for self-recognition that is perhaps constrained by derivation from a gametic function selected to reduce inbreeding. The possibility of a common genetic system, or linked systems, governing fusion and gametic compatibility deserves further investigation, promising to elucidate the early evolutionary history of allorecognition itself (Oka 1970; Burnet 1971; Weissman et al. 1990).

Acknowledgments

This work was funded by Natural Environment Research Council grants GR/12896 (to R. N. H., J. D. D. B., P. H. M.), GR3/11355 (to R. N. H., D. Atkinson, S. M.), and GR9/03614 (to R. N. H., S. F. C.). P. H. M. is indebted to M. T. Baiges for help with rearing colonies. We thank Rick Grosberg and Michael Feldgarden for constructive comments. Any errors remain ours.

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