usually four in *I. temusirotis* and up to five in *E. huenei*. The forefin is about half the length of the mandible, as in *I. temusirotis*, whereas in *E. huenei* it is relatively longer, often exceeding the length of the mandible. There are 12 elements in the longest digit (commencing with the epipodial13), which is within the observed range for *I. temusirotis*, but there are usually more in *E. huenei* (up to 23). Fin structure, however, is often variable within species, especially in *E. huenei*.

Aside from its longer snout, the skull of *E. costini* is similar to that of *I. temusirotis* (compare Figs. 2b, a); this results in their having similar orbital ratios, but their other mandibular ratios, and their cranial ratios, are disparate (Table 1). However, if the snout of *I. temusirotis* is increased in length until the ratio of skull length/mandible length corresponds to that of *E. costini*, their mandibular and mandibular ratios converge (Table 1). *E. costini* could therefore be derived from *I. temusirotis* by an elongation of the snout.

*E. costini* is cranially similar to *E. huenei*, except in having a relatively longer mandible (Fig. 2c); this is reflected in the similarity of their cranial ratios, and the dissimilarity of their mandibular ratios (Table 1). However, if the mandible of *E. costini* is reduced to 300 mm, giving the same value for the ratio mandible length/skull length as in *E. huenei* (0.51), its mandibular ratios converge with those of *E. huenei* (Table 1). *E. huenei* could therefore be derived from *E. costini* by mandibular reduction.

If *E. huenei* evolved from a long-snouted ancestor like *I. temusirotis*, through an intermediary like *E. costini*, what mechanism might have been involved? A change in growth rates of the rostrum and mandible during ontogeny seems plausible, and some insight is gained by studying ontogeny in *Xiphias*. Larval swordfish have mandibles extending to the tip of the skull17-19 whereas in adults the mandible is only about one-third of the skull length. These profound changes are effected by relatively small differences in growth rates between the mandible and rostrum (C. McGowan, in preparation). A small increase in the growth rate of the rostrum in an ichthyosaur like *I. temusirotis* could therefore give rise to a descendant like *E. costini* with an elongated rostrum. This, in turn, could give rise to *E. huenei* by a reduction in the growth rate of the mandible. Such minor modifications would presumably require relatively small genetic changes, but the morphological, and consequent biological, changes are, he feels, greater.

I thank M. A. Taylor for bringing the new material to my attention and also thank him, S. Swansborough and M. L. K. Curtis for their help in Bristol. I thank J. Mulock for his drawings; P. Purves for typing; R. Johnson and J. Thomason for reading the manuscript; C. E. McGowan for nomenclatural inspiration; and J. Campise for classical advice. This research was supported by the Natural Sciences and Engineering Research Council of Canada, grant A 9550.

The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate

Richard K. Grosberg* & James F. Quinn

*Department of Zoology and Division of Environmental Studies, University of California, Davis, California 95616, USA

The evolution of altruism, cooperation and sociality should be favoured by mechanisms promoting interactions amongst relatives. In turn, the opportunity for such interactions should be enhanced where related individuals are spatially associated. The simplest explanation for association of kin involves philopatry, or limited, dispersal. Alternatively, kin recognition—which is known from a broad array of taxa—can produce similar associations. Neither the prevalence of kin recognition, nor aggregations of kin, are by themselves sufficient to demonstrate that kin recognition plays an important role in the production of nonrandom associations of relatives. To have such a role, kin recognition must promote or inhibit associations of kin beyond the effects of other processes, notably dispersal, that modify spatial patterns. Here we report the results of field experiments showing that sibling planktotonic larvae of the sessile colonial ascidian *Botryllus schlosseri* settle in aggregations that are much stronger than expected from dispersal distance effects alone. Laboratory experiments indicate that larvae distinguish kin on the basis of shared alleles at a highly polymorphic histocompatibility locus known to regulate fusion between adult colonies. This kin recognition mechanism, along with limited dispersal of larvae, promotes co-settlement of histocompatible individuals. Consequently, the probability of fusion between adult colonies is far greater than that expected if larvae settled randomly.

*Botryllus schlosseri* colonies are founded after a sexually produced planktotonic tadpole larva attaches to a firm substratum and metamorphoses. Repeated cycles of asexual multiplication produce a colony of morphologically and genetically identical zooids, connected by a blood vascular system. As in many colonial marine invertebrates, the larvae of several oviparous ascidians can metamorphose soon after release into the plankton. In the case of *B. schlosseri*, our laboratory and field observations, as well as those of others10, demonstrate that larvae can metamorphose—and many do—soon after escaping from their natal colony.

To determine the contribution of larval settlement behaviour to the spatial arrangement of sibling adult colonies, we examined the recruitment pattern of sibling colonies (<4 weeks old) that were founded by larvae, all derived from the same cross, and carrying a rare genetic marker. We identified the marker from an electrophoretic survey of 512 colonies living on the side of the Marine Biological Laboratory Supply Dock in the Eel Pond at Woods Hole, Massachusetts, USA. At the phosphoglucom isomerase locus (PGI), five electrophoroms occurred, one of which (PGI-fast) was present in only two of the colonies surveyed. We cross-fertilized these two colonies, then assayed the F1 colonies for PGI-fast homoygotes. One PGI-fast homozygote was selected as the source colony for marked larvae, and was positioned at the centre of a 1-m diameter circular asbestos-cement panel horizontally suspended 0.5 m below the dock. All zooids in a sexually mature *B. schlosseri* colony ovulate synchronously approximately weekly. Just before each ovulation, we returned the source colony to the laboratory, where we mated it with a sibling colony homoygous for the same marker allele, then repositioned the source colony on the panel. We left the panel in position for 4 weeks, then mapped and removed all *B. schlosseri* colonies that had recruited. Colonies were frozen at -80 °C, then analysed electrophoretically. We mapped the panel before any recruited colonies had reached sexual maturity; thus,
colonies homozygous for the PGI marker allele were assumed to be siblings originating from the source colony at the centre of the panel. Recruits not carrying the marker allele must have immigrated.

Figure 1a and b show respectively maps of marked and unmarked colonies. To test for spatial association among siblings, we computed the Clark and Evans' index of aggregation \( R \) based on nearest-neighbour distances between sibling colonies. \( R \) is the mean nearest-neighbour distance observed, divided by that expected if dispersion were random. Thus, randomly arranged individuals are expected to yield a value for \( R \) of 1.0. Values of \( R \) significantly <1 indicate aggregation, whereas values >1 indicate overdispersion. The sibling colonies were aggregated as tested by Clark and Evans' significance criteria \( R = 0.769; P < 0.001 \). The unmarked immigrant colonies were significantly overdispersed \( R = 1.227; P < 0.001 \). This overdispersion could result from larval behaviour or could simply be an artefact of spacing due to colony growth. In any case, the degree of aggregation of siblings differs even more from that of immigrants than it does from random (t-test; \( P < 0.001 \)).

Figure 1a shows that sibling colonies are clustered around the source colony, perhaps because B. schlosseri larvae can settle upon release into the plankton. To separate any aggregation caused by sibling recognition from that due to limited dispersal, we constructed a null model based on a bootstrap analysis of the nearest-neighbour data. The locations of colonies were expressed in polar coordinates with the origin at the centre of the source colony: pseudosamples with the same distributions of distances and directions were chosen independently and randomly, with replacement, from the actual coordinates of sibling colonies. The randomized distance and direction coordinates are thus independent in the pseudosamples. This randomization procedure should preserve in the pseudosamples the overall aggregating effect of dispersal distance alone. However, smaller-scale aggregations due to interactions between immediate neighbours will be broken up by the randomization of the angular coordinates. For 500 randomizations, we calculated the value of \( R \) produced by the random model. In all cases, the values of \( R \) under the random model were substantially greater (mean = 0.897; s.d. = 0.022; range 0.824–0.964) than the observed \( R = 0.769 \). This analysis demonstrates that limited dispersal explains only about half the deviation of mean nearest-neighbour distance from that expected if settlement were random.

The histocompatibility system known to control fusion and rejection between adult Botryllus colonies may provide a mechanism by which larvae recognize their siblings. Histocompatibility is controlled by a single mendelian locus such that two colonies sharing one or both alleles can fuse by their blood vascular systems. When colonies do not share an allele, rejection, accompanied by necrosis at the site of allogenic contact, occurs. Fusion frequencies among pairs of Botryllus colonies collected in the field have been reported to range from 4 to 8% (refs 18, 19). In our studies on the Marine Biological Laboratory Supply Dock, of 500 pairs of colonies collected haphazardly along a 20-m transect, 22 pairs (4.2%) fused. According to the method of Curtis et al., at equilibrium, approximately 100 equally frequent histocompatibility alleles would produce the fusion frequency observed in the Eel Pond population. Given such a high level of polymorphism at the histocompatibility locus, the likelihood that any two colonies will share a histocompatibility allele should depend primarily on their pedigree relatedness.

Because the settled colonies in the field experiments were all full siblings, shared alleles at loci other than the histocompatibility locus could have been used for sibling recognition. Accordingly, we designed laboratory experiments to test whether larval settlement location is better predicted by the histocompatibility type or the relatedness of resident individuals. With a breeding programme using colonies of known histocompatibility genotypes (determined by fusion assays), in a factorial design, we varied independently the effects of pedigree relatedness and histocompatibility genotype of larvae and residents on distance between settled larvae and previously attached residents. Thus, there were four experimental treatments: (1) the residents and larvae were inbred, and shared one histocompatibility allele; (2) the residents and larvae were inbred, but did not share a histocompatibility allele; (3) the residents were fourth-generation outbred descendants of the parents of the larvae, with one shared histocompatibility allele; and (4) the same as (3), but residents and larvae did not share a histocompatibility allele.

In outline, the breeding programme was as follows: for treatments (1) and (2), homozygous resident colonies of known histocompatibility genotype (AA) were derived from crosses between heterozygous sibling colonies (AB). Unfusible homozygous colonies (BB) were isolated from the same cross. In treatment (1), introduced larvae (AB) were derived from a cross between the AA and BB full-sibs. In treatment (2), introduced larvae (BB) were derived from a BB × BB cross from the same sibship used to generate the larvae in treatment (1). Therefore, the introduced larvae in treatments (1) and (2) bear the same pedigree relatedness to the residents, but differ in the presence of AA or BB alleles.
Table 1 Two-way analysis of variance on the distance between settled larvae and the nearest resident colony as a function of resident/larval relatedness and histocompatibility types

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>F value (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatedness</td>
<td>572</td>
<td>1</td>
<td>1.35 (&gt;0.25)</td>
</tr>
<tr>
<td>Histocompatibility type</td>
<td>3,708.5</td>
<td>1</td>
<td>87.46 (&lt;0.001)</td>
</tr>
<tr>
<td>Replicate</td>
<td>59.8</td>
<td>1</td>
<td>0.47 (0.63)</td>
</tr>
<tr>
<td>Relatedness/histocompatibility</td>
<td>174.1</td>
<td>1</td>
<td>4.10 (0.04)</td>
</tr>
<tr>
<td>Error</td>
<td>9,964.6</td>
<td>235</td>
<td></td>
</tr>
</tbody>
</table>

n = Number of observations for each treatment.

Table 2 Summary of the mean distances between settled larvae and the nearest resident colony according to experimental treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (mm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings, no shared alleles</td>
<td>14.70</td>
<td>60</td>
</tr>
<tr>
<td>Siblings, one shared allele</td>
<td>7.50</td>
<td>60</td>
</tr>
<tr>
<td>Outbred, one shared allele</td>
<td>5.15</td>
<td>60</td>
</tr>
<tr>
<td>Outbred, no shared alleles</td>
<td>13.92</td>
<td>60</td>
</tr>
</tbody>
</table>

of an A histocompatibility allele. For treatments (3) and (4), either AA or BB larvae were introduced into Petri dishes with outbred residents carrying an A allele. These residents were derived after sequential outcrossing with different colonies taken from the wild, none of which carried either an A or B allele. After each generation, a colony carrying the A allele was identified (with a fusion assay) and used for subsequent matings. After four generations of outcrossing, single zooids from a heterozygous colony carrying the A histocompatibility allele, and an unknown second allele (differing from B), were used as residents.

For each treatment, we established three replicates, each with 20 resident colonies. The resident colonies were found by attaching single zooids from a colony of known genotype to random positions on the submerged undersurfaces of the tops of 150-mm plastic Petri dishes. Twenty-four hours later, the dishes were filled with seawater, sealed and kept in the dark. We then introduced 20 larvae through a port in the side of each dish according to the above treatments. One day later, we measured the distance from each settled larva to the nearest resident.

The results of an analysis of variance show that the relatedness of residents and larvae has no discernible effect on nearest-neighbour distances (Table 1). In contrast, where larvae and residents shared a histocompatibility allele, the larvae settled significantly closer to the residents (Table 1). Indeed, the mean nearest-neighbour distance between larvae and residents sharing a histocompatibility allele is roughly half that observed when no allele is shared (Table 2). Table 1 shows a marginally significant interaction between the two main effects that is difficult to interpret. However, the minor contribution that this interaction makes to the overall sum of squares suggests that the interaction is biologically unimportant in these experiments.

Our experiments do not eliminate the possibility that larval kin recognition and histocompatibility are closely linked, rather than identical, genetic traits. However, because larval kin recognition increases the likelihood of adjacent colonies being fusible—an otherwise improbable event given the polymorphism of the histocompatibility locus—there are functional grounds for expecting the two processes to be controlled by a single locus. The promotion of co-settlement of histocompatible colonies, coupled with the restriction of fusion to closely related genotypes, indicate that colony fusion may be beneficial, but only among kin. In colonial organisms such as Botryllus, fusion may benefit one, or both, members of a chimaera in several ways. First, colony fusion immediately increases colony size. Survivorship is known to be size-dependent in several colonial ascidians (refs 21, 22 and our unpublished observations), sponges23,24, ctenophores25-27 and ectoparasites28-30; hence, fusion may reduce the likelihood of total mortality26,29. Second, if the onset of reproduction is size-dependent—as it appears to be in several ctenophores27 and ascidians30—then fusion may lower the age at first reproduction of both members of the chimaera29. In colonial organisms where growth rate is size-dependent23,33, fusion may also increase the probability of subsequent survival and reproduction. Indeed, fusion among juvenile colonial organisms is well known30,33. Third, a chimaera may have different physiological attributes from either component colony, potentially increasing the range of environmental tolerance33. Finally, fused botryllid ascidians (like many other clonal taxa33,35) freely exchange cells that can differentiate into gametes22, and the rate of differentiation may vary genetically35.

Under these conditions, somatic cell parasitism, in which one member of a chimaera increases its reproductive output at the expense of the other, can occur22. However, because the polymorphism and genetics of the Botryllus histocompatibility system limit fusion almost entirely to closely related individuals, the parasitic losses, in evolutionary terms, would be considerably less than if the fused colonies were unrelated39,79.

Another potential consequence of close association of kin is increased inbreeding. Laboratory studies suggest that self-fertilization is deleterious in B. schlosseri and, in some congeners, symbiosis may not occur if sperm and egg carry the same histocompatibility allele40,42. However, there is no evidence of inbreeding depression in the Eel Pond population of Botryllus schlosseri43. Indeed, studies of other marine invertebrates in which closely related individuals are likely to cross-fertilize provide no evidence for inbreeding depression41,44.

Botryllus schlosseri appears to be one of the first species where a functional—and perhaps evolutionary—link between kin recognition, historecognition and the enhancement of colony fusion has been experimentally demonstrated. The prevalence of limited dispersal and historecognition systems among sessile clonal marine invertebrates32 suggests that such a link may be found in other clonal organisms. Recent studies of congeneric mice show that haplotypic differences in the H-2 region affect mating behaviour45. The presence of such markers could mediate kin recognition in other taxa. Whether the study of marine invertebrates will provide useful insights into the more complex dynamics of kin recognition in mobile, social vertebrates is unclear, but the analogies remain intriguing.

This research was funded by NSF grant OCE 84-07158 to R.K.G. The Committee on Research of the University of California at Davis provided additional support. We thank P. Sherman and M. Marthas for their useful comments.

Received 17 January; accepted 24 June 1986.

Genetically restricted suppressor T-cell clones derived from lepromatous leprosy lesions

Robert L. Modlin*, Hideyuki Katô, Vijay Mehra†, Erica E. Nelson**, Fan Xue-dong†, Thomas H. Rea†, Paul K. Pattengale* & Barry R. Bloom†

Section of *Dermatology and †Department of Pathology, University of Southern California School of Medicine, Los Angeles, California 90033, USA
‡Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, USA

Leprosy is a spectral disease in which immune responses to Mycobacterium leprae correlate with the clinical, bacteriological and histopathological manifestations of disease1,2, so study of its pathology provides insights into immunoregulatory mechanisms in man. At the tuberculoïd pole, patients have few lesions in the skin which contain rare organisms and are able to mount strong cell-mediated immune responses to M. leprae antigens. In contrast, at the lepromatous pole, patients have disseminated skin lesions containing large numbers of acid-fast bacilli and are selectively unresponsive to antigens of M. leprae. M. leprae-induced suppressor clones derived from peripheral blood have been reported to be active in vitro3, yet their in vivo significance has remained unclear. Because the focal point of the immune response to M. leprae is the skin lesion consisting of lymphocytes and macrophages, we have recently developed methods for isolating lymphocytes from skin biopsies of leprosy patients. We report here that two T8 clones derived from lepromatous leprosy skin biopsies, in the presence of lepromin, suppress concanavalin A (Con-A) responses both of peripheral blood mononuclear cells and of T4 clones in an HLA-D (HLA, histocompatibility locus antigen)-restricted manner. Moreover, these T8 clones suppressed responses of HLA-D-matched, but not HLA-D-mismatched antigen-responsive T4 clones to M. leprae antigens, indicating that T-cell suppression is major histocompatibility complex (MHC)-restricted at some level in man.

Initial immunohistological studies of skin lesions from patients across the spectrum revealed that cells bearing the T8 (Leu 2a) phenotype were present in excess in lepromatous granuloma as compared with tuberculosis3–7, because the surface markers do not necessarily reflect lymphocyte function within lesions8, simple procedures were developed to extract $10^5$–$10^7$ lymphocytes from biopsies of leprosy skin lesions12. The phenotypic distribution of these cells as determined by fluorescence-activated cell sorter (FACS) analysis was similar to that obtained using immunoperoxidase staining of the tissue sections (T4: T8 = 0.5:1 in lepromatous and T4: T8 = 2.0:1 from tuberculoïd lesions). In lepromatous patients, the T4/T8 ratio of extracted cells was significantly different from that in blood, indicating that contamination from peripheral blood was negligible. Because we assumed that the lymphocytes in the lesions may be involved in specific immune reactivity and were likely