Evolution, 39(4), 1985, pp. 724–732

THE PRECISION OF HISTOCOMPATIBILITY RESPONSE IN CLONAL RECOGNITION IN TROPICAL MARINE SPONGES

JOSEPH E. NEIGEL[1] AND JOHN C. AVISE
Department of Genetics, University of Georgia, Athens, GA 30602

Abstract.—Recently discovered histocompatibility-like phenomena in sponges (Phylum Porifera) have prompted attempts to measure the precision with which allogeneic grafts are recognized and rejected. The results of these investigations have been extremely varied, ranging from suggestions that allorecognition does not occur to suggestions that every genetically distinct individual may be unique in histocompatibility type. Interpretation of these findings is complicated by the variation in methods and species used by different workers. Here we compare various measurements of allorecognition precision for several species of tropical marine sponges. From our results we conclude that 1) tissue implant grafts are more prone to artifact than grafts between intact sponges; 2) the possibility of clonal propogation should be considered when graft acceptances are observed between sponges selected from a single population; and 3) allozyme variation in *Niphates erecta* shows that occasional grafts between genetically different individuals may be accepted.

Received September 13, 1984. Accepted April 15, 1985

The capacity of sponges to distinguish cells of their own species from those of other species has been studied in the context of cell reaggregation for more than 75 years. However, only in the last 15 years has it generally been recognized that sponges may also be capable of much finer distinctions within species. Reports of precise discrimination in intraspecific grafts have led some to interpret allorecognition in sponges as an immunological phenomenon homologous to vertebrate histocompatibility (Hildemann et al., 1979, 1980). The immunological interpretation of graft rejection suggests a working hypothesis: in sponges (as in vertebrates) virtually every genetically different individual may possess a unique histocompatibility type (Hildemann et al., 1980; Hildemann and Linthicum, 1981; Kaye and Ortiz, 1981; Curtis et al., 1982). The question of allorecognition precision has assumed added significance with recent applications of histocompatibility bioassays in attempts to identify clones in natural sponge populations (Jokiel et al., 1982; Curtis et al., 1982; Neigel and Avise, 1983; Neigel and Schmahl, 1984).

Allorecognition precision is defined here as the proportion of grafts between individuals that produces a response visibly distinct from that seen in grafts between parts of the same individual, or between individuals known to be genetically identical. In this operational definition, the ability of the observer to distinguish graft responses can be a critical factor. Furthermore, allorecognition precision defined in this way is a property dependent on relationships between grafted sponges as well as on the particular molecular basis of the underlying recognition system. That the precision of allorecognition cannot be separated from the context of population structure has not always been fully appreciated.

Whereas some previous studies have indicated little or no capacity of sponges for allorecognition (Moscona, 1968; van de Vyver, 1970; Curtis, 1979; Curtis et al., 1982), others have suggested a very high degree of precision (Hildemann et al., 1979, 1980; Jokiel et al., 1982; Neigel and Avise, 1983). Choices of both species and techniques have varied among these studies, so it has not been possible to eliminate either factor as the source of conflicting results. The experiments reported here were specifically intended to address the problem of measuring precision of allorecognition in grafts between tropical marine sponges. Since

[1] Present address: Department of Microbiology and Immunology, University of California, Los Angeles, CA 90024.

several different concepts and methodological approaches were involved, we have organized methods, results, and discussion together under four separate topic headings. The sponges used in our investigations were *Agelus scraptrum, Aplysina fistularis, Ectyoplasia ferox, Haliclona rubens* and *Iotrochota birotulata* in Discovery Bay, Jamaica; *Aplysina cauliformis* in St. Croix, U.S. Virgin Islands; and *Niphates erecta* in Upper Matecumbe Key, Florida. Critiques of previously published information on precision of graft response are also presented, where appropriate.

Grafting Methods

In general, two grafting techniques have been employed in investigations of allorecognition precision in sponges. Some studies have used *implant grafts,* in which a block of tissue is removed from a donor and implanted into an incision or cavity in the recipient. Other studies have employed *parabiotic grafts,* in which the intact external surfaces (pinacoderms) of sponges are placed in contact, either by allowing sponges to grow together from explants or gemmules, or by tying together intact parts of sponges. In such parabiotic grafts, there is usually no real distinction between donor and recipient. Reports of imprecise allorecognition in sponges, in most instances, have involved the implant technique (Moscona, 1968; Curtis et al., 1982), while results suggesting precise allorecognition have usually been obtained with parabiotic methods (Hildemann et al., 1979, 1980; Jokiel et al., 1982; Neigel and Avise, 1983; Neigel and Schmahl, 1984). In one case, this contrast in outcomes is evident in experiments with a single species (Moscona, 1968; Simpson, 1973; McClay, 1974).

In our experiments, we have compared results of implant grafts and parabiotic grafts for three reef sponges: *Iotrochota birotulata, Agelus scraptrum,* and *Ectyoplasia ferox.* Implants were made by removing a plug of tissue with a one-half inch diameter brass cork borer and inserting the plug into a hole bored in the same manner. Parabiotic grafts were made by tying together intact segments of branches (see Neigel and Avise, 1983). All grafts were scored "blind" with respect to expected outcomes.

Iotrochota birotulata.—A total of 29 implant grafts were made; each was replicated by a parabiotic graft. Both types of grafts were scored after four days. Fourteen of the parabiotic grafts were scored as "fusions" or acceptances, and 15 were scored as "non-fusions" or rejections (Neigel and Avise, 1983). Five of the implant grafts apparently became dislodged and lost. Of the remaining 24, all appeared healthy and could be readily scored. The responses assigned to the implant grafts corresponded exactly to those assigned the parabiotic grafts.

Agelus scraptrum.—When scored after 13–19 days, parabiotic grafts yielded two responses: acceptance ($N = 9$), in which the pinacoderms of donor and host fused; and rejection ($N = 34$), in which no fusion occurred and the opposed surfaces of donor and host hardened relative to the surrounding tissue. Implant grafts generally died within a few days, and could not be scored.

Ectyoplasia ferox.—Highly anomalous results in graft allorecognition have been reported for this species by Curtis et al. (1982). In seven of 62 cases, non-reciprocity in implant graft response was observed (i.e., sponge A accepted a graft from sponge B, but B rejected a graft from A). Unfortunately, none of the grafts was replicated to allow distinction between true non-reciprocal compatibility and technical artifact.

For our experiments with *E. ferox,* we located the group of sponges studied by Curtis et al. (1982) in Discovery Bay, Jamaica, using the map provided in their paper and by finding tags that remained attached to a few of their grafts. Our grafting experiments with *E. ferox* were conducted in August, as were the experiments of Curtis et al. (1982).

Both implant grafts and replicate parabiotic grafts of *E. ferox* were made to

provide two sets of autografts and 13 reciprocal combinations between sponges. At 5–7 days, the grafts were scored independently by each of the authors. At that time, it was very difficult to recognize distinct graft responses. The appearance of about half of the grafts seemed unchanged since they were first made. Both implant and parabiotic grafts of one of the autograft combinations were tentatively scored as non-fusions. For seven grafts between sponges, the two scorers disagreed on provisional assignment of response. Among the remaining experiments, the responses designated for two pairs of grafts were non-reciprocal; and for two combinations implant grafts were scored as non-fusions while parabiotic grafts were scored as acceptances.

The grafts were scored again at 15–27 days. At this time, results of the parabiotic grafts were internally consistent: autografts were scored as acceptances, and all reciprocal responses were in agreement. Changes in scores included grafts that initially appeared to be fusions rescored as rejections, and initial rejections later scored as fusions. Implant grafts yielded different results. In the later scoring period, four of the implant grafts appeared to have died. One was a self-graft (autograft), and another involved two sponges that had formerly yielded graft acceptances in the reciprocal implant and replicate parabiotic grafts.

Overall, these results with several species of sponges suggest that parabiotic grafts are generally more reliable than are implant grafts, although in some species (e.g., *Iotrochota birotulata*) either technique may be adequate. The implant graft technique involves exposing relatively large areas of tissue that are normally beneath the pinacoderm to the external environment. This treatment may severely traumatize tissues near the graft interface. Because rejection responses in sponges are often characterized by a lack of fusion, effects of trauma may be mistaken for graft rejection. The implant method used by Curtis et al. (1982) appears to be especially prone to artifact. The length of time allowed by Curtis and his co-workers for graft responses to develop in *E. ferox* (maximum of 7–9 days) appeared to be insufficient with either parabiotic or implant grafts. In our experiments with this species, failure of some implant grafts to fuse with host after longer periods of time probably resulted from tissue trauma rather than allogeneic interactions.

Allografts and the Problem of Population Structure

The most commonly used approach for estimating allorecognition precision in sponges has been to observe the proportion of rejection responses in artificial grafts between individuals (Moscona, 1968; Curtis, 1979; Hildemann et al., 1979, 1980; Kaye and Ortiz, 1981; Jokiel et al., 1982). Because the dispersal of progeny is likely to be limited, the size of the area over which individuals are selected for grafting may be critical. However, the spatial extent of the population sampled has varied considerably among investigations of this type. For example, Hildemann and co-workers (1979, 1980) used grafts between sponges collected from "widely separated areas of Kaneohe Bay, Hawaii" and found no acceptances in 1,400 grafts. In contrast, Kaye and Ortiz (1981) grafted sponges 10 meters or less apart and found many acceptances. Least informative are those results reported without any specification of the spatial extent of the population sampled (e.g., Moscona, 1968; Curtis, 1979).

Asexual reproduction by colony fragmentation is common in sponges (Bergquist, 1978) and is expected to result in the formation of localized clones of individuals, members of which would presumably accept grafts from one another. Under the hypothesis that allorecognition is perfectly precise, the proportion of rejection reactions should approach 100% as the distance between grafted sponges becomes great enough to exclude individuals belonging to the same clone. We have examined the relation-

TABLE 1. Frequency of graft rejection as a function of donor-recipient distance in several species of marine sponges. Numbers of scored grafts are indicated in parentheses.

Species	Distance (meters)			Maximum distance for observed acceptance (meters)	N
	<1	1–10	>10		
Haliclona rubens	0.36 (83)	0.65 (88)	0.99 (99)	17	(270)
Aplysina fistularis	0.41 (22)	0.91 (33)	1.00 (9)	2.1	(64)
Aplysina cauliformis (St. Croix)	0.69 (36)	0.90 (84)	0.88 (17)	12.9	(137)
Aplysina cauliformis (Jamaica)	0.09 (22)	0.46 (68)	1.00 (68)	10.0	(158)
Agelus screptrum	0.33 (9)	0.91 (32)	1.00 (2)	1.7	(43)
Iotrochota birotulata	0.45 (94)	0.99 (96)	1.00 (30)	2.7	(220)
Niphates erecta	0.30 (20)	0.77 (61)	— (0)	5.0	(81)

ship between the proportion of parabiotic grafts rejected and the distance between grafted individuals for six species of sponges (Table 1).

For these species, the observed proportion of grafts accepted declined to zero with distances ranging from 1.7–17 meters. At lesser distances, both acceptances and rejections were observed. Little is known about typical dispersal distances of larvae for most sponge species, but duration of the free swimming stage is typically on the order of a day or longer for subtidal sponges (Bergquist et al., 1970; Bergquist, 1978). Unless larvae were released in unusually still water, a free planktonic stage of this duration should disperse larvae considerably more than 17 meters from point of release. Thus, it is unlikely that the graft acceptances between nearby sponges represent a sibling or close genetic relationship other than clonal identity. The demonstration that acceptances take place primarily or exclusively between spatially adjacent individuals is necessary, but not sufficient, evidence that they are clonemates.

Genetic Models and Transitivity of Graft Acceptances

A second prediction of the hypothesis that allorecognition in sponges is precise is that relationships defined by graft acceptances will be transitive (if A = B and B = C, then, by transitivity, A = C). Individuals that accept grafts from one another because histocompatibility determinants exactly match (whether or not they differ at genetic loci not involved in histocompatibility) should agree in responses to grafts from additional individuals. In contrast, relationships of similarity (rather than identity) may be intransitive. One example of relationships defined by similarity involves the genetic control of histocompatibility in a colonial tunicate, *Botryllus schlosseri.* Two colonies are "similar" enough to fuse if they share one allele at a single genetic locus controlling histocompatibility (Scofield et al., 1982).

Grafts that define all relationships among sets of three or more individuals can be used to test for transitivity. The strength of this test is that it does not require a priori judgments regarding which individuals are likely to be clonemates. In this study we tested for transitivity in three species (*Agelus screptrum, Haliclona rubens,* and *Niphates erecta*), and results are shown with other information from the literature in Table 2. The data for the Barbados population of *Aplysina cauliformis* were retrieved

TABLE 2. Transitivity of histocompatibility relationships in sponge populations.

Population	Transitive			Intransitive	Reference
	A = B A = C B = C	A = B A ≠ C B ≠ C	A ≠ B A ≠ C B ≠ C	A = B A = C B ≠ C	
Agelus screptrum (Jamaica)	2	3	6	0	present study
Haliclona rubens (Jamaica)	15	27	4	0	present study
Niphates erecta (Florida)	4	3	5	0	present study
Iotrochota birotulata (Jamaica)	5	12	58	0	Neigel and Avise (1983)
Aplysina cauliformis[1] (Barbados)	192	383	1,443	0	Kaye and Ortiz (1981)
Aplysina cauliformis[1] (Jamaica)	10	24	148	0	Neigel and Schmahl (1984)
Aplysina cauliformis[1] (St. Croix)	2	38	200	0	Neigel and Avise (1983)

[1] Formerly *Verongia longissima.*

from a matrix of graft comparisons given in Kaye and Ortiz (1981). In a total of 2,584 tests with these five species, no instances of intransitivity have been observed.

Tests for the transitivity of identity relationships cannot detect all forms of imprecision in allorecognition, but they are potentially quite powerful. Any "noise" in the expression of allorecognition, such as might be generated by errors in scoring grafts, inhibition of graft rejections due to acquired tolerance, or other factors that alter individual graft responses would be expected to create some intransitive relationships. A histocompatibility system characterized both by a high frequency of graft rejection among individuals, and by transitivity, is likely to be highly precise.

Allozyme Variation as an Independent Measure of Relatedness

A more direct approach to the measurement of allorecognition precision involves comparison of grafting results with some independent indicator of genetic identity. Unfortunately, the traditional approach to genetic analysis, inheritance study, cannot easily be carried out with sponges because most species fail to complete the reproductive cycle in the laboratory.

Detection of allozyme variation by protein electrophoresis represents a promising approach to establishing an independent measure of genetic relatedness against which graft responses can be compared. Since many sponges carry a rich assemblage of other organisms in symbiotic, commensal or parasitic relationships, some criterion is needed to distinguish proteins produced by the sponge from those of the community it harbors. One convention of allozyme work routinely applied to organisms for which inheritance studies are not feasible is to compare observed genotype frequencies with expectations based on Hardy-Weinberg equilibria. This criterion for verification of the genetic basis of electrophoretic variation is conservative: selection, inbreeding, or other natural processes (as well as contamination or scoring difficulties) can also cause deviation from Hardy-Weinberg proportions.

In a serious challenge to the concept of allorecognition precision in sponges, Curtis et al. (1982) compared the responses of grafts in *Ectyoplasia ferox* with a protein electrophoretic diagnosis of their genetic identity. They observed considerable variation in the electrophoretic mobilities of proteins in different individuals and reported that some sponges

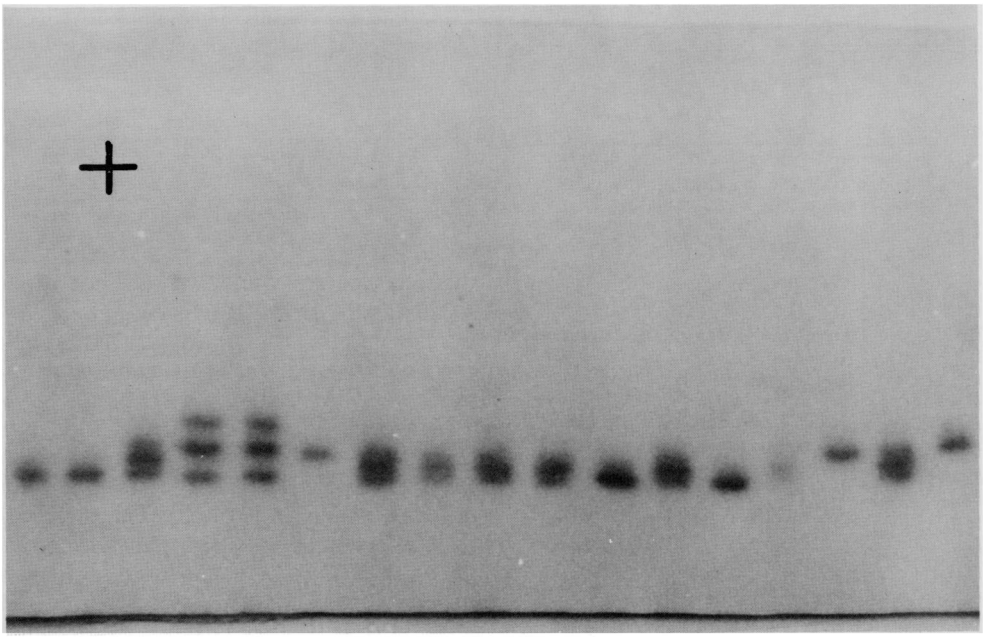

FIG. 1. Gel patterns for phosphoglucoisomerase in 17 individuals of *Niphates erecta*. Diploid genotypes are as follows: 100/100 (lanes 1, 2, 11, 13); 120/100 (lanes 3, 7–10, 12, 14, 16); 120/120 (lanes 6, 15, 17); 140/100 (lanes 4, 5).

which accepted grafts from one another differed in electrophoretic banding pattern. They concluded that "graft acceptance is in no way a demonstration of clonal identity."

Even apart from concerns about the correct scoring of the implant grafts themselves (see above), we feel that the empirical basis for the conclusion of Curtis et al. (1982) is open to serious question. In their study, sponge tissues were homogenized and a cell membrane fraction prepared by differential centrifugation. Electrophoresis involved separation by molecular weight on polyacrylamide SDS gels, and proteins were elucidated with a non-specific stain. This procedure differs in significant ways from assays of allozyme variation usually associated with population genetic applications, and yields results that are difficult to interpret in genetic terms. Genetically-based variation in the structure of a protein encoded by a particular gene usually involves a few amino acid substitutions that alter electrical charge but have little effect on molecular weight. The large molecular weight differences reported by Curtis et al. (1982) must have some basis other than conventional allelic variation at homologous loci. The banding patterns on gels were not interpreted in Mendelian or biochemical terms, nor were replicates produced to show that the differences were not artifacts of sample preparation, such as cleavage of proteins or sample contamination.

To circumvent this kind of ambiguity we have employed a conventional analysis of allozyme variation in which the genetic basis of electrophoretic banding patterns can be confirmed from both biochemical and Mendelian expectations. A preliminary electrophoretic survey of about 20 enzymes in the species for which we have the largest body of grafting results (*Iotrochota birotulata*) unfortunately revealed no reliably scorable protein polymorphisms. However,

TABLE 3. Allozyme variation in *Niphates erecta*.

Locus	Genotype[1]	Number of individuals: Observed	Number of individuals: (Expected)[2]	Probability
Hk	100/100	82	(75.7)	
	100/160	35	(47.8)	$P < 0.01$
	160/160	14	(7.5)	
Pgi	80/80	0	(0.0)	
	80/100	1	(1.9)	
	80/120	1	(0.9)	
	80/140	1	(0.0)	
	100/100	64	(59.9)	ns
	100/120	45	(52.8)	
	100/140	3	(2.7)	
	120/120	16	(11.6)	
	120/140	0	(1.2)	
	140/140	0	(0.0)	
Sod[3]	100/100	116	(116.2)	
	100/175	9	(8.7)	ns
	175/175	0	(0.1)	

[1] Alleles designated by percent mobility relative to the most common allele designated 100.
[2] According to Hardy-Weinberg equilibrium.
[3] *Sod* not scored in six individuals.

extracts from the sponge *Niphates erecta* did run exceptionally well. Three enzyme systems—hexokinase (HK, Enzyme Commission number 2.7.1.1), phosphoglucoisomerase (PGI, E.C.5.3.1.9), and superoxide dismutase (SOD, E.C.1.15.1.1)—produced zymogram patterns evidencing clear allelic variation. Individuals exhibiting one band were scored as homozygotes, and those with three bands (representing a heterodimer and two homodimers) were scored as heterozygotes. Gel patterns for PGI are shown in Figure 1.

Parabiotic grafts of *Niphates erecta* were made in September 1983 and scored three months later. All grafts were between sponges less than 10 meters apart. Branches from each sponge were also collected and frozen in liquid nitrogen. Tissue samples were later homogenized in cold buffer, centrifuged to remove particulate material, and loaded onto horizontal starch gels. The methods of gel electrophoresis and histochemical staining were essentially those given in Ayala et al. (1972) and in Selander et al. (1971). Four alleles were observed at *Pgi*, and two each at *Sod* and *Hk*. Genotype frequencies at *Pgi* and *Sod* did not differ significantly from Hardy-Weinberg expectations, but the genotype frequencies at *Hk* showed a mild but significant deficiency of heterozygotes (Table 3).

TABLE 4. Graft response and multilocus allozyme genotype[1] in 65 assayed pairs of *Niphates erecta*.

Multilocus allozyme genotype	Graft response: Acceptance	Graft response: Non-fusion
Same	23	5
Different	5	32

[1] Based on the unambiguously scorable loci *Hk*, *Pgi*, and *Sod*.

Grafts of *Niphates erecta* yielded two responses: fusion, in which the tissues of the participants became confluent over an area consisting of most of the graft interface; and non-fusion, in which the sponges could be easily separated, although they were sometimes connected by a fibrous external sheath. A total of 28 grafts were scored as fusions and 37 as non-fusions. Frequency of graft non-fusion was higher between individuals that were located further apart (Table 1), and no instance of intransitivity was observed in 12 tests (Table 2).

The concordance between observed graft response and response predicted from allozyme genotype was strong (Table 4): in a total of 65 grafts, most acceptances (82%) were between sponges with same allozyme genotype, and most non-fusions (86%) involved sponges differing in allozyme genotype. In five cases, graft non-fusions occurred between sponges identical in assayed allozymes, suggesting that in these situations the histocompatibility assay was a more refined discriminator of putative clones. (From observed allozyme frequencies [Table 3], the expected probability that two unrelated sponges share identical electrophoretic genotype is 15%.) However, five grafts between sponges with different allozyme genotype were scored as acceptances. Four of these differed at the *Pgi* locus and one differed at *Hk*. Thus, 14% of the allogeneic relationships predicted by allozyme genotype remained undetected in the histocompatibility bioassay.

Conclusions

Allorecognition responses to grafts in sponges and other invertebrates often appear to be controlled by fairly polymorphic genetic systems. These allorecognition phenomena may be useful as bioassays of population structure if the relationships implied by the responses can be identified. In situations where allorecognition is extremely precise, such that virtually every genetically distinct individual is distinguished by the recognition system, bioassays that distinguish lineages within populations can be developed. Where allorecognition is less precise, it may be possible to identify relationships of similarity that are also of interest. For example, in the colonial ascidian, *Botryllus,* individuals that share one or both alleles at a self-recognition locus will fuse on contact. Thus, parent-offspring relationships in *Botryllus* will be characterized by histocompatibility.

The hypothesis of extreme precision in allorecognition may be empirically tested in several ways. First, since relationships of equality must conform to certain rules of logic, falsification may follow from a demonstration of internal inconsistencies (such as non-reciprocity or intransitivity) in the identity relationships implied by graft responses. In any test of allorecognition precision, the outcome may depend critically on the reliability of the grafting methodology. Technical artifacts, which represent a loss of information, are more likely to create the impression that allorecognition is imprecise than to change the apparent behavior of an imprecise system so that it resembles precision. Because implant grafting techniques may yield unreliable results with some sponges, studies that employ these techniques should be interpreted with caution.

As a second approach to testing allorecognition precision, the results of a self-recognition bioassay may be evaluated against the known or suspected biology of a particular species. For example, for a species in which dispersal of asexual propagules is obviously limited, the frequency of graft rejection in a precise histocompatibility system should approach 100% as the distance between grafted individuals becomes sufficient to exclude clonemates. Allorecognition responses may also be compared with relationships assigned by some independent measure of genetic relatedness, such as protein electrophoresis. In these comparative tests, apparent discrepancies could result from actual imprecision in allogeneic recognition, artifacts inherent in the grafting methodology, or from errors in whatever criteria are used independently to establish genetic relationships. For an assay of genetic identity such as protein electrophoresis, the genetic basis of the assayed variation should be well documented.

Allorecognition in the populations of marine sponges investigated here appears to be quite refined. No evidence of imprecision was found in tests of transitivity or in grafts between remote individuals. For *Niphates erecta,* there was also a strong concordance between observed graft response and the response predicted from allozyme genotype. Nonetheless, in a few instances, graft acceptances were observed between sponges differing in allozyme genotype. Conversely, several instances of graft nonfusion were observed between sponges identical in genotype at three or four moderately polymorphic allozyme loci. As a first approximation, these results suggest that histocompatibility in *Niphates erecta* may have a genetic basis of complexity roughly comparable to that of a few moderately polymorphic genes.

These results suggest that histocompatibility bioassays of clonal identity in tropical marine sponges are in general fairly accurate, although not invariably so. This conclusion is not surprising, because any assay method (such as protein electrophoresis or histocompatibility bioassay) which utilizes a subset of the genome to distinguish entire genotypes will inevitably leave a finite number of clones unresolved. There has been an unfortunate tendency in the literature to

perceive the value of histocompatibility bioassays in population studies as critically dependent on total and taxonomically universal allorecognition precision. However, absolute precision is not essential for all applications, and where extreme precision is an essential premise it may be subject to empirical tests. Furthermore, for those situations in which histocompatibility responses leave some allogeneic differences unrecognized, study of the biological significance of the phenomenon may eventually lead to applications which reveal components of population structure other than clonal lineages. Clearly, the approaches used in this study to assess allorecognition precision should be extended to additional species.

Literature Cited

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourao, and S. Perez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics 70:113–139.

Bergquist, P. R. 1978. Sponges. Univ. California Press, Berkeley.

Bergquist, P. R., M. E. Sinclair, and J. J. Hogg. 1970. Adaptation to intertidal existence: Reproductive cycles and larval behavior in Demospongiae. Symp. Zool. Soc. Lond. 25:247–271.

Curtis, A. S. G. 1979. Individuality and graft rejection in sponges, or a cellular basis for individuality, pp. 39–48. *In* G. Larwood and B. R. Rosen (eds.), Biology and Systematics of Colonial Organisms. Academic Press, N.Y.

Curtis, A. S. G., J. Kerr, and N. Knowlton. 1982. Graft rejection in sponges. Genetic structure of accepting and rejecting populations. Transplantation 30:127–133.

Hildemann, W. H., C. H. Bigger, I. S. Johnston, and P. L. Jokiel. 1980. Characteristics of transplantation immunity in the sponge, *Callyspongia diffusa*. Transplantation 30:362–367.

Hildemann, W. H., I. S. Johnston, and P. L. Jokiel. 1979. Immunocompetence in the lowest metazoan phylum: Transplantation immunity in sponges. Science 204:420–422.

Hildemann, W. H., and D. S. Linthicum. 1981. Transplantation immunity in the Palaun sponge *Xetospongia exigua*. Transplantation 32:77–80.

Jokiel, P. L., W. H. Hildemann, and C. H. Bigger. 1982. Frequency of intercolony graft acceptance or rejection as a measure of population structure in the sponge, *Callyspongia diffusa*. Mar. Biol. 71:135–139.

Kaye, H., and T. Ortiz. 1981. Strain specificity in a tropical marine sponge. Mar. Biol. 63:165–173.

McClay, D. R. 1974. Cell aggregation: Properties of cell surface factor from five species of sponge. J. Exp. Zool. 188:89–102.

Moscona, A. A. 1968. Cell aggregation: Properties of specific cell-ligands and their role in the formation of multicellular systems. Devel. Biol. 18:250–277.

Neigel, J. E., and J. C. Avise. 1983. Histocompatibility bioassays of clonal population structure in marine sponges: Clonal structure in *Verongia longissima* and *Iotrochota birotulata*. J. Hered. 74:134–140.

Neigel, J. E., and G. P. Schmahl. 1984. Phenotypic variation within histocompatibility-defined clones of marine sponges. Science 224: 413–415.

Scofield, V. L., J. M. Schlumpberger, L. A. West, and I. L. Weissman. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. Nature 295:499–502.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI. Univ. Texas Publ. 7103:49–90.

Simpson, T. L. 1973. Coloniality among the Porifera, pp. 549–565. *In* R. S. Boardman, A. H. Cheetham, and W. A. Oliver, Jr. (eds.), Animal Colonies. Development and Function through Time. Dowden, Hutchinson and Ross, Stroudsburg, PA.

van de Vyver, G. 1970. La non-confluence intraspecifique chez les spongiaires et la notion d'individu. Ann. Embryol. Morph. 3:251–262.

Corresponding Editor: R. J. MacIntyre