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CLONAL DIVERSITY AND POPULATION STRUCTURE IN A REEF-BUILDING CORAL, *ACROPORA CERVICORNIS*: SELF-RECOGNITION ANALYSIS AND DEMOGRAPHIC INTERPRETATION

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Description of genetic variation in a population, recognized by Hubby and Lewontin (1966) as providing "the fundamental datum of evolutionary studies," is now routinely accomplished by electrophoretic characterization of allozyme variation and by other molecular techniques. These studies have revealed considerable variation at the molecular level, as well as the structure of this variation within and among populations. Although the value of the molecular approach in population genetics is beyond dispute, there remain interesting aspects of population variation not directly assayable by these methods.

Biological self-recognition phenomena, exemplified by self-sterility in flowering plants and immune systems in animals, have been utilized in a population genetic context for only a few species. An analysis of genetic population structure based upon a self-recognition phenomenon requires a relatively straightforward assay incorporating the phenomenon, and an established relationship between the detectable polymorphisms and a more general aspect of population structure.

Harberd used a combination of morphological characters and self-sterility relationships to deduce clonal structure in populations of the grasses, *Festuca rubra* and *F. ovina* (Harberd, 1961, 1962*a*) and the clover, *Trifolium repens* (Harberd, 1962*b*). In most vertebrate populations nearly every individual possesses a unique histocompatibility type, but some vertebrate population structures have been characterized by a departure from this condition of complete diversification. Kallman (1964) interpreted the occurrence of duplicated histocompatibility types within isolated populations of platyfish as an indication of inbreeding. For populations in which asexual reproduction occurs, some authors (Maslin, 1967; Cuellar, 1976, 1977; Angus and Schultz, 1979; Angus, 1980) have assumed that each clone is distinguished by a unique histocompatibility type, thus equating the diversity of incompatible strains with clonal diversity. In one such study (Angus and Schultz, 1979), a tissue graft analysis applied to populations of the unisexual fish, *Poeciliopsis monacha-lucida,* resolved more clones than had been detected in an earlier electrophoretic survey (Vrijenhoek et al.,

1977). Sebens (1982) has used intraspecific aggression in the sea anemone, *Anthopleura elegantissima,* as an indicator of genetic identity. Agonistic encounters apparently occur only between members of different clones (Francis, 1973).

Asexual lineages, or clones, represent clearly defined units of population structure that can be resolved by a sufficiently precise self-recognition system. Although, with the exceptions cited above, vertebrate populations are generally not structured into clones, invertebrates often employ modes of asexual reproduction that lead to clonal propagation. Self-recognition phenomena in invertebrates, which have been described only recently but which appear to be analogous to vertebrate histocompatibility, can provide a biological assay to determine genetic population structure. Here we report the development of such an approach, its application to population structure in the reef-building coral *Acropora cervicornis* (Linnaeus), and a theoretical exploration of the demographic processes that determine population structure in this system.

Biology and Natural History

Demography

Scleractinian corals possess several biological features that facilitate analysis of demographic processes. Because corals are sessile and lack hidden root systems, demographic events may be observed directly by monitoring specific individuals (Porter et al., 1981*a,* 1981*b*). A natural record of growth patterns and the incidence of vegetative colony fissioning is also provided in the calcareous skeletons secreted by reef-building corals (Hughes and Jackson, 1980).

Staghorn coral, *A. cervicornis,* is a reef-building coral common in the 3 to 30 m depth range of Caribbean reefs. Its success in escaping competition for space with other sessile benthos, which enables it to spread over extensive areas of the reef, has been attributed to an open branching morphology, rapid growth rate, and a propensity for vegetative propagation and dispersal (Tunnicliffe, 1981). The typical scleractinian life cycle includes both asexual proliferation of polyps for colony growth, and sexual reproduction leading to the development of free-swimming planula larvae. The planulae of most species represent a dispersal stage, remaining planktonic for up to two months, after which settlement on a hard substratum occurs and a new colony is initiated from a single polyp (Connell, 1973). Census of recently settled juvenile corals within a community can provide estimates of sexual recruitment rates. Even in areas where *A. cervicornis* is more abundant than all other corals combined, relatively few juveniles of this species are found (Rylaarsdam, 1980). It has been suggested that in populations of *A. cervicornis* the most prevalent mode of reproduction is a form of vegetative propagation involving fragmentation and regeneration of colonies (Gilmore and Hall, 1976). Tunnicliffe's (1980) extensive study of *A. cervicornis* populations in Discovery Bay, Jamaica provides a detailed account of the processes of breakage, vegetative propagation, and mortality, and supports the view that these populations are maintained primarily by asexual reproduction.

An adult *A. cervicornis* colony grows most actively from apical polyps at the distal termini of branches, while the proximal portion of the colony is generally devoid of living tissue. Boring organisms infiltrate the exposed section of skeleton which eventually erodes. Adult colonies may be supported by several branches, particularly in dense stands where neighboring colonies interlock. Death of the entire colony may occur if it slips down into rubble or sediment. A recently settled juvenile colony is usually cemented to hard substratum by a still living basal section, which is typically flared to encrust a larger area of attachment.

Self-Recognition Phenomena

Self-recognition phenomena, analogous to vertebrate histocompatibility, have been described for the scleractinian genera *Acropora* (Hildemann et al., 1975), *Porites* (Hildemann et al., 1975, 1977*b*), *Fungia, Pavona, Pocillipora* (Hildemann et al.,

1977*b*), and *Montipora* (Raison et al., 1976; Hildemann, 1977; Hildemann et al., 1977*b*; Johnston et al., 1981), and are perhaps common to all members of the order. These studies have shown that contacts between fragments propagated vegetatively from one isogenic colony (isografts) always result in a fusion of both tissue and skeletal elements. Contacts between conspecific colonies from different locations, very likely derived from separate sexual recruits (allografts), invariably result in a chronic "rejection" response, characterized by the formation of a morphologically distinct bridge separating the normal tissue and skeletal elements of the colonies. A memory component to the rapidity with which allogenic tissue is recognized was demonstrated in experiments using *Montipora verrucosa* (Hildemann et al., 1977*a*). Colonies receiving repeated allografts from the same donor clone exhibited an acceleration of the rejection response with successive grafts.

Although the presence of immunoglobin-like substances in cnidarians has not been demonstrated, Hildemann and coworkers (1977*a*) have argued that the properties of specificity and memory imply a direct phylogenetic homology between scleractinian and vertebrate histocompatibility. For the purpose of developing an assay for clonal population structure, the mechanism underlying the self-recognition phenomenon is less relevant than the operational properties that appear to govern the behavior of the system. Furthermore, because the operational properties of a self-recognition system are population phenomena (i.e., the specificity of vertebrate histocompatibility is reduced in inbred populations, although the mechanism remains unchanged) necessary controls should be performed for each population before assuming the validity of an assay requiring these properties.

Materials and Methods

Study Sites

Field studies were conducted at two locations in the West Indies: Discovery Bay, on the north coast of Jamaica, in August of 1979 and June of 1980, and Tague Bay, on the north coast of St. Croix, United States Virgin Islands, in November of 1980 and July of 1981.

Prior to the passage of Hurricane Allen in August 1980, densely interlocked stands of *A. cervicornis* built up as "haystacks" extended over much of the fore reef terrace of Discovery Bay. Our study site was located on the west side of the bay where the West Fore Reef terrace slopes gently from the shallow reef crest seaward for several hundred meters to a steeper escarpment, with a sheer drop-off beginning at about 55 m depth. The haystack formations alternate with sand channels, which funnel sediment down the fore reef slope toward the drop-off. Bare substratum free of sediment, available for the settlement of scleractinian larvae, was abundant. The observations reported here were made within a section of haystack, designated LTS, in the depth range of 10 to 15 m. The study area was bounded by two sand channels about 25 m apart and extended for about 30 m along the length of the haystack. Dense *A. cervicornis* cover was present on LTS between depths of 3 and 30 m. The morphology of Discovery Bay reefs has been described in detail by Goreau and Land (1974).

In Tague Bay, the shelf corresponding to the reef terrace is much broader. The deep water drop-off is at least 5 km from any point on the reef crest. An apparent consequence of this topography is a noticeable increase in sedimentation relative to Discovery Bay. Visibility is lower due to suspended material in the water column, and a layer of sediment covers most of the hard substratum. Sediment may have interfered with larval settlement, accounting for the fact that we did not observe juvenile *A. cervicornis* colonies in the Tague Bay population. At the fore reef location where the Tague Bay study was conducted, *A. cervicornis* was restricted to patches from about 1 m to tens of meters across surrounded by sand plains. Grafting experiments were performed in an area within 50 m of the seaward margin of the reef crest and about 50 m across. Depths ranged from 10 to 15 m.

Self-Recognition Assay

Histocompatibility relationships among colonies of *A. cervicornis* were determined by observing contact responses in both natural interactions and experimental grafts. Natural interactions result when adjacent colonies passively topple together and their tissues come into contact. Experimental grafts were performed in situ as follows: branch segments (10 to 50 cm in length) from donor colonies were tied onto recipient colonies with 40 lb test nylon monofilament line. Each experimental graft was labeled with a plastic tag identifiable by a specific configuration of punched holes. Grafts were scored after nine months, allowing sufficient time for the reactions to fully mature (Hildemann et al., 1975).

Grafts were considered scoreable only if both donor and recipient branches were living and firmly cemented together by a contact zone at least a centimeter in length. To eliminate subjective bias in scoring different types of grafts, scoring was performed in situ on the reef; the identification tag was then collected and later cleaned and decoded in the laboratory.

Two types of response are observed in contacts between *A. cervicornis* colonies. An acceptance response (Fig. 1-A) is characterized by the complete fusion of the calcareous skeletons and external soft tissues of the participants into an unbroken juncture. A rejection response (Fig. 1-B) is always characterized by an undulated suture line at the skeletal interface separating the tissues of the participants, although the skeletons become firmly cemented together. Varying degrees of bleaching (loss of pigmented algal symbionts), anomalous growth near the area of contact, and incomplete polyp development are also generally evident around the suture line in a rejection response. These responses have been described previously by Tunnicliffe (1980) for natural interactions between *A. cervicornis* colonies in Discovery Bay. They appear to be identical to the isograft and allograft responses described by Hildemann and co-workers (Hildemann et al., 1975).

Contact responses observed in natural interactions and experimental grafts were scored to (1) establish operational properties of the self-recognition bioassay with respect to clonal lineage relationships, (2) estimate levels of clonal diversity, and (3) examine the spatial extent of clones, and develop maps of their distributions. Estimates of clonal diversity were summarized by a statistic, the Neighbor Index (*N.I.*), which we define as the estimated proportion of spatially adjacent colonies that belong to different clones. Thus, *N.I.* can range from zero to one and assumes higher values for higher levels of microgeographic clonal diversity. This statistic was chosen because its expected value is independent of the total area included in sampling a population. All in situ work was performed with the use of SCUBA.

Simulation Models

Computer simulations were employed to extend our analysis of *A. cervicornis* population structure. Simulation programs were run on the Tektronix 4054 Graphics Display System.

In these models, each colony occupies a position within a 2-dimensional square lattice representing the population. Mortality is introduced by deleting colonies selected by a random number function. These open positions are immediately filled by either a new clone (sexual recruit) or a clone represented in one of the eight neighboring positions. The occurrence of sexual recruitment at a fixed mean rate is also controlled by a random number function. Inputs required by the model are the rates of mortality, sexual recruitment and vegetative propagation, and the initial population composition.

Realistic input parameters for these simulations were derived from two sources of demographic data available for the pre-Hurricane Allen *A. cervicornis* population in Discovery Bay. (Demographic processes have not been monitored in Tague Bay populations.) An annual mortality rate was estimated from a currently in-progress photographic monitoring study of a fore reef community on the reef section im-

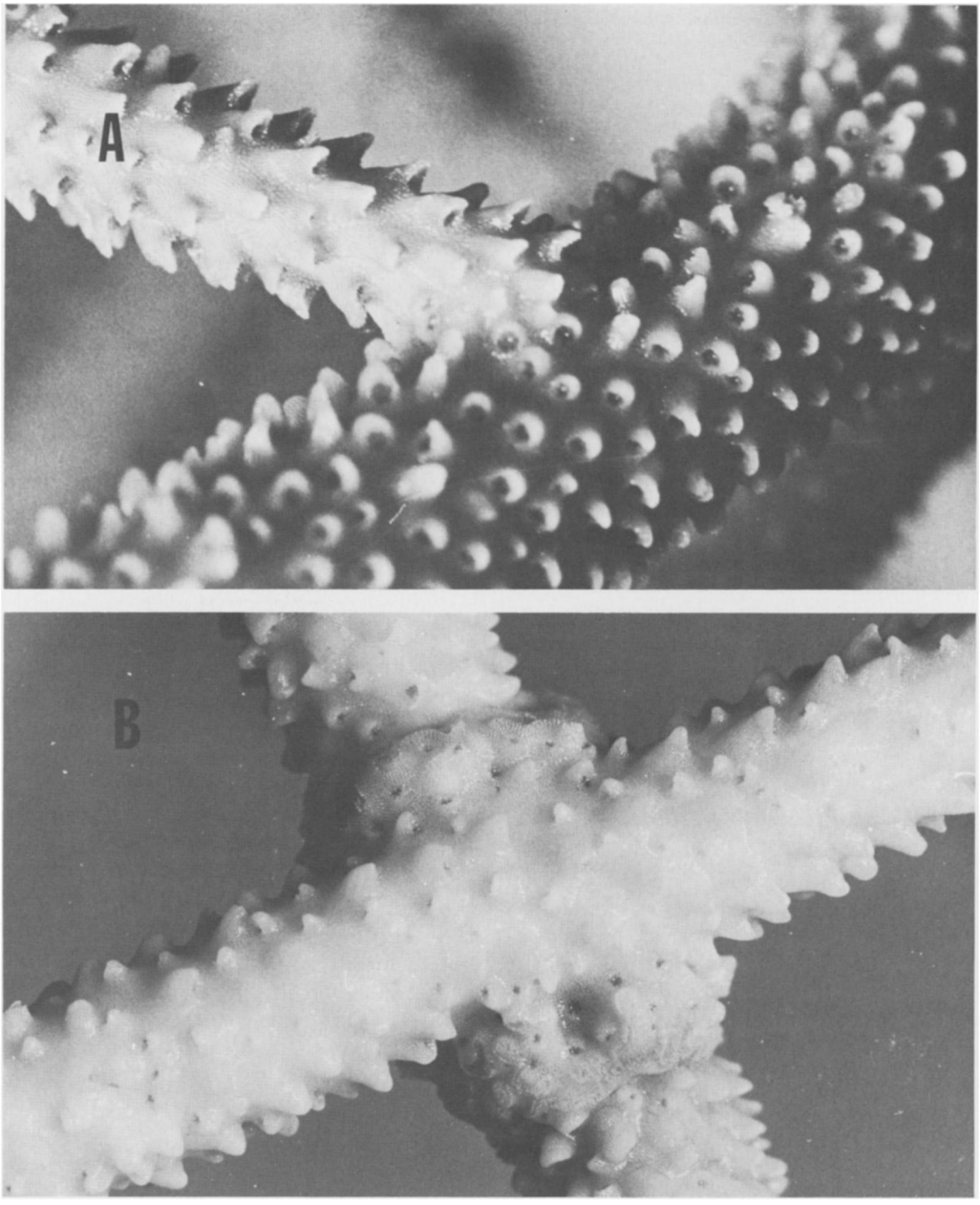

FIG. 1. Contact responses of *Acropora cervicornis* colonies. A. Acceptance response. B. Rejection response.

mediately west of our LTS study site (see Porter et al., 1981). Rate of sexual recruitment was estimated from Tunnicliffe's (1980, 1981) data on the frequency of juvenile *Acropora* colonies encountered on a line transect on the West Fore Reef of Discovery Bay. Neighbor Index values of clonal diversity were calculated by ex-

TABLE 1. *Natural interactions observed in Discovery Bay that define operational properties of the self-recognition bioassay for* A. cervicornis.

Colony relationships and response	*N*	Significance
Colony exhibiting different responses to different colonies	24	Colonies are not limited to a single mode of interaction at one time
Colonies in contact at multiple points		Responses are reproducible over multiple grafts with the same pair of colonies
All accept	38	
All reject	44	
Different responses	0	
Same colony (selfs)		Isogenic contacts result in acceptance responses
Accept	22	
Reject	0	
Contacts between colonies interconnected by segments of dead skeleton		Colonies likely to be vegetatively derived "sisters" retain equivalent identities
Accept	42	
Reject	0	
Interconnected colonies in common contact with other colonies		Probable sister colonies respond alike in contacts with other colonies
All accept	13	
All reject	4	
Different responses	0	

haustively comparing all pairs of adjacent colonies in the simulation generated populations.

RESULTS

Operational Properties of the Self-Recognition System

The proposition that a self-recognition system precisely discriminates clonemates from non-clonemates requires that certain operational properties characterize the system. Our observations of natural interactions and experimental grafts have indicated that these necessary properties are indeed features of *Acropora cervicornis* contact responses. These results are summarized in Tables 1–3, and their significance is evaluated below. The specific observations of natural interactions tabulated here were made during a single systematic search of each population. However, many additional casual observations of this type have been made during our investigations, and these were fully consistent with the tabulated results.

1) *A single colony is not limited to a single mode of interaction at one time.* Interactions were observed in which a single colony simultaneously exhibited both acceptance and rejection responses in contacts with different colonies (Table 1). This independence of response in multiple tissue contacts is a required property of a system which responds specifically to the relationship of the interaction participants.

2) *Responses are reproducible.* Where the same pair of colonies was in contact at multiple points, the response was consistent for all contacts (Table 1).

3) *Self-contacts exhibit acceptance responses.* Frequently, different branches from the same colony grow into contact with one another. The results of 22 such contacts were tabulated, and many more have been casually observed. In addition, the responses of 22 experimental self-grafts were scoreable. In all cases, the acceptance response was observed (Tables 1, 2).

4) *Physiologically separated clonemates retain equivalent self-recognition identities.* Interconnection of physiologically isolated colonies by sections of dead skeleton may indicate the colonies are isogenic "sisters," separated by the loss of intervening tissue from a single parent colony. Only acceptance responses were observed

TABLE 2. *Experimental grafts performed in Tague Bay that define operational properties of the self-recognition bioassay for* A. cervicornis.

Colony relationships and response	*N*	Significance
Same colony (selfs)		Isogenic contacts result in acceptance responses
Accept	21	
Reject	0	
Contacts between colonies interconnected by segments of dead skeleton		Colonies likely to be vegetatively derived "sisters" retain equivalent identities
Accept	23	
Reject	1*	
Contacts between colonies approximately 600 meters apart (long distance grafts)		Grafts between colonies likely to be derived from distinct (allogenic) clonal lineages result in rejection responses.
Accept	0	
Reject	18	

* Two alternative explanations for the anomaly. (1) the colonies were in fact non-clonemates, cemented together near their bases by a previous rejection response, or (2) the colonies were clonemates that had diverged in histocompatibility identity.

for natural interactions between interconnected colonies. Furthermore, colonies interconnected by dead skeleton responded alike in all natural interactions with other colonies (Table 1). However, among 24 experimental grafts between interconnected colonies in Tague Bay, a single rejection response was observed (Table 2). Although the anomalous rejection response may represent divergence in histocompatibility identity among clonemates, it is likely that colonies of separate origin were in this case mistakenly identified as sister colonies. Dead branches of allogenic colonies that had been connected by cementation in a rejection response could appear to be interconnected remnants of the same colony; the characteristic features of a contact interaction are generally not distinguishable in dead portions of *A. cervicornis* colonies.

5) *Grafts of colonies separated by large distances exhibit rejection responses.* The vegetative dispersal of *A. cervicornis* clones by fragmentation is limited by the dense calcareous skeleton and by the tendency for unattached branches to interlock with neighboring branches. The probability of two colonies belonging to the same clone should therefore decrease with the distance between them. In Tague Bay, 26 colonies from our study site were grafted with colonies from a distant location (600 m west of our study site). All 18 scoreable grafts exhibited the expected rejection response (Table 2).

6) *Self-recognition identity relationships are transitive.* An important property of self-recognition identity relationships results from exact discrimination of self from non-self: identities will be transitive (if A is identical to B, and B is identical to C, then A is always identical to C). An assay utilizing a transitive self-recognition system defines discrete units of population structure that are independent of the particular subset of pair-wise comparisons that delimit them. Identity relationships defined by a self-recognition system with absolute precision would necessarily be transitive; however if the system were imprecise, intransitivities could result (i.e., if identity were indicated for individuals within a threshold of similarity, the pairs A-B and B-C could be within the threshold, while the pair A-C was outside the threshold).

A set of grafting experiments was performed in Tague Bay to test for the transitivity of *A. cervicornis* self-recognition. Because there is no category intermediate between rejection and acceptance in our scoring of *A. cervicornis* contact responses, any intransitive relationship within a group could be isolated as an intransitivity involving only three colonies. In 11 sets of grafts that defined identity relationships among groups of three colo-

TABLE 3. *Transitivity test. Experimental grafts which define self-recognition identity relationships among sets of three colonies: A, B, and C, in Tague Bay.*

Relationships	Number observed
Transitive	
A = B B = C A = C	3
A = B B ≠ C A ≠ C	6
A ≠ B B ≠ C A ≠ C	2
Intransitive	
A = B B = C A ≠ C	0

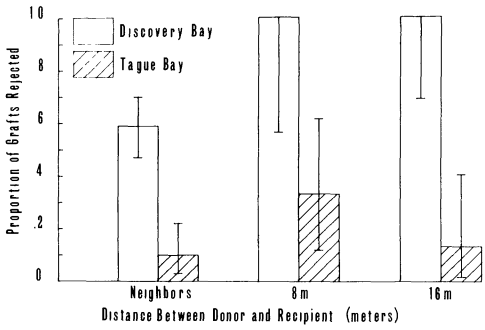

FIG. 2. Frequency of rejection response with 95% confidence limits for contacts between neighboring colonies, 8 m grafts, and 16 m grafts in *A. cervicornis* populations from Discovery Bay and Tague Bay. Sample sizes for Discovery Bay are neighbor interactions, $N = 70$; 8 m grafts, $N = 12$; 16 m grafts, $N = 10$. For Tague Bay, neighbor interactions, $N = 41$; 8 m grafts, $N = 15$; 16 m grafts, $N = 15$.

nies, no intransitivities were revealed (Table 3).

Applications of Self-Recognition Analysis

1) *Clonal Relationship as a Function of Distance.*—A total of 443 experimental grafts was initially set up in Tague Bay; 316 grafts were located during the scoring operation, and of these, 247 (78%) were alive and scoreable after nine months. In Discovery Bay, we scored a total of 16 experimental grafts from a pilot project, which was begun in 1979. An additional 450 grafts, set up in June 1980, were destroyed by Hurricane Allen in August of 1980. Thus our data for Discovery Bay are limited to observations of natural interactions and the results of the pilot project.

In both Discovery Bay and Tague Bay, experimental grafts were scored between colonies 8 m apart and 16 m apart; these colonies were selected at regular intervals along parallel line transects. In Figure 2, the two populations are compared with respect to frequencies of rejection responses for these distances. There is a dramatically lower rejection response frequency for the Tague Bay colonies, suggesting a lower microgeographical diversity of clones in this population. This conclusion is supported by results from natural and experimental interactions among physically adjacent colonies (Table 4). For Discovery Bay and Tague Bay, the observed neighbor index (*N.I.*) values were 0.59 and 0.10, respectively. This difference is highly significant ($P < .0001$, Sokal and Rohlf, 1969 p. 607).

In Tague Bay, a total of 194 experimental grafts was scored for which the distance between donor and recipient was recorded. There is a pronounced increase in rejection response frequency along the series of increasing donor-to-recipient distance classes: "neighborhood" (less than 1 m), "local" (1 to 24 m) and "long distance" (greater than 24 m) (Fig. 3). However, no significant trend was discernible for the frequency of rejection responses in more finely graded series within the "local" distance category. The largest donor-to-recipient distance for a graft acceptance observed in this study was 20 m.

2) *Clone Distribution Maps.*—Although the quantitative assessments of population structure presented above are useful for statistical and comparative purposes, a more direct representation of spatial patterns of population structure is provided by actual maps of the distributions of particular clones within a population. Four especially ramose colonies in the Tague Bay study area were selected as mul-

TABLE 4. *Neighbor index (*N.I.*) estimates of clonal diversity of* A. cervicornis *populations in Discovery Bay and Tague Bay.*

Location	Assay	# of responses		N.I.	95% conf. limits
		Accept	Reject		
Discovery Bay	Natural interactions[1]	29	41	0.59	0.47–0.70
Tague Bay	Natural interactions[2]	27	3	0.10	
	Experimental grafts	47	5	0.10	
	Total	74	8	0.10	0.03–0.22

[1] Among the 199 colonies along four line transects of total length 59 m.
[2] Includes interactions among the 29 colonies along three line transects of total length 65 m.

tiple graft donors in order to sample the spatial distributions of histocompatible colonies. From each donor colony, 25 to 45 branch fragments were grafted to recipients in the vicinity. The resulting clone maps are shown in Figure 4. A variety of clone distribution patterns was observed. Clone I had no clonemates within the surrounding mapped area. In contrast, clones II and III predominated in the areas around the map donors, although outlying colonies were non-clonemates. Clone IV is especially interesting because it alone occupies the southwest half of the mapped area, and is completely absent from the northeast half. These data, although limited, do provide a sampler of the actual spatial distributions of particular clones in Tague Bay. Some clones may be confined to a single colony, while others predominate among all colonies in an area of at least 10 m diameter. Clones appear to be spatially discrete, with fairly distinct boundaries. It is of course possible that mapping on a larger scale would have revealed other areas in which these clones also occur.

In general, the distributions of individual clones did not follow closely the patchy physical distribution of the population. Grafting experiments revealed that even small *A. cervicornis* patches (less then 2 m across) could be composed of several clones, while the distributions of abundant clones could extend beyond a single patch. These qualitative features of clonal structure were not evident from summary measures such as the *N.I.*, or graft response as a function of donor-to-recipient distance.

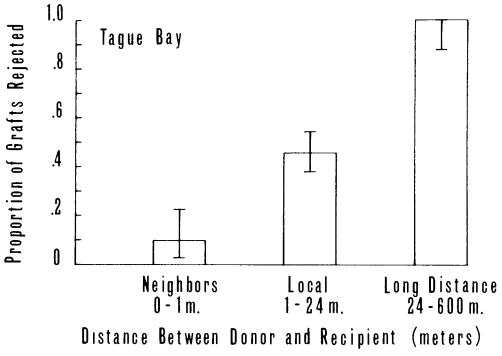

FIG. 3. Frequency of graft rejection with 95% confidence limits vs. donor-to-recipient distance, Tague Bay. Sample sizes are neighbor interactions, N = 41; local grafts, N = 147; long distance grafts, N = 28.

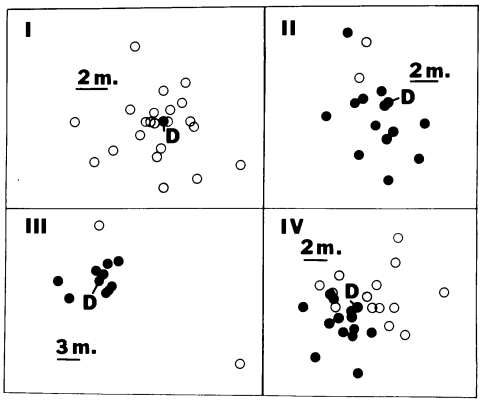

FIG. 4. Clone distribution maps, Tague Bay. Positions of colonies which accepted grafts from donor indicated by solid circles; colonies that rejected grafts from donor indicated by open circles; position of donor indicated by solid circle and "D."

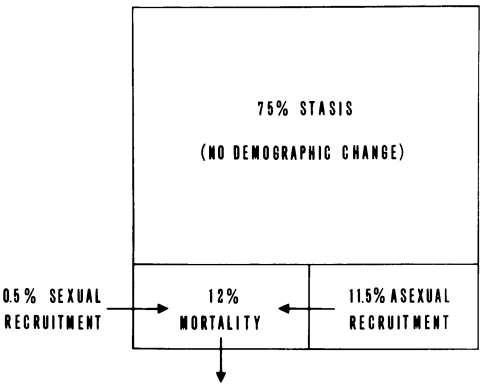

FIG. 5. Demographic budget for Discovery Bay *Acropora cervicornis* population.

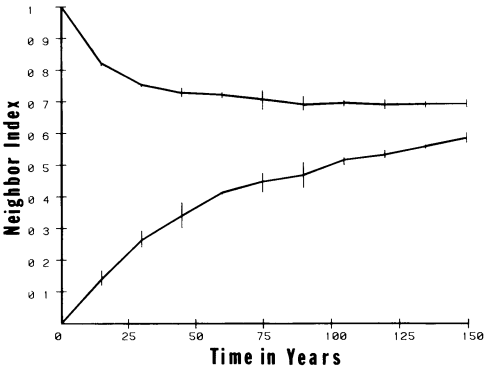

FIG. 6. Neighbor Index vs. time in a population simulation with the Discovery Bay demographic budget; the mean responses and range of three runs with different random number sequences are shown for populations initialized with minimum ($N.I.$ = 0) and maximum ($N.I.$ = 1) clonal diversity.

Simulation Models

The initial concern of the modeling study was to determine if the high $N.I.$ estimated for the Discovery Bay population is compatible with a demographic scenario in which reproduction is almost entirely vegetative. An intuitive consideration of this demography might suggest that individual clones should cover relatively large areas, implying an expected $N.I.$ lower than our observed value of 0.6.

In our initial models, asexual recruitment was assumed to occur at a rate that with sexual recruitment, balances total recruitment with mortality. Without a knowledge of the history of the population, this constant population size model is the simplest to adopt. In Figure 5, the annual "demographic budget" derived from observations on the Discovery Bay population (see Methods) is portrayed.

Using values from this demographic budget as input parameters for the computer simulations, runs of the model with three different random number sequences were made, and the $N.I.$ was calculated after successive periods of iteration. Results are shown in Figure 6. Neighbor Index is plotted through time (time is calibrated to a mortality rate of 12% per year) in populations initially composed of a single clone ($N.I.$ = 0), and in populations initially consisting of all unique clones ($N.I.$ = 1). The $N.I.$ trajectories in the two sets of populations converged toward similar values. Within 150 years, an $N.I.$ of between 0.56 and 0.70 was reached, regardless of the initial level of clonal diversity. This result is in excellent agreement with our observed $N.I.$ value of 0.6 for the Discovery Bay population.

We can further characterize the pattern of clonal structure in simulations by examining sizes of individual clones. Using input parameters representative of the Discovery Bay population, the effects of the initial population composition on $N.I.$ are mostly lost within about 100 to 150 years. However, the influence of initial population composition cannot be assumed to be entirely lost until all clones originally present in the population have been eliminated by drift. This occurs after approximately 1,350 years with Discovery Bay input parameters. After this point, a pattern of clonal structure has developed such that no clone's distribution is beyond 15 lattice elements in linear extent (Fig. 7). This quantity corresponds to an actual distance of 4.4 m in the Discovery Bay population, where each colony occupied an average of 0.29 m along line transects. This predicted scale for clonal distributions is in accord with the results of the 8 m and 16 m donor-to-recipient experimen-

6397	4458	5716	5244	4458	4614	4614	6396	6396	4614
4990	5860	4990	4990	4614	5244	6481	6396	4614	5865
5856	5856	4990	4990	5244	5244	5244	6034	6375	4614
6331	5394	5244	5244	5503	5244	6316	6375	5244	6421
5394	6430	5244	5244	5503	5244	5244	6316	6421	6034
5394	6331	5118	5118	5194	5194	6393	5194	5194	5865
5856	5394	6358	5118	5194	5194	6393	6393	5194	5503
5394	5856	1737	6468	6208	6393	6393	5194	6480	6450
5856	5394	5394	5394	6489	6208	6393	5194	5194	6295
1737	5394	5394	5394	6208	6393	6393	5503	5194	5194

FIG. 7. Population composition lattice generated by simulation after a period of iteration corresponding to 1,350 years with Discovery Bay demographic budget. The central 100 positions of a 30 × 30 lattice are shown. All clones present in the initial population have been lost. The clonal identity numbers at each position correspond to the temporal order of clonal recruitment. The Neighbor Index for the entire lattice was 0.65; for the portion shown alone, 0.74.

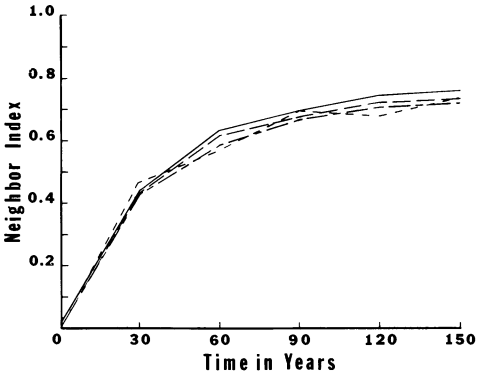

FIG. 8. The mean and range of the Neighbor Index response with variants of the demographic model are shown for sets of three runs with different random number sequences. Basic model parameters are a 12% annual mortality rate and a 1.2% annual sexual recruitment rate with a 40 × 40 lattice. Solid line is original version of model; short dash is model run with 10 × 10 lattice; long dash is model run with hexagonal lattice; alternating short and long dash is for runs with population following an annual cycle of mortality and recruitment.

tal grafts in Discovery Bay, which resulted solely in rejection responses.

Demographic processes in the Tague Bay population have not been monitored. However, qualitative inferences can be made regarding the rates of these processes. The proportion of scoreable experimental grafts (0.78) reflects the survival rate of donor and recipient pairs over a 9-month period. On this basis, the annual mortality rate in the Tague Bay population does not appear to differ markedly from that observed in the Discovery Bay population. However, in contrast to our Discovery Bay site, no juvenile *Acropora* were encountered in surveys of the Tague Bay study area. Evidently the rate of sexual recruitment there is extremely low. In simulation runs where the ratio of sexual recruitment to total recruitment is reduced to 0.001, a *N.I.* value below 0.10 is the eventual outcome of the model. The *N.I.* of 0.10 observed in the Tague Bay population may therefore be explained as a consequence of low rates of sexual recruitment. Demographic simulation also predicted the spatial segregation of different clones and the extreme variance in clone size evident in the clonal distributions mapped by self-recognition analysis in Tague Bay.

In order to assess the robustness of the simulations, various assumptions of the demographic model were altered in additional runs. By modifying the basic model structure, we have determined: (1) the mean response of the *N.I.* as a function of input parameters shows almost no dependence on population size (simulations were generally run with a 30 × 30 lattice, although similar mean responses could be obtained for the range of lattice sizes tested, from 6 × 6 to 40 × 40). However, there is a greater variance in the response with smaller lattices; (2) the behavior of the model was not detectably altered by substituting a six-neighbor hexagonal array for the original eight-neighbor square array; and (3) the model's behavior in terms of the *N.I.* appeared to be unaffected when an annual quota of mortality deletions was allowed to accumulate in the population

before recruitment (with this cyclic recruitment modification, the number of "living colonies" in the population follows an annual cycle, and so the assumption of constant population size is relaxed). Representative outputs for simulations run with these modifications are shown in Figure 8.

These simulations have helped to identify parameters most likely to influence clonal population structure. The rate of sexual recruitment certainly plays a major role in determining equilibrium levels of clonal diversity. Historical factors may also exert a lasting influence on clonal diversity. For example, in populations receiving sexual recruits at a rate of less than 5% of the rate of total recruitment, the initial population composition may be a major determinant of $N.I.$ for a subsequent period of 100 years or more. In the model, the rate of approach to an equilibrium $N.I.$ value over time slows considerably after the initial period of roughly 100 years, so that the $N.I.$ value determined by the demographic parameters and initial population composition tends to remain at a relative plateau for durations on the order of hundreds of years. In contrast, population size, the precise geometry of vegetative spread, or the temporal pattern of recruitment do not appear to be important in predicting clonal population structure.

Discussion

Self-Recognition Analysis Method

Hildemann and Johnston (1979) have suggested that antecedents of the vertebrate histocompatibility system originated early in metazoan evolution, and are probably universally present among extant invertebrate groups. In this study we have developed an assay of clonal population structure that utilizes this biological resource, potentially available for the study of the many invertebrate groups in which self-recognition systems are developed. Our method of validating the self-recognition assay, by application to known lineage relationships, establishes a measure of genetic identity independent of other techniques.

The experimental evidence furnished by this study of *Acropora cervicornis* is consistent with our working postulate of precise discrimination of clones by the self-recognition assay. However, before discussing results in terms of clonal population structure, we consider several caveats that apply to equating assay-defined histocompatibility strains with vegetative clones.

It has been suggested that intraspecific interactions between corals, like the rejection response, may represent mechanisms of competition (Potts, 1976; Johnston et al., 1981). If the rejection response is in fact an interference mechanism, and the survival rate of allograft participants is reduced as a result, a bias against allografts would be expected in the proportion of scoreable grafts. This possibility can be addressed for our Tague Bay data by comparing the proportion of allograft controls (grafts between colonies at least 30 m apart) that were unscoreable due to mortality or other causes, with the proportion of unscoreable isografts (self-grafts and grafts between putative sister colonies). Among the set of 28 presumed allografts, 7.1% were unscoreable, while 16.4% of the 55 presumed isografts were unscoreable. Thus, there is no indication of a reduction in the survival rate of colonies involved in rejection interactions. However, it should be noted that the scoreability of these two sets of grafts may also have been biased by other factors associated with the locations where each set of experiments was conducted.

Another reservation is that a finite number of controls cannot definitively prove that self and non-self relationships will invariably be distinguished by particular experimental outcomes. However, in our studies of *A. cervicornis* populations in Discovery Bay and Tague Bay, we have explicitly recorded the outcomes of 62 known isogenic or inferred allogenic interactions, without observing any exceptions to precise clonal discrimination. Additional controls for the operational

properties expected of a precise self-recognition system (Tables 1–3), as well as casual observations made in the field lend further support to our postulate of precise recognition. It may also be of relevance to note that for a related Pacific coral species (Acroporidae: *Montipora verrucosa*), Hildemann (1977) observed only rejection responses in some 300 grafts between distantly located (and therefore probably allogenic) colonies.

An additional caveat applies to the use of spatially separated colonies as allogenic controls. Clones within local subpopulations may be more genetically related than the clones chosen for allogenic controls if gene flow within the population is restricted. A biased rate of allogenic rejections between distant clones could result. We have assumed that the degree of polymorphism at the presumed self-recognition loci and the dispersal of gametes and larvae are sufficient to prevent such inbreeding effects. However, this possibility could be directly addressed by determining if grafts between juvenile, sexually derived colonies and their neighbors are rejected as often as grafts with distant colonies.

It must also be acknowledged that the exact genetic basis of histocompatibility specification in scleractinian corals remains unknown. Even our use of the term "histocompatibility" in describing these phenomena is predicated on an analogy to vertebrate histocompatibility systems, which exhibit similar operational properties. However, the genetic basis of an extremely precise self-recognition system need not be unreasonably complex. Curtis et al. (1982) showed, for example, that a genetic control system of only four loci, each with five equally frequent alleles, will lead to less than 0.2% allogenic acceptance responses in a panmictic population. The caveat that some clones could remain unrecognized in a histocompatibility system of moderate genetic complexity also applies to any assay method which utilizes a subset of the genome's loci to distinguish entire genotypes.

Finally, as a possible explanation of the variation in severity of rejection responses observed in natural interactions between *A. cervicornis* colonies, Tunnicliffe (1980) suggested that weak rejection responses might occur between clonemates which have diverged in histocompatibility identity after physiological separation. Experimental work by Hildemann and co-workers (Hildemann et al., 1977*a*; Johnston et al., 1981) with *Montipora verrucosa* suggested a more conservative explanation, consistent with the postulated genetic determination of histocompatibility identity. The rate of development and severity of the rejection response in allografts with this species depended on the specific allogenic combination involved, "pre-immunization" from previous allogenic contacts, and ambient temperature. However, if new histocompatibility identities are in fact acquired by individual colonies, they would appear as distinct clones in our analysis. Only if the rate of such occurrences approached the rate of sexual recruitment would they be likely to significantly alter assessments of clonal population structure.

Demographic Interpretation of Clonal Structure

Populations from a broad taxonomic array of plant and animal species have been shown to be structured into electrophoretically or morphologically distinguishable clonal lineages. The respective roles of demographic processes, historical factors, and selection in establishing clonal structure are generally unknown (see Parker, 1979, for a recent review). In our study we have examined in particular the role of demography and historical factors in structuring populations. An attempt was made to develop the most parsimonious models incorporating parameters estimated from direct observation, before considering additional factors that could be regarded as modifications of basic demographic processes. The predictions of these simple models were entirely consistent with the patterns of clonal structure observed in natural populations of *A. cervicornis*.

The influence of historical factors is predicted to be of long duration by demographic simulations. Although the theoretical predictions for populations of constant size and equilibrium genetic structure are consistent with our empirical population structure data, an event likely to have drastically altered existing clonal structure occurred during our study of the Discovery Bay population. In August of 1980 Hurricane Allen brought extensive damage to coral reefs on Jamaica's north coast. The impact of this event on benthic communities in Discovery Bay has been well documented. Over 90% of the *A. cervicornis* colonies in the West Fore Reef population were killed by immediate physical damage, and further mortality has resulted from ecological after-effects (Knowlton et al., 1981; Porter et al., 1981; Woodley et al., 1981).

Mass mortality and the subsequent recovery of *Acropora* populations can be expected to have a major effect on clonal diversity and population structure. Shinn (1972) described two modes of recovery observed in *Acropora* populations. After being devastated by Hurricane Donna in 1960, an *A. cervicornis* population in the Key Largo Dry Rocks area of the Florida reef tract was re-established by the vegetative spread of surviving colony fragments and the settlement of planula larvae on newly created substrate. Recovery was complete within five years. A population of a morphologically similar *Acropora* species in the Persian Gulf also recovered rapidly after a cold front in 1965 resulted in nearly 100% mortality. However in this case, the population was re-established entirely by larval settlement. The latter mode of recovery would maximize clonal diversity in the population, with each recruit representing a unique clone.

Disturbance events which decimate *Acropora* populations may occur frequently enough to prevent attainment of equilibrium patterns of clonal diversity. Our models, which assume no fitness differences among clonal genotypes, predict that the random loss of clones from a population with low rates of sexual recruitment may be an extremely slow process. For a population with all colonies initially unique in clonal identity, a mortality rate of 12% per year, and no sexual recruitment, several hundred years would elapse before a $N.I.$ less than 0.5 would be attained, and over 5000 years would be required to reach the $N.I.$ of 0.10 observed in Tague Bay. It is almost certain that major disturbance events would occur before such a population exhibited $N.I.$ values this low. Extending this result to our field studies, we can conclude that if the assumptions of the model are appropriate, then any major past disturbances of the Tague Bay population were not followed by entirely sexual recolonizations. Although extremely high rates of mortality and replacement would accelerate the stochastic reduction of clonal diversity, there is no evidence of rapid population turnover from our study. A population mortality rate of 80% per year would be required to bring the $N.I.$ down to the value of 0.1 within 1,000 years of a phase of sexual recolonization.

Williams (1975) and others have speculated about the importance of selection in shaping clonal diversity and population structure. We cannot evaluate the importance of selection from our data without a specific model of its action. However, we can conclude that the general patterns of clonal structure observed could be formed in the absence of any differences in fitness among clonal genotypes. Studies which purport to demonstrate selection in clonally structured populations often use as evidence phenotypic variation of potential adaptive significance among clones. Often the presumed genetic component of phenotypic variation could not have been distinguished from variation induced secondarily by the environment. However, a more serious limitation of an approach which simply demonstrates the potential for selection to operate is that a consideration of interclonal variation in fitness by itself does not lead directly to predictions of clonal population structure. The demographic approach yields models with pre-

dictions testable by virtue of their formulation in terms of directly measurable parameters.

Summary

Biological self-recognition phenomena, analogous in many respects to vertebrate histocompatibility, are apparently widespread in scleractinian corals. Here we exploit the operational properties of a self-recognition bioassay to estimate clonal diversity and population structure in a common reef-building coral, *Acropora cervicornis*.

When branches of *A. cervicornis* come into contact, within several months the tissues and calcareous matrix exhibit either an acceptance or rejection response. Through a variety of controlled experimental grafts, and observations of natural interactions, we demonstrate that these responses accurately distinguish clonemates (the products of asexual fragmentation of colonies) from non-clonemates (the products of sexual recruitment).

This self-recognition bioassay was subsequently used to analyze the clonal structure and diversity in *A. cervicornis* populations in Discovery Bay, Jamaica, and Tague Bay, St. Croix, U.S. Virgin Islands. A total of nearly 500 experimental grafts was scored in situ and additional information was obtained from observations of naturally occurring contacts. In comparison to the Tague Bay population, the Discovery Bay population has a significantly greater microgeographic clonal diversity that is reflected in a much higher Neighbor Index, the proportion of rejection responses in contacts among adjacent colonies. In Tague Bay, spatial maps of *A. cervicornis* clones were constructed; they show that clones are variable in size (from a single colony to assemblages up to 10 m or more in diameter), and are distributed in discrete patches.

A computer model was used to simulate the development of clonal population structure from simple demographic processes (colony mortality, asexual and sexual recruitment). Using empirically derived rate estimates of these demographic processes we found that the quantitative predictions of Neighbor Index values from the model corresponded closely to our bioassay estimates of this parameter for real populations. Qualitative predictions of the model were also in good agreement with our empirical assessments of the size range of an individual clone's spatial distribution in the Discovery Bay population, and the degree of segregation and variance in size observed for spatial distributions mapped in the Tague Bay population.

The model indicated that the relatively low diversity of clones apparent in the Tague Bay population is expected when the input of new clones into the population by sexual recruitment is extremely low. Recently settled *Acropora* colonies did in fact appear to be virtually absent in this population. Sedimentation on the Tague Bay reef may interfere with the establishment of juvenile scleractinia on the available substrata.

The simulations also predict that occasions of catastrophic mortality and subsequent recovery of *A. cervicornis* populations may be major determinants of clonal diversity and population structure, especially when the rate of sexual recruitment is relatively low. Major disturbances, such as severe hurricanes, may prevent populations of *A. cervicornis* from attaining equilibrium patterns of clonal structure.

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ANNOUNCEMENT

CONFERENCE ON COPEPODA

The Second International Conference on Copepoda will be held in Ottawa, Canada from August 13 to 17, 1984.

In addition to contributed papers there will be four symposia (Biogeography of Copepoda; Behaviour Ecology; Growth, Life History and Culture; Morphology and Anatomy) and a panel discussion on phylogeny of Copepoda. Persons interested in receiving future announcements and other informations about the Conference are asked to write to:

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