remains of a mechanism that had been necessary for the elimination of intercalary heterochromatin. In either case, the cytological data support the electrophoresis data, which show that, although there is an almost identical morphology, the separation of *P. univalens* from *P. equorum* is ancient (about 10 million years) (*3*). A similar situation has been found in the twin species of nematodes *Caenorhabditis elegans* and *C. briggsae*, which by nucleotide sequence divergence appear to have separated 10 million years ago although they are almost identical morphologically (*13*).

With respect to the DNA sequences eliminated in somatic cells during early embryogenesis, we have determined that the amount of heterochromatin in *P. univalens* metaphases is about 70 percent of the total length of all the chromosomes. The amount of germ line–limited satellite DNA is about 85 percent of the total DNA (*9*). If one assumes a similar DNA content per unit chromosome length in euchromatin and heterochromatin, the near equivalence between these two measures supports the view that in these species the germ line–limited DNA is composed almost exclusively of highly repetitive DNA (*9*).

CLARA GODAY*
SERGIO PIMPINELLI†
Dipartimento di Genetica
e Biologia Molecolare,
Universita di Roma, 00185 Roma, Italy

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* Permanent address: Instituto de Genetica, C.S.I.C Madrid, Spain.
† To whom correspondence should be addressed.

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Phenotypic Variation Within Histocompatibility-Defined Clones of Marine Sponges

Abstract. *Nongenetic phenotypic variation can be identified by its occurrence within genetically uniform clones. A histocompatibility bioassay of clonal identity was used to ascertain the extent of phenotypic variation within natural clones of two species of marine sponges. Multiple morphological forms of the sponge* Aplysina fistularis *were found to occur within single clones, indicating a nongenetic polymorphism. In contrast, a genetic basis is suggested for a polymorphism of* Aplysina cauliformis; *within single clones of this species, individuals were uniform in color and morphology.*

A necessary concern of evolutionary biology is the distinction between genetic and nongenetic components of phenotypic variation in nature. Conventional heritability studies are limited to those organisms that can be bred under controlled conditions. Ideally, sets of genetically equivalent individuals should be examined to isolate environmental and developmental influences on phenotypic expression. Among plants and lower invertebrates, replicates of single genotypes in natural populations are produced by clonal propagation. If such clones can be identified, the natural range of phenotypic expression displayed by single genotypes can be observed. Self-recognition phenomena have potential use for the identification of clonal lineages. The capacity for self versus nonself discrimination in intraspecific contacts has been demonstrated for various lower invertebrates, including sponges and cnidarians. Distinct behavioral responses (*1*) and histocompatibility-like responses (*2*) distinguish allogeneic from isogeneic contacts between individuals. Where asexual modes of reproduction have structured populations of sessile marine invertebrates into clonal lineages, it has been possible to utilize these phenomena as bioassays of clonal identity. Self-recognition bioassays have been used to assess the spatial distributions of clones which were then analyzed with respect to demographic and ecological factors for sponges (*3, 4*), reef-building corals (*5*), and sea anemones (*6*). We report here an extension of this approach to examine variation in phenotypic expression for individual clones of two species of marine sponge.

Aplysina fistularis (*Verongia fistularis*) and *Aplysina cauliformis* (*Verongia longissima*) are common Caribbean reef sponges occurring at depths from 5 to 30 m (*7*). The typical growth form of *A. fistularis* is a hollow cylinder, occurring either individually or in clusters up to a meter in height and 10 cm in diameter. This form has been designated *A. fistularis fistularis* by van Soest (*8*). Other morphotypes have been characterized, in part, by the presence of structures that either extend from the apices of the tubes as slender digitate processes (less than 2 mm in diameter) or arise from a massive or encrusting base as erect branches (up to 3 cm in diameter). These forms have been designated as *A. fistularis insularis* and *A. fistularis fulva*, respectively (*8*). *Aplysina cauliformis* is a ropelike or branching sponge, with a typical diameter of 1 to 2 cm and ranging in color from dark blue to yellow (*7*).

Grafting bioassays were used (i) to assess the accuracy of the bioassays in distinguishing clones and (ii) to determine the range of phenotypic expression for selected traits within single clones. Field work was undertaken at the Discovery Bay Marine Laboratory, in Jamaica, and at the NOAA Hydrolab facility in St. Croix, U.S. Virgin Islands.

Grafting experiments were performed on sponges that were selected from relatively large areas so that many different clones would be included. A total of 80 *A. fistularis* grafts were performed in situ on Arena Reef, in Discovery Bay, over an area of approximately 1000 m^2. A disk of tissue was removed from a donor sponge with a cork borer (1/2-inch diameter) and inserted into a hole in the recipient sponge made in the same manner. For *A. cauliformis*, 188 grafts were made on the outer eastern slope of Salt River Canyon, St. Croix, in an area of approximately 600 m^2. A segment of branch 2 to 5 cm in length was cut from a donor sponge and tied to a branch on a recipient sponge with nylon monofilament. For both species, a numbered identification tag was attached to each graft. Within 3 to 5 days, two distinct graft responses could be observed. A graft acceptance was characterized by the fusion of donor and recipient tissues. No tissue fusion occurred in rejection responses; instead a pronounced cuticle developed on the interfacing surfaces of the donor and recipient tissues. Scoring of grafts was coded "blind" with respect to expected outcomes.

Experimental controls indicated accurate identification of clones by the histocompatibility bioassay. (i) All autografts between parts of the same sponge, 9 for

A. fistularis and 11 for *A. cauliformis*, were accepted. (ii) Grafts between sponges separated by more than 2.1 m for *A. fistularis* (N = 30) and 13 m for *A. cauliformis* (N = 12) were all rejected. Because vegetative dispersal is limited by distance, the rejection of all grafts between sufficiently distant sponges is expected if grafts are accepted only between clone mates. (iii) All relationships defined by graft acceptances within sets of three or more individuals—three sets for *A. fistularis* and 40 sets for *A. cauliformis*—were transitive, as is predicted for clonal identity relationships (*9*).

Considerable morphological variation was observed in the Discovery Bay population of *A. fistularis*. Individual sponges were assigned to one of four phenotypic classes: (i) Tubes without additional structures (*fistularis* form), (ii) tubes with thin, digitate extensions (*insularis* form), (iii) massive forms with thick erect branches (*fulva* form), and (iv) small (less than 5 cm) encrustations without additional structures (juveniles). The juvenile form can be produced by asexual propagation and may be observed budding from the projections that characterize the *insularis* and *fulva* morphologies.

The spatial distribution of *A. fistularis* on Arena Reef was investigated for its relation to genetic population structure. An area of 600 m^2 was sampled along six 50-m line transects. All *A. fistularis* occurring within contiguous quadrats (1 by 1 m) on either side of the transect line were located and morphologically characterized. Other *A. fistularis* in the area were also inspected. The pattern of distribution was extremely clumped (*10*). Of the 45 sponges inspected, only four were isolated, with no other *A. fistularis* in the immediate vicinity (within 8 m). All others occurred in groups of two to eight sponges with a mean of 3.23 (S.D., 1.83) individuals per group. All of the 17 encrusting juveniles were associated with a group that included at least one large specimen. The mean patch diameter for all groups encountered was 1.03 m (S.D., 0.95), and it was 0.84 m (S.D., 0.85) for those groups including encrusting forms. This pattern of distribution would be expected if larger sponges were undergoing vegetative propagation, giving rise to patches of clonal descendants.

Many spatially well-defined groups of *A. fistularis* were polymorphic. Of the 14 groups examined, only two showed consistent growth form among their members. Seven groups were composed of a single large individual and juveniles. The remaining five groups included large individuals of differing morphologies.

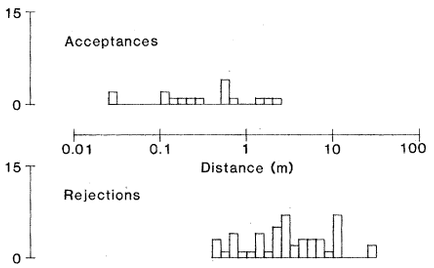

Fig. 1. Graft responses versus distance between donor and recipient for *A. fistularis*.

These observations suggested that, if patches of *A. fistularis* represent clones, considerable morphological variation may be expressed within these clones.

Graft response in *A. fistularis* was dependent on the distance between donor and recipient sponges (Fig. 1). Acceptances were confined to grafts between neighboring sponges less than 2.1 m apart. Grafts were not accepted between spatially discrete groups of sponges, although in some cases more than one clone was detected in what appeared to be a single large group. Similarity of morphology was not essential for graft acceptance in *A. fistularis*. Twenty grafts were made between sponges that were assigned to different phenotypic classes. One of eight grafts was accepted between a sponge of the typical *fistularis* morphology and a sponge characterized as *fulva*, two of two were accepted between *fistularis* and *insularis* type sponges, and three of three were accepted between sponges designated *fulva* and *insularis*. Grafts were also accepted (five of seven) between adult *fistularis* and encrusting juveniles. These results indicate that individual clones of *A. fistularis* are capable of expressing the entire range of morphological phenotypes that we have described for the population. The projecting structures that differentiate these phenotypes appear to effect vegetative propagation and may therefore reflect a developmental state specialized for this mode of reproduction.

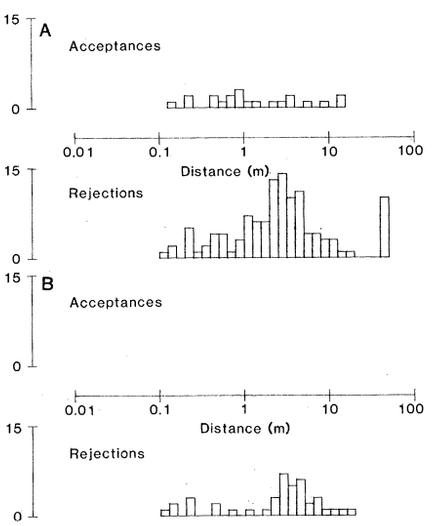

Fig. 2. (A) Graft responses versus distance between donor and recipient for *A. cauliformis*, all grafts. (B) Graft responses versus distance between donor and recipient for *A. cauliformis*, grafts between phenotypes.

Both morphology and color (as it appeared in situ) were variable in the Salt River Canyon population of *A. cauliformis*. Individual sponges were assigned to one of three phenotypic classes: (i) Light blue, with branches of a uniform diameter less than 2 cm; (ii) light green to yellow, with uniform branches less than 2 cm in diameter; and (iii) dark green to violet, with branches of irregular diameter (2 to 4 cm). These forms typically occurred in mixed assemblages. Juvenile *A. cauliformis* were not observed. Previous grafting studies with *A. cauliformis* in Barbados (*11*) and Jamaica (*3*) have indicated that the phenotypes designated above as (i) and (ii) may be characteristic of specific clones. Grafting bioassays indicated that clones of *A. cauliformis* in Salt River Canyon tended to be more spatially dispersed than *A. fistularis* clones in Discovery Bay. Graft acceptances were observed between donor and recipients up to 13 m apart. However, all 41 grafts between individual *A. cauliformis* that had been assigned to different phenotypic classes were uniformly rejected over a range of donor to recipient distances (Fig. 2). These results suggest that the members of an individual clone of *A. cauliformis* were restricted to a single class of phenotypic expression.

Phenotypic variation within histocompatibility-defined clones, as demonstrated here for *A. fistularis*, suggests several possible explanations. First, genetic variation within clones may arise through somatic mutation. However, exceptionally high mutation rates would be required to produce the extensive variation within small clones that was observed in this study. A second possibility is that histocompatibility-defined clones are actually small groups of closely related, although not genetically identical, individuals. This explanation presupposes that (i) larval dispersal is extremely limited (that is, larval dispersion is usually less than 2 m); and either (ii) gamete dispersal is also extremely limited, so that siblings are often related through both parents, and therefore frequently share all histocompatibility determinants, or (iii) grafts are accepted between individuals that only partially share histocompatibility determinants.

An example of an invertebrate self-recognition system characterized by these conditions is found in the colonial tunicate, *Botrylus* (*12*). However, in *Aplysina* and other sponges, a requirement for complete rather than partial sharing of histocompatibility determinants in graft acceptances is indicated by the observation that histocompatibility identity is always transitive (*3, 11*). Therefore, unless both gamete and larval dispersal are extremely limited, it is unlikely that histocompatibility-defined clones of *A. fistularis* are often multiple-related clones. Phenotypic variation within histocompatibility-defined clones of *A. fistularis* can be attributed to nongenetic influences. Aside from the taxonomic significance of such findings, the recognition of phenotypic variation as nongenetic carries basic evolutionary implications. Phenotypic plasticity may imply differential response to environmental conditions or, as we suggest for *A. fistularis*, may represent developmental alternation of specialized morphologies. For *A. cauliformis*, the complete association between a visible polymorphism and individual clones implies a genetic basis for the polymorphism, although somatic inheritance of nongenetic determinants cannot be ruled out. The three forms of *A. cauliformis* may represent either a polymorphism within a single species or distinct sympatric species.

Self-recognition bioassays provide a new approach for the study of variation. Exploitation of the natural replication of genotypes present in many invertebrate populations may lead to isolation of nongenetic components of variation.

JOSEPH E. NEIGEL
Department of Molecular and Population Genetics, University of Georgia, Athens 30602
GEORGE P. SCHMAHL
Department of Zoology, University of Georgia

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9. Transitivity is a necessary property of all relationships of equality, that is, If $A = B$, and $B = C$, it follows that $A = C$. Relationships of similarity can be intransitive. Sets of three are sufficiently large to isolate and characterize all intransitive relationships; demonstration that intransitivity does not occur in sets of three is a sufficient demonstration that it does not occur in sets of any size.
10. The variance to mean ratio of the quadrat counts was significantly greater than one, and the Morisita index of dispersion indicated a contagious distribution that was significantly different from random ($\chi^2 = 1929.8$, $Z = 27.5$, d.f. = 599, $P < 0.005$); J. M. Elliot, *Freshwater Biol. Assoc. Sci. Publ.* 25 (1977).
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Ingestive Behavior Evoked by Hypothalamic Stimulation and Schedule-Induced Polydipsia Are Related

Abstract. *Some, but not all, rats eat or drink in response to electrical stimulation of the lateral hypothalamus. Similarly, some, but not all, rats given food intermittently display schedule-induced polydipsia. In this experiment, animals that ate or drank during electrical stimulation tended also to be those displaying polydipsia. Thus, individual differences in predisposition to engage in ingestive behavior are consistent under two very different conditions.*

Rats become more active during electrical stimulation of the lateral hypothalamus (ESLH), but only some of them eat, drink, or exhibit other specific behaviors (*1, 2*). The explanation of why animals should eat or drink during ESLH has been disputed. Some investigators have suggested that ESLH activates specific motivational states such as hunger and thirst when electrodes are in the appropriate location (*3*). Others have maintained that stimulation evokes an increase in activity that may be channeled into eating, drinking, or other behaviors, depending on environmental factors and the predisposition of the animal (*1*). The evidence supporting these competing interpretations has been reviewed (*4, 5*).

We have addressed this question by trying to understand why only some animals eat or drink during ESLH even though electrodes seem to be in identical locations. The most obvious explanation is small, but critical, differences in electrode placements between animals. This explanation, however, seems to have been ruled out by a number of experiments. Many animals, for example, engage in the same behavior in response to stimulation at very different brain sites (*2*), and rats that eat or drink in response to ESLH continue to display the same behaviors after the electrode position has been changed (*6*). The same behavior is also evoked after the neural tissue surrounding the electrode tip has been destroyed and higher currents are used to stimulate more distal areas (*7*). Moreover, strains that normally consume equal amounts of food and water differ significantly in the probability that ESLH will evoke eating or drinking. This suggests that factors other than hunger and thirst must be considered (*8*). Thus, the earlier idea that individuals differ in predisposition to display specific behaviors during ESLH has gained support (*1, 2*).

To pursue the question of individual differences, we are exploring other experimental procedures in which animals consume excessive amounts of food or water. The experiment reported here was undertaken because of the many similarities between ESLH-evoked ingestive behaviors and the phenomenon of schedule-induced polydipsia (SIP). When food-deprived animals are given small amounts of food reward, intermittently, they consume excessive amounts of water—under some conditions, as much as half their body weight in 3 hours (*9*). In common with ELSH, intermittent delivery of food to hungry animals produces an increased activation that can be channeled into other responses besides drinking, including aggression, wheel running, and ingestion of nonnutritive substances such as sawdust (*10*). Moreover, the behaviors evoked by ESLH and SIP normally get stronger over repeated trials (*10, 11*). Finally, the behaviors evoked under both conditions may depend on dopamine pathways (*5, 12, 13*).

Schedule-induced polydipsia has been called an "adjunctive" or "displacement" behavior because it occurs when a desired goal cannot be obtained (*10, 14*). The increases in general activity may be accompanied by stress, as ele-