grains supplied by Whittaker gave electron-diffraction patterns [and energy-dispersive, x-ray emission spectra (EDS)] indistinguishable from those obtained from talc (*1*). These crystals could not be spurious contaminants as they were identified in micrographs provided to us by Whittaker. Incidentally, the observation of Whittaker that *c*-axis patterns from chaoite show rhombohedral symmetry suggests an incomplete understanding of diffraction theory. An unknown compound that has a hexagonal pattern of spots may be indexed according to either hexagonal or rhombohedral symmetry, depending on the assumed orientation of the a^* and b^* axes.

Finally, Whittaker mentions the recent work of Heimann *et al.* (*11, 12*). The approximately linear relation found by these investigators between the crystallographic *c* parameter and the chain length for seven carbynes is to be expected, since the chain length is inferred from the *c* parameter in the first place. The scatter in this plot is then reduced by arbitrarily assigning the seven carbynes to two classes (cumulene and polyyne), giving three points on each of two lines and one point midway between them. The relationship between the *a* parameter and chain length appears to be little better than random.

The suggestion that surface contamination could account for our observed EDS is apparently based on a mistaken interpretation of our work. Heimann *et al.* (*11*) reported that we found only small amounts of silicon, aluminum, and iron, implying that this was the case for all samples studied. However, such low concentrations apply only to the nontronite-graphite intergrowth, where the nontronite is a minor component; for the other three samples that we described, the EDS signals were very strong and could not have arisen from minor surface contaminants. We remain confident of our identification of sheet-silicate minerals in these samples by means of EDS and single-crystal electron diffraction. We find nothing new in this technical comment, and we see no reason to doubt the data and interpretations in our 1982 report.

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Graft Compatibility and Clonal Identity in Invertebrates

Neigel and Schmahl (*1*) reported that morphological variation among conspecifics of the tropical sponge *Aplysina fistularis* is probably due to phenotypic plasticity (or somatic mutation) rather than to genetic variation for these traits. To determine the genetic identity of individuals, they used histocompatibility assays. They assumed that sexually produced individuals, because of genetic differences at the locus (or loci) controlling histocompatibility, were unfusible (that is, incompatible); thus, only clone mates were fusible. However, what little is known of the formal genetics of histocompatibility in clonal invertebrates—for example, in the colonial ascidian *Botryllus* (*2*), in the athecate hydroid *Hydractinia echinata* (*3*), and in the sponge *Ephydatia fluviatilis* (*4*)—shows that clonal identity is not a prerequisite for fusion. Therefore, in the absence of (i) transplants of the phenotypic variants into a common environment, (ii) reciprocal transplants between the source environments of the phenotypic variants, and (iii) formal genetic analysis of histocompatibility, their argument rests on the unproved assumption that graft compatibility (that is, fusibility) demonstrates clonal identity.

Verification of the assumption that only clone mates are fusible requires that clones (not just fusions and rejections) be distinguished unambiguously. On the basis of three criteria, Neigel and Schmahl suggested that histocompatibility assays between tissue grafts were reliable tests of clonal identity in sponges. First, fusions between randomly chosen individuals were rare, whereas all autografts fused. Second, the probability of fusion between individuals was inversely correlated to the distance between individuals. This correlation was presumed to arise from limited dispersal of clonal fragments from their source. Third, fusibility relationships were transitive (individual A fuses with B, B fuses with C, and C always fuses with A). From the absence of intransitivities, they concluded that complete genetic matching was a prerequisite for fusion. We believe, however, that these lines of reasoning do not adequately support the conclusion that fusibility implies clonal identity.

The reliability of the first criterion, that of rare fusibility among random grafts, has been questioned on theoretical grounds (*5*). Neigel and Schmahl infer from the paucity of fusions (that is, no fusions in 30 grafts separated by more than 2.1 m) that it is unlikely that any two sexually produced individuals will share histocompatibility determinants, and therefore be fusible. However, a single locus, complete genetic matching model (grafted individuals must hold both alleles in common to fuse), with as few as eight equally frequent alleles, produces fusion frequencies consistent with those reported by Neigel and Schmahl. Because the number of genotypes at any locus is defined by $[n(n + 1)]/2$, where n is the number of alleles, there need be only 36 histocompatibility genotypes to account for their results. Therefore, if the population contains more than 36 sexually produced individuals, it is highly probable that two of these individuals would be fusible. Unless the number of fusibility tests greatly exceeds the actual number of histocompatibility genotypes in a population, fusion frequency data alone cannot be used to support the assumption that only clonemates are fusible.

The second criterion, that of attenuated probability of fusion with increasing distance, is not met by one of the species in Neigel and Schmahl (*1*). We find no statistically significant negative trend, by parametric or nonparametric methods, of graft acceptance as a function of distance for *Aplysina cauliformis* [figure 2 in (*1*)]. Even the observations of no graft acceptances from pairs separated by more than 13 m is nonsignificant (binomial test; $P = 0.121$) given the small sample size ($n = 13$) and the low overall

acceptance rate (estimated at 15 percent from their figure 2).

There appears to be attenuated fusion frequency with distance in *A. fistularis*, and this could well be produced by limited dispersal of clonal fragments. However, such a pattern could also arise from a combination of limited dispersal of both clonal fragments and sexually produced offspring. According to Neigel and Schmahl, fusions between relatives would be too rare to generate the observed cline in fusibility. They give two reasons for this rarity of fusions: (i) the histocompatibility system of sponges precludes fusion between relatives and (ii) dispersal of larvae and gametes is widespread. Nevertheless, a high probability of fusion between related individuals is predicted by histocompatibility models that are based on either full or partial matching systems. For example, with eight alleles, the full matching model predicts a fusion rate of 25 percent between full sibs, and 7 percent between half-sibs. If the magnitude of dispersal of sibling larvae is limited [as appears to be the case for a number of clonal marine invertebrates (*6*)], or gamete dispersal is restricted (or both), then a cline of decreasing fusibility with distance from the larval (or gametic) source should result (*7*). There appear to be no data which indicate the mode of larval dispersal, or the mechanism of fertilization in either *A. fistularis* or *A. cauliformis*. Thus, the negative relation between distance and fusion frequency observed by Neigel and Schmahl does not provide evidence that fusibility implies genetic identity.

Finally, the transitivity data do not substantiate the proposition that a complete matching histocompatibility system is operating in either *A. fistularis* or *A. cauliformis*. Only three trials for transitivity were conducted in *A. fistularis*, and the complete transitivity observed is easily explained by chance alone. Data on *A. cauliformis* show fully transitive fusibility relationships with larger sample sizes ($n = 40$). Because fusions occurred only among proximate individuals (*1*), the transitivity data do not statistically demonstrate the clonal identity of fusible individuals (that is, the complete matching hypothesis). Consider a simple example: suppose the histocompatibility system in *Aplysina* was like that of *Botryllus* in which only one allele at a single, highly polymorphic locus needs to be shared for individuals to fuse (= partial matching). Following Neigel and Schmahl (who suggest that asexual propagation is the predominate mode of colonization in *Aplysina*), we can further suppose that fusible individuals within a patch are composed of 80 percent clonal individuals (from the founding clone and its derivatives) and 20 percent sexually produced progeny from this clone. If all of these offspring were half-sibs, and if the number of histocompatibility alleles were large, then the probability of a transitive relationship is 0.98, and the probability of 40 such transitive relationships is 0.446. Thus, the transitivity data are potentially consistent with a partial matching, as well as a complete matching, histocompatibility system.

Given these qualifications, and the absence of independent determination of genotypic identity, we conclude that the reliability of fusibility assays as indicators of clonal identity should be viewed with some caution.

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Grosberg *et al.* (*1*) question the interpretation of our histocompatibility experiments with sponges (*2*) as assays of clonal identity. No assay of genetic identity, except for one that would compare the entire genomes of organisms, can be absolutely accurate. However, this does not invalidate the use of histocompatibility as a genetic marker in this or in similar applications. If controls can be devised that demonstrate the accuracy of an assay within statistical limits, then it can be applied correctly in accordance with those limits. The use of controls in our study should be evaluated in this statistical sense. Sets of grafts were made to test the accuracy of the assays in controlled combinations, and then the assumption was made that these controls were representative of the experimental grafts between sponges of same or differing phenotypes.

First, Grosberg *et al.* used our *Aplysina fistularis* graft data to estimate the minimum number of histocompatibility alleles consistent with no graft acceptances between 30 nonneighboring individuals, with the assumption of a single locus model of histocompatibility. They use the estimate as if it were an estimate of the actual number of alleles to conclude that "if the population contains more than 36 sexually produced individuals, it is highly probable that two of these individuals would be fusible."

This theoretical argument is superfluous because the statistical limitations of our data can be defined much more simply by determining the 95 percent confidence limits for our estimate of the frequency of fusion between nonneighboring individuals of *A. fistularis*. These limits are 0 and 0.095 (*3*). There is no need to assume a genetic model or to require that every individual in the population has a different histocompatibility type. If the acceptances observed between phenotypically distinct individuals have occurred by chance, as suggested (*1*), their frequency should not differ significantly from that observed for the controls. The differences, however, are statistically significant at the 0.05 percent level. We observed 11 acceptances in 20 grafts between individuals of different phenotypes with juveniles considered a distinct phenotype, and 6 acceptances out of 13 grafts that do not include juveniles. The 95 percent confidence limits for these proportions are 0.36 and 0.77, and 0.16 and 0.68, respectively (*3*), and do not overlap with the confidence limits for the controls.

Second, Grosberg *et al.* state that one criteria of accuracy, attenuated probability of acceptance with increasing distance, is not met by *A. cauliformis*. They do not describe their statistical tests; however, I find, to the contrary, that the trend is significant with the Wilcoxon Rank Sum test ($P = 0.02$). Yet this point is irrelevant in view of the results of the assay itself. None of the histocompatibility-defined clones contained more than one phenotypic variant. This conclusion would stand even if it were known that the histocompatibility assay failed to resolve all clones. Clones "hidden" within the phenotypically uniform groups of histocompatible individuals would still be phenotypically uniform.

Regarding the third concern expressed

by Grosberg *et al.*, each point raised was treated in detail (*2*). If the dispersal of larvae is extremely limited, histocompatibility-defined clones of *A. fistularis* may actually be small groups of closely related, although not genetically identical, individuals. We did not suggest that either (i) the histocompatibility system of sponges precludes fusion between relatives or (ii) dispersal of larvae and gametes is widespread. However, although no dispersal data are available for *A. fistularis*, data on larval behavior for other sponges suggest that dispersal is not limited. The larvae with the shortest known free swimming period are those of sponges in the order Clathriidae; these larvae swim actively for 2 to 3 hours (*4*). Sponges with larvae that do not swim at all, but rather creep along the substrate, have only been found in the order Halicondrida (*4, 5*). Furthermore, the species that exhibit these dispersal-limiting behaviors are found only in intertidal environments, where restriction of dispersal is believed to be adaptive (*4, 6*). Duration of the larval stage for subtidal sponges is typically on the order of a day or more (*4*). *A. fistularis* is a subtidal species in the order Dictyoceratida. The larvae from other species in this order are active swimmers (*5*), and a congeneric species, *Aplysina gigantea*, is believed to produce larvae of relatively long planktonic life (*7*).

Related to the question of larval dispersal is the question of transitivity. Sibling individuals, either dispersed or not, are unlikely to be histocompatible if compatibility requires that they share all their histocompatibility determinants, and as a result of gamete dispersal, they share only one parent. If acceptance requires complete sharing of histocompatibility determinants, the relationships defined by graft acceptances will always be transitive. Intransitive relationships may occur if partial sharing is sufficient (in an intransitive relationship, individual A is compatible with B and C, but B and C are not compatible with each other). The arguments (*1*) against the sufficiency of the transitivity data for *Aplysina* are wanting. Grosberg *et al.* suppose a group of individual sponges, in which 80 percent belong to one clone, and the remaining 20 percent are half-sib progeny of that clone. They conclude that, in 40 trials, there is a probability of 0.446 that all the compatibility relationships examined would be transitive (a result of the high proportion of clonal individuals), even though the group would include some histocompatible half-sibs. The actual probability of a transitive relationship is not 0.98 as they state, but 0.952 (*8*). The probability of 40 transitive relationships is therefore not 0.446 as stated (*1*), but 0.140.

In addition, they ignore other findings regarding transitivity, which we cited (*2*). The data for the genus *Aplysina* alone are considerable. In a Jamaican population of *A. cauliformis*, among 182 trials of transitivity, there were 148 groups of three with no compatible pairs, 34 with transitive acceptances, and none with intransitive acceptances (*9*). From a chart of histocompatibility relationships for a Barbados population of the same species (*10*), I identified 575 groups of three with transitive acceptances, and none with intransitive acceptances. The scenario proposed (*1*) is inconsistent with these data.

The use of histocompatibility bioassays in invertebrate population biology is a new method, and certainly it should initially be employed with caution. The important virtue of this method is that its assumptions, while reasonable, can be subjected to further experimental tests.

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