Asexual Reproduction, Population Structure, and Genotype-Environment Interactions in Sea Anemones

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SYNOPSIS. Our studies of sea anemones reveal that asexual reproduction can lead to the amplification of particularly successful genotypes. Populations of *Haliplanella luciae* studied to date are characterized by exclusively asexual reproduction and typically are dominated by one or a few clones. A field translocation experiment suggests that this population structure may result from differential mortality among colonizing clones, most of which are not preadapted to local conditions. Asexual reproduction by survivors leads to extensive multiplication of one or a few genotypes. *Metridium senile* reproduces sexually and asexually, and we offer evidence that there is significantly less vegetative proliferation, but larger individual body size, in areas of low tidal current velocity than in areas of moderate to high velocities. This may indicate that small individuals (produced asexually) are at a particular feeding disadvantage in slowly moving water, leading to an emphasis on maintaining individual body size at the expense of asexual reproduction. Individuals heterozygous for a phosphoglucose isomerase (PGI) locus appear to be more successful than homozygotes in maximizing body size, independent of current regime, and in maintaining large clone biomass in low velocity habitat. Members of heterozygote clones are significantly more dispersed, some clonemates being separated by 9 meters or more, and are overrepresented in the low velocity habitat. Selection against (small) homozygotes, active choice of habitat, and passive, differential dispersal of larvae and adult anemones may all contribute to this pattern.

INTRODUCTION

A major consequence of ameiotic asexual reproduction is the creation of many organisms in a population, each bearing the same genotype. Potential beneficial results of copying a genome without recombination have been inadequately studied, particularly at an empirical level. Yet it is clear that asexual reproduction can lead to pronounced effects on population genetic structure of organisms making use of it. For example, Hebert (1974) found that in permanent populations of *Daphnia magna*, heterozygotes at certain enzyme loci became relatively more abundant than homozygotes through parthenogenesis. The implication is clear. If a particular genotype is well adapted to a given environment, it may well pay to reproduce that genotype without recombination and without the loss of the 50% of the genome involved in union of haploid gametes from different genotypes (Maynard Smith, 1971; G. C. Williams, 1975). Further, asexual amplification of a genotype will eventually lead to enhanced contribution of that genotype to succeeding sexual generations. In short,

asexual proliferation has potential for enhancing fitness of successful genotypes.

Different species of sea anemones exhibit a variety of reproductive strategies. A particular species may reproduce primarily asexually, while others reproduce only sexually; still others regularly exhibit both (Chia, 1976). This feature has attracted us from somewhat different directions to the Anthozoa in order to evaluate the effects of asexual reproduction on the structure of populations. We independently had been concentrating primarily on two species, one of which is strictly asexual in populations studied thus far, and the other of which always exhibits a mixture of the two reproductive strategies.

HALIPLANELLA LUCIAE

We have studied the ecological determinants of distribution and asexual reproduction of the colonizing sea anemone, *Haliplanella luciae* (Verrill) (Shick, 1976; Johnson and Shick, 1977; Shick and Lamb, 1977). At Blue Hill Falls, Maine, this species extends into the high intertidal, its uppermost limit being set by physiological tolerances and its lower limit by predation (Shick and Lamb, 1977). Unlike *Anthopleura elegantissima*, studied by Sebens (1977), *H. luciae* decreases the incidence of asexual fission in response to food scarcity (Minasian, 1976; Johnson and Shick, 1977), and laboratory experiments by the latter authors demonstrated that maintenance under intertidal conditions also reduces the frequency of longitudinal fission relative to subtidal specimens, consistent with field observations by Uchida (1936).

Electrophoretic analyses (Shick, 1976; Shick and Lamb, 1977) have revealed that individual populations of this introduced species on the U.S. Atlantic coast consist of one or a very few clones, with one clone typically dominating in numbers. We have sought to explain the well-known ephemeral nature of *H. luciae* populations (cf., Stephenson, 1935) in terms of lack of genetic variability in these populations; although *H. luciae* is phenotypically plastic and tolerant of a wide range of environmental conditions, once the genotypic lim-

its for survival are exceeded, entire populations may disappear, since typically only a single genotype is present.

The observed genetic structure of *H. luciae* populations could have several origins: Successful clones may aggressively exclude others from suitable habitat; a single individual may be introduced into unoccupied habitat and proliferate asexually; and/or an area may be colonized by several individuals of different clones, only one of which is preadapted to local conditions. At present there is little evidence for the first of these possibilities. Although *H. luciae* does possess "catch tentacles" used in interclonal aggression (R. B. Williams, 1975; Shick and Lamb, 1977), aggressive behavior has not been ovserved in the field.

An unpublished study by Hieb (1977) provides evidence for a simple founder effect, the second possibility. A female population of one morph of the species occupies a horizontal range of 25 m in the intertidal zone at Bodega Harbor, California. The density of anemones at the center of the distribution is about 500 per m^2 and that at the lateral extremes is 10-20 per m^2. Such a structure likely results from the introduction of one or a few uniclonal individuals with subsequent asexual proliferation spreading away from the point of introduction, although this evidence does not exclude the third possibility.

Field experiments of our own, in which members of a single clone from Charlestown Salt Pond, Rhode Island, were introduced at Blue Hill Falls, Maine, provide evidence of differential survival among introduced clones in a habitat. Anemones (Type 2 anemones in Shick and Lamb, 1977, morphologically and electrophoretically distinguishable from the local population) were allowed to attach to the upper and lower surfaces of a brick, which we then placed in an area of the Blue Hill Falls intertidal zone cleared of other anemones. A brick with members of the local uniclonal population was included as a control. We monitored survival of the anemones from October, 1977, through March, 1978, and the results, shown in Figure 1, indicate greater survival among the Blue Hill Falls animals.
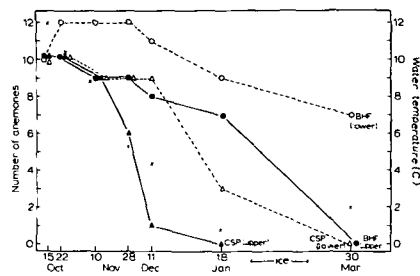
FIG. 1. Survival of clones of *Haliplanella luciae* from Charlestown Salt Pond (CSP) Rhode Island, and from Blue Hill Falls (BHF), Maine, in the intertidal at Blue Hill Falls. "Upper" and "lower" refer to surface of brick. Water temperature on each observation date is indicated by an "x."

The 100% mortality of specimens on the unprotected upper surface of both bricks is probably due to ice abrasion. The differential mortality between the groups on the lower surface likely reflects real physiological differences between the populations. However, the differences are not interpretable simply in terms of the respective habitat temperatures experienced by the two populations, since winter water temperatures at Charlestown Salt Pond (Myers, 1977) are comparable to those at Blue Hill Falls, and the survival of Charlestown individuals in laboratory cultures at 0°-2°C equals that of Blue Hill Falls animals.

One pertinent difference between the populations, however, is their vertical distribution: *H. luciae* at Charlestown Salt Pond ranges from just above mean low water into the subtidal, whereas the species at Blue Hill Falls is strictly intertidal. Laboratory survival under *tidal* conditions (regular immersion and emersion) at 0° – 2°C was excellent in both clones, but one important difference is apparent: whereas all of the Blue Hill Falls anemones remained attached to the culture dishes, 80% of the Charlestown Salt Pond animals tested detached, and the remaining few anemones were barely attached by mucus. At least one cause for the extinction of the Charlestown Salt Pond clone at Blue Hill Falls appears to be its inability to maintain attachment to the substrate at low temperatures under conditions of alternating immersion and emersion. If a *Haliplanella* at Blue Hill Falls releases its hold on intertidal substrate in

winter, the animal will be washed into the subtidal zone, inhabited by the predatory nudibranch *Aeolidia papillosa*. Indeed, a subtidal replicate of the translocation experiment at Blue Hill Falls resulted in *all* anemones disappearing within three weeks at a time when *A. papillosa* was increasing in abundance.

It is clear from the foregoing and from our earlier reports of the enormous success of *H. luciae* at Blue Hill Falls (Shick, 1976; Shick and Lamb, 1977), that differential selection may occur among colonizing clones in the field, and that asexual reproduction can indeed lead to a remarkable amplification of a well adapted genotype. In spite of the success of this clone, we must admit that it represents an evolutionary dead end, since there is no opportunity for sexual reproduction and dispersal of larvae. Because only one genotype is present (we are ignorant of the features of the genotype which led to the success of this clone), an environmental catastrophe or long-term change might well result in the extinction of the entire population, a common phenomenon among populations of this species (Stephenson, 1935; Shick and Lamb, 1977).

METRIDIUM SENILE

Metridium senile (L.) is a conspicuous and particularly well studied anemone found in most of the cold oceans of the world. Sexual reproduction occurs by shedding of gametes into the water, and a typical swimming planula ensues, although metamorphosis has not been observed (Gemmill, 1920). Asexual reproduction occurs by tearing pieces of the basal disk free from the parent, a process known as pedal laceration (Stephenson, 1935). Reports of fission (*e. g.*, Parker, 1899) probably stem from observations of animals with more than one pharynx as a result of injury or abnormal development (Hahn, 1905). There have been repeated references to the asexual capacities of the species and to the clonal structure of populations that results (*e. g.*, Torrey, 1902; Hahn, 1905; Stephenson, 1935; Hoffman, 1976), but there has been no work on environmental factors affecting

pedal laceration, nor an evaluation of the quantitative importance of asexual reproduction. Torrey (1902) and others have noted that asexual reproduction often leads to large aggregations of identically colored polyps, and Purcell (1977a) has recently described aggressive interactions between nonclonemates that may tend to stabilize clonal integrity in California populations. We have not .commonly observed catch tentacles in Atlantic material, and we do not know whether interclonal aggression is important there. Clones frequently sit side-by-side in the field with no evidence of separation and members of one clone commonly move short distances into the midst of adjacent clones (Hoffmann, 1976).

We hoped to take advantage of the mixed sexual and asexual reproductive modes of *Metridium* to examine contributions of asexual reproduction to the population structure of an anemone regularly showing both reproductive methods. We also had reason to believe that we could identify at least one feature of the genotype that would be involved in part in determining fitness.

Significance of a phosphoglucose isomerase polymorphism

Metridium senile populations along the New England coast exhibit a one-locus, two-allele polymorphism for phosphoglucose isomerase (PGI) (Hoffmann, 1976). There is evidence to suggest that the polymorphism is selectively important and that the electrophoretically fast allozyme is a warm water form, while the slow allozyme is better suited to colder waters. There is a cline in allele frequencies such that the putative warm water allele is fixed or nearly fixed in populations south of Cape Cod, while the cold water allele becomes increasingly common north of Cape Cod, up to a frequency of 0.48, in the Gulf of Maine. There are also biochemical differences between allozymes which may help explain in part the basis for this association of allele frequency with temperature (Hoffmann, in preparation). For these reasons, additional details of which will be published elsewhere, PGI may assume added importance as a marker in studies of *Metridium senile* population structure.

The Blue Hill Falls environment

Blue Hill Falls, Maine, is the tidal outflow of Salt Pond, a large, shallow tidal pond that warms considerably during the summer months. The area around the falls itself is alternately influenced by the warm water of Salt Pond on ebbing tides and by the cold water of Blue Hill Bay on flooding tides. Continuous recordings during August, 1978, revealed that water temperature regularly fluctuates between about 17.5°C on ebbing tides and 12°C on flooding tides (Fig. 2). This short-term variation is nearly as great as the difference in August mean water temperature over the range from south of Cape Cod to Eastport, Maine (Coast and Geodetic Survey, 1968), the extreme points in the measured cline of PGI allele frequencies.

We also measured current velocities 10–12 cm above the substrate at numerous locations in the study area on both ebb and flood tides over several days. We have used the maximum observed current velocity as an index of the flow regime. Due to unusual hydrographic features (the cove studied is seaward of the outflow of Salt Pond and slightly higher in elevation than another shoreward cove), flow is unidirectional regardless of whether the tide is ebbing or flooding. We noted this direction of flow at the location of each measurement (see below).

Procedures

We chose a subtidal cove of approximately 9 × 9 m which is exposed only a few times each year during the lowest spring tides, and systematically mapped the positions of all specimens of *M. senile* larger
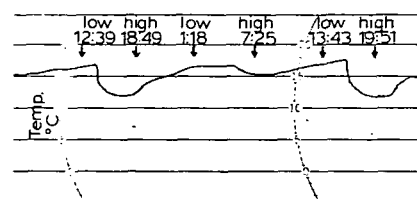

FIG. 2. Continuous recording of water temperature in the subtidal zone at Blue Hill Falls on 13 and 14 August, 1978. Times of high and low tides are shown.

than about 1 cm basal diameter. We purposely ignored very small animals for two reasons. First, they were not always conspicuous enough to provide us with confidence that we could collect all individuals. Second, as very small animals, their successful establishment had not yet been determined. In fact, most of these anemones are gone as of this writing, probably as a result of predation by *Aeolidia papillosa*, which is seasonally abundant in the area.

Collected anemones were numbered by map location and returned to the laboratory where they were weighed and genotyped electrophoretically for four polymorphic enzyme loci (PGI, phosphoglucomutase, and two leucine aminopeptidase loci). Thus, for each of the 169 anemones representing 33 clones (see below) we recorded its map location, ash-free dry weight (estimated from wet weight by a regression of ash-free dry weight on wet weight, $n=31$, $r=0.995$), and genotype at four loci.

Assignments of anemones to clones were based on genotypic identity at the four polymorphic enzyme loci. The probability that a given four-locus genotype would be encountered in a random sample of sexually produced animals is given by the product of the genotype frequencies at each locus (ignoring linkage). Obviously, the more polymorphic loci involved, the less probable will be the occurrence of a given genotype and the more probable that two genetically identical animals are clonemates.

One of the sample populations for PGI geographic variation (Hoffmann, in preparation) came from Blue Hill Falls. In that collection we attempted to minimize the collection of clonemates by taking only widely spaced individuals to obtain an estimate of allele frequencies for the sexually produced settling planulae. As we shall see below, we have not been able to eliminate completely clonemates from such collections, and the result is some uncertainty in our estimates of allele frequencies. In an effort to deal with this uncertainty, we have made two limiting assumptions and made separate estimates of allele frequency based on each.

The first assumption is that none of our animals are clonemates, since we did not collect more than a single animal from an aggregation. The second is that all members of the collection bearing the same four-locus genotype are clonemates. We estimated allele frequencies at each locus based on each of these assumptions and considered the range in frequencies to represent the uncertainty of our estimates. This uncertainty is not large.

To be as conservative as possible, we have calculated the probability of encountering each genotype in the present collection on each of the above assumptions and then have chosen the higher of the two as our confidence estimate for clonal assignments. The genotype with the highest probability of occurrence has $P=0.075$. That is, we are 92.5% confident that any two animals of this genotype are clonemates. This is the lowest confidence for any clone we encountered. The least probable genotype in the present collection has $P=5 \times 10^{-5}$. The mean is $P=0.021$, so that on average we are about 98% confident of our clonal assignments. In short, we are reasonably certain that most of the animals in our collection bearing the same four-locus genotype are clonemates, and we have proceeded on this assumption. We are aware that a few of our "clones" are possibly of mixed origin, but we believe that most are not.

For some purposes we divided animals into small and large size classes based on a break in the size-frequency histogram of all ash-free dry weights. Large anemones are ≥ 9.75g, while small anemones are < 9.75g.

The location, PGI genotype, and clone assignment number of each animal is shown in Figure 3. Circled animals are ≥ 9.75g. Current velocities are also shown, as well as current direction. Double-headed arrows for current measurements represent eddies. The study area is not uniform with respect to tidal current velocity, and the cove can be divided into two habitats— one of low current velocity (longitudinal map zones G through J, in which the maximum measured velocities do not exceed 5 cm sec^{-1}) and the other of moderate to high velocity (longitudinal zones A–F, in which the maximum velocities range from 10–85
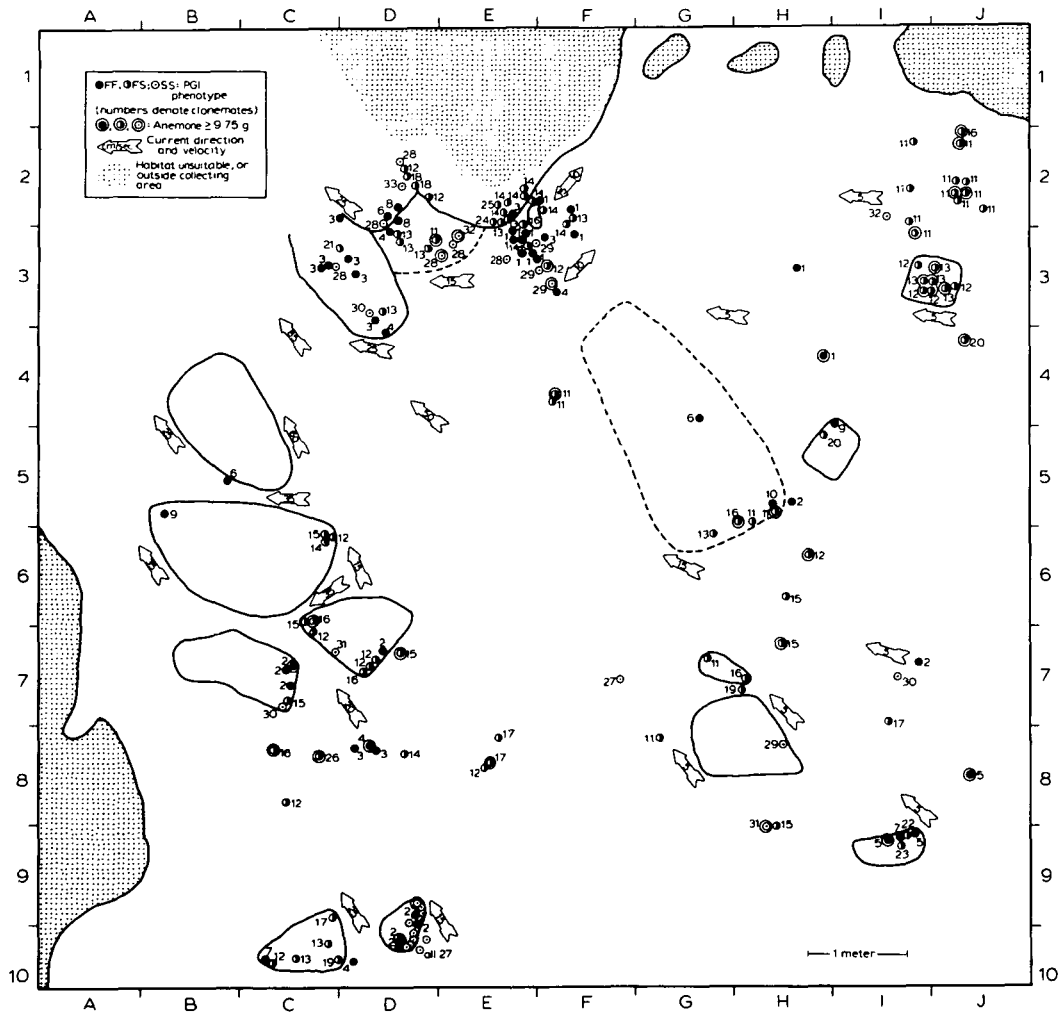
FIG. 3. Map of study site for *Metridium senile* at Blue Hill Falls. Shown is the location and PGI genotype of each individual. Numbers next to each anemone represent clone assignment numbers. Anemones ≥ 9.75 g are circled. Direction and maximum velocity of tidal currents are indicated by arrows. See text for details of collection and analysis.

cm sec^{-1}). Table 1 summarizes the weight data for anemones as related to their PGI genotype, clone assignment number, and current regime in which they were collected.

Spatial distribution of clones

Some of the clones are compact, as one would expect for sessile animals which reproduce vegetatively. The best example of this is clone 27 (quadrats D9 and D10). Surprisingly, however, many of the clones have representatives distributed widely over the sampling area (*e.g.*, clones 11, 12, 13, and

15), and some clonemates are separated by distances of 9 m. This wide dispersion of clonemates was unanticipated in view of accounts in the literature of the process of pedal laceration and formation of large aggregations of *M. senile* (*e.g.*, Torrey, 1902). Sea anemones, including *M. senile*, are known to be capable of pedal locomotion (McClendon, 1906), but the few quantitative studies available indicate that longterm net movement occurs only over rather short distances (*e.g.*, Fleure and Walton, 1907; Osburn, 1914; Parker, 1917; Ottaway and Thomas, 1971; Ottaway,

1973; Dunn, 1977; Ottaway, 1978).

The broad dispersion of clonemates is not unique to the Blue Hill Falls population. Sufficient spatial data are available from populations of *M. senile* inhabiting rock jetties on Cape Cod, Massachusetts, to demonstrate that other clones are distributed over comparable distances. Further, collections of single animals from each of 22 pilings supporting the Lagoon Pond Bridge on Martha's Vineyard during two successive summers contained representatives of only three clones, the same three both years.

We partially understand the bases for the dispersal of *Metridium* at Blue Hill Falls. Much of it is passive. Many of the anemones are attached to mussels (*Modiolus modiolus* and *Mytilus edulis*) and to single mussel valves, and we have observed anemones on unanchored valves being transported by tidal currents. After our clearing of the cove in July, 1978, two adult immigrants were found in August, and 11 more in November; several of these immigrants were attached only to small pieces of mussel shell. Further, we have observed *M. senile* to release its hold on the substrate in response to an attack by *Aeolidia papillosa*, the free-floating anemone being swept away by the current. Other workers have also noted this phenomenon, for example, Harris (1973), who emphasized that this response to predation may be of general importance in sea anemone distribution. Several of the anemones found in November had settled in a low current velocity area and were completely unattached; *A. papillosa* was rampant at the site. It seems more reasonable to attribute dispersal of the magnitude that we observe to these current-related processes than to crawling by anemones, although as we will suggest below, this too may be important. Interestingly, Fleure and Walton (1907) noticed the sudden appearance of large specimens of *Metridium* (= *Actinoloba dianthus*) in an area from which they were previously absent. This may well have resulted from similar processes.

Dispersal of anemones is more commonly associated with the sexually-produced planktonic planula larva. The greatest density (in terms of number of clones, as well as individual anemones) oc-

curs in the region at the top of the map in quadrats C3; D2,3; E2,3; and F2,3. In this small area of 6 m², 18 of the total 33 clones are represented, suggesting that it may be an initial settlement site for planulae or a "sink" for drifting adults. This supposition is supported by the general current pattern in the cove. That planulae are involved is indicated by the absence of any unattached adult colonists in this area, and by a simple experiment in which small styrofoam beads were released in the center of the cove; most of the beads, representing planktonic larvae, were carried out of the cove, but those that remained were trapped among the crevices, densely-packed mussels, and current eddies in the "sink" region. Sebens (1977) has observed initial settlement of *Anthopleura elegantissima* larvae in mussel beds and subsequent emigration of juveniles from the beds.

The role of current in structuring the population

Current velocity has both obvious and subtle effects on the spatial structure of the *Metridium* population, since not all of the seemingly available substrate is occupied by anemones. At one extreme, there are no anemones 1 cm diameter or larger in areas of very high current velocity ($\geq$ 50 cm sec^{-1}), likely due to mechanical constraints of the animal's body plan (Koehl, 1977b).

Referring to Table 1, there are three pertinent observations relative to the low velocity habitat (map zones G-J): (1) there is a significant tendency for *Metridium* to produce fewer individuals per clone in this area than in the moderate velocity area (Wilcoxon signed ranks test; $P < 0.005$), so that 22 of the 33 clones (67%) are represented, but only 52 of 169 individuals (31%); (2) anemones of the FF and FS PGI genotypes are significantly larger than in the moderate velocity area (t-tests; $P < 0.001$ in both cases), and the SS individuals also tend to be larger in the low velocity area; (3) there is a disproportionate representation of PGI heterozygotes in the low velocity area (70% of the anemones present there are heterozygotes as opposed to 40% in the moderate velocity habitat), and the difference is significant ($P < 0.001$).

Taken together, (1) and (2) suggest that in low current velocity habitats, more effort is put into individual growth than into asexual proliferation. Chia (1976) has noted that *M. senile* in shallow water (where current velocities will be relatively high) typically is small and forms aggregations by pedal laceration, whereas deep-water (lower current velocity) specimens tend to be large and solitary. Although we did not collect small anemones resulting from recent pedal laceration, we observed them to be common in the moderate velocity area and all but non-existent in the low velocity area. (These observations are from field notes made before the cove was partitioned according to flow velocity.) Although it has been observed that small specimens of *Metridium* are preferentially consumed by *Aeolidia papillosa* except when they occur in large aggregations (Harris, 1973), our study was conducted in July, when the nudibranch is not present and when the incidence of pedal laceration is high.

Because of the active and passive dispersal of adult anemones, no one hypothesis is likely to explain the observed size distribution pattern at our study site. Nevertheless, the apparent maximization of individual body size at the expense of asexual reproduction in low velocity habitats may be an accommodation to the constraints of acquiring adequate food in these passive suspension feeders. Wainwright and Koehl (1976) and Koehl (1976; 1977a) have discussed the problems of flow and the morphological adaptations of sea anemones to it. When water moves across the substrate, velocity is at a minimum immediately adjacent to the substrate (the low velocity boundary layer) and increases with distance from the substrate, up to a point (the mainstream current velocity). A passive suspension feeder such as *M. senile,* which inhabits areas of low velocity tidal currents and yet relies on current to bring its planktonic food, maximizes the effect of flow by being large (tall), which provides an escape from the boundary layer and maintains the feeding apparatus in the current mainstream (Koehl, 1977a). Coelenteron content analyses by Purcell (1977b) indicate that both large and small specimens of *Met-*

ridium primarily use planktonic food; based on limited field observation of *M. senile* populations, she further suggests that large individuals may be favored in habitats providing only planktonic food.

Sebens (1977, and this symposium) argues that longitudinal fission in *Anthopleura elegantissima* will increase the prey capture surface relative to body volume and therefore increase clone (and gonad) biomass. This presupposes that prey are readily available to all individuals, which is likely the case in the wave-swept habitat of *A. elegantissima,* but may not be true for small pedal lacerates of *M. senile* beginning their independent existence in the boundary layer of an already low velocity environment. Here there is a premium on maintaining large (tall) individual size to maximize prey capture.

Although it is difficult to envision any significant cost in terms of reduction of body size to a 10 g *Metridium* of producing a 10 mg pedal lacerate, it is common for numerous asexual progeny to be produced at a single laceration event, so that the total biomass involved may be substantial. Also, the constraint of food availability may apply to the pedal lacerate itself. Calow (1977) has correctly argued that the partitioning of a given quantity of biomass into a large number of small units (*e.g.,* asexual propagules) results in greater catabolic output per unit weight than does partitioning the same biomass into fewer large units. If small polyps in the boundary layer of low velocity habitats are unsuccessful in prey capture (that is, if asexual proliferation does not increase energy input for the clone), Calow's argument that maximization of individual body size will allow the maintenance of a greater biomass at the level of the population *per unit of energy flow* may be applicable to *Metridium* clones without justification of the "metabolic altruism" of large individuals by group selectionist arguments (Calow, 1977, p. 13). Earlier data of our own (Johnson and Shick, 1977) for uniclonal *Haliplanella luciae* support this suggestion: There was an inverse correlation between number of individuals and clone biomass when cultures were maintained on a limiting ration. Data on the re-

levance of these hypotheses to *M. senile* may be forthcoming from other workers. M. Koehl (University of California, Berkeley) is investigating prey capture by *Metridium* of different sizes in different current velocities in laboratory flow tanks, and K. Sebens (Harvard University) is assessing plankton availability in different flow regimes in nature.

Genotype-related "success"

Our original objective was to examine the possibility of differential success among clones of *Metridium senile*. In this context, we assume that a given clone will have potentially greater fitness if its members collectively produce more gametes than do other clones. Thus, the success of a clone involves not only numbers of individuals, but, in animals capable of indeterminate growth and of degrowth, also includes individual body weight, because gonad production is directly proportional to body weight or volume (Sebens, 1977).

Because the large population of *Metridium* is twice daily subjected to pronounced thermal variation (Fig. 2), PGI heterozygotes might be more successful here, perhaps due to a metabolic flexibility afforded by having both the warm and cold water allozymes, as well as the heterodimer. We of course do not contend that PGI genotype alone determines success, but it is an identifiable character that varies predictably with temperature and which may be one of many significant contributors to fitness. The two PGI alleles occur with almost equal frequency in this habitat, so there was hope for examining a reasonable number of clones of each genotype.

Perhaps because of the imprecision inherent in associating "success" with the single character of PGI genotype, and because PGI heterozygotes tend to occur in a current regime which minimizes asexual proliferation (see previous section), there is not a significant difference between homozygotes and heterozygotes in the number of individuals per clone ($F_{[1,31]}$= 0.127; $P > 0.50$). This lack of significance is also due in part to the relatively large number (six, or 37.5%) of heterozygote

clones represented by single anemones, individuals which may be recent immigrants into our study area (see above).

Because both numbers of anemones per clone and clone biomass are potential components of fitness, we have combined the two measures in a regression of total clone biomass on numbers of individuals per clone. A larger individual body size of the heterozygotes is indicated by this regression (Fig. 4). The slopes of the regressions (*i.e.*, biomass per individual) for the homozygotes do not differ significantly ($q_{[13]}$= 1.24°· $P <0.50$) (Zar, 1974) but the slope of the regression for the heterozygotes is significantly greater than that of the FF homozygotes ($q_{[22]}$ = 6.946; $P <0.001$) and that of the SS homozygotes ($q_{[19]}$ = 3.748; $P < 0.025$). However, this apparently greater individual weight of the heterozygotes may be partly due to their overrepresentation in the low velocity area, which promotes large body size. When homozygotes and heterozygotes occurring in the moderate velocity area (which does not promote large size) are compared (Table 1), no significant difference emerges ($t = 0.900$; df = 115; $P > 0.20$), nor indeed are there significant differences between these groups within the low velocity area ($t = 0.561$; df = 50; $P > 0.50$).

The divergence between the regression line for the heterozygotes and those of the homozygotes is not apparent until a thresh-
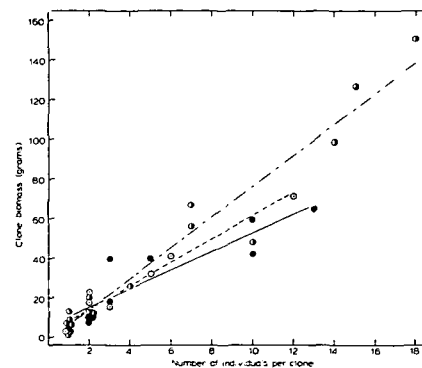
FIG. 4. Regressions of clone biomass on number of anemones per clone for the three PGI genotypes. ●, FF homozygotes; ◑, heterozygotes; ○, SS homozygotes. Each point represents a single clone. The slope for heterozygotes is significantly steeper than that for either homozygote.

TABLE 1. Asexual reproduction frequency (number of individuals), individual body weight and clone biomass (grams ash-free dry weight) in Metridium senile related to PGI genotype and tidal current regime occupied.*

PGI FF homozygotes

Clone number	Number of individuals	Number in low velocity area	Body weight ($\bar{X} \pm$ S.E.)	Clone biomass	Number in moderate velocity area	Body weight ($\bar{X} \pm$ S.E.)	Clone biomass
1	13	2	8.90±3.27	17.80	11	4.27±0.56	46.97
2	10	2	6.71±1.98	13.42	8	5.80±1.28	46.40
3	10	0	—	—	10	4.22±0.69	42.20
4	5	0	—	—	5	8.03±2.68	40.15
5	3	3	13.45±3.94	40.35	0	—	—
6	3	1	9.13	9.13	2	4.72±0.38	9.44
7	2	1	4.58	4.58	1	5.75	5.75
8	2	0	—	—	2	3.97±3.00	7.94
9	2	1	8.36	8.36	1	1.99	1.99
10	1	1	4.18	4.18	0	—	—
	LOW VELOCITY AREA:	TOTAL NUMBER = 11	MEAN BODY WEIGHT = 8.89±1.46	MEAN CLONE BIOMASS = 13.97±4.76	MODERATE VELOCITY AREA:	TOTAL NUMBER = 40	MEAN BODY WEIGHT = 5.02±0.51	MEAN CLONE BIOMASS = 25.11±7.20

TABLE 1. (continued)

PGI FS heterozygotes

Clone number	Number of individuals	Number in low velocity area	Body weight ($\bar{X}\pm$ S.E.)	Clone biomass	Number in moderate velocity area	Body weight ($\bar{X}\pm$ S.E.)	Clone biomass
11	18	14	8.28±0.87	115.92	4	8.50±2.88	34.00
12	15	5	12.47±2.45	62.35	10	6.44±1.02	64.40
13	14	5	11.97±1.64	59.85	9	4.29±0.73	38.61
14	10	0	—	—	10	4.75±0.47	47.50
15	7	3	7.00±1.55	21.00	4	8.84±3.05	35.36
16	7	3	12.07±2.74	36.21	4	7.57±2.71	30.28
17	4	1	6.90	6.90	3	6.27±1.85	18.81
18	2	0	—	—	2	6.35±0.35	12.70
19	2	1	8.82	8.82	1	3.51	3.51
20	2	2	10.02±1.34	20.04	0	—	—
21	1	0	—	—	1	8.19	8.19
22	1	1	7.40	7.40	0	—	—
23	1	1	3.77	3.77	0	—	—
24	1	0	—	—	1	5.99	5.99
25	1	0	—	—	1	1.72	1.72
26	1	0	—	—	1	13.23	13.23
		LOW VELOCITY AREA: TOTAL NUMBER = 36	MEAN BODY WEIGHT = 9.50±0.66	MEAN CLONE BIOMASS = 34.23±11.32	MODERATE VELOCITY AREA: TOTAL NUMBER = 51	MEAN BODY WEIGHT = 6.07±0.52	MEAN CLONE BIOMASS = 24.18±5.33

PGI SS homozygotes

Clone number	Number of individuals	Number in low velocity area	Body weight ($\bar{X}\pm$ S.E.)	Clone biomass	Number in moderate velocity area	Body weight ($\bar{X}\pm$ S.E.)	Clone biomass
27	12	1	4.65	4.65	11	6.03±0.97	66.33
28	6	0	—	—	6	6.97±2.06	41.82
29	5	1	7.62	7.62	4	5.99±1.88	23.96
30	3	1	6.59	6.59	2	4.69±1.31	9.38
31	2	1	15.97	15.97	1	6.78	6.78
32	2	1	8.21	8.21	1	9.75	9.75
33	1	0	—	—	1	3.45	3.45
		LOW VELOCITY AREA: TOTAL NUMBER = 5	MEAN BODY WEIGHT = 8.61±1.94	MEAN CLONE BIOMASS = 8.61±1.94	MODERATE VELOCITY AREA: TOTAL NUMBER = 26	MEAN BODY WEIGHT = 6.21±0.69	MEAN CLONE BIOMASS = 23.07±8.78

* See text for tests of significance.

old of three or four anemones per clone is achieved (Fig. 4). This may reflect the contribution of longevity to apparent success: Marginal differences among genotypes may be magnified through time. We emphasize that we have no data on the ages of individuals or clones, but longevity would nonetheless be an indication of temporal success, which provides an indication that PGI heterozygotes tend to be more successful in this particular environment.

Dispersion of anemones may be an index of longevity, older clones having longer to disperse. One might further imagine that more widely dispersed clones escape competition in dense aggregations of clonemates and are better colonizers of new areas. They would be less subject to complete devastation through predation or physical catastrophe. As a result, the spatial extent of clones may also measure their success. We estimated the extent of clones of three or more individuals by calculating the center of distribution (Hayne, 1949) for each clone and then measuring the distance (d) from each member of a clone to its clonal centroid. If we compare these distances between homozygotes and heterozygotes, summing across all polyps of the appropriate genotype, the heterozygotes are significantly more removed from their clonal centers than are pooled homozygotes. This is true whether the measure tested is d ($F_{[1,142]} = 29.489; P < 0.001$) or d^2 ($F_{[1,142]} = 15.984; P < 0.001$), which is proportional to area occupied.

Genotype-environment interaction

We have shown in *Metridium senile* that individual body size and the tendency for the anemone to proliferate asexually are dependent on the current velocity which the anemone experiences (Table 1). Therefore, the analysis of any genotypic effect on clonal success is not straightforward, and is further complicated by the overrepresentation of the heterozygotes in the low velocity area. For these reasons, total clone biomass, which combines the measures of numbers *and* individual size, might be a more appropriate index of clonal success.

Although there is a tendency for *Met-*

ridium to produce clones having larger biomass in the moderate velocity area (Table 1), current regime does not significantly affect total clone biomass within a genotype (*t*-tests: FF-$t = 1.250$, df = 13, $P > 0.20$; FS-$t = 0.866$, df = 21, $P > 0.40$; SS-$t = 1.356$, df = 10, $P > 0.20$). Viewed together with the other data in Table 1, this suggests that the different responses of body size and asexual proliferation to current velocity result in a convergence of fitness (clone biomass) across habitats. Within the moderate velocity area, there is likewise no difference between heterozygote and (pooled) homozygote clones in their total biomass ($t = 0.003$; df = 26; $P > 0.90$). Importantly, however, heterozygote clones do produce significantly more biomass in the low velocity habitat than do the homozygotes ($t = 2.089$; df = 20; $P < 0.05$).

Thus, in the region of moderate to high current flow, where the amount of food available per unit time is likely to be great, all clones appear to achieve comparable success. In the low flow area, where planktonic food is more limited and selection pressures are therefore intensified, differential success among genotypes is detectable.

The greater biomass per clone in the heterozygotes in the low flow area may involve individual size differences despite our inability to demonstrate significant differences among genotypes in *mean* body weight. The size-frequency histogram of all individuals in the sample reveals a nonrandom distribution, and analysis of this histogram by the method of Cassie (1954) indicates a distinct break in the distribution, such that there is a class of body weights continuously distributed up to about 9.75 g (small anemones) and a second class of individuals above this weight (large anemones). A three-way G-test (Sokal and Rohlf, 1969) reveals significant overall heterogeneity ($G_{[4]} = 29.283; P < 0.001$) among the factors of PGI genotype (homozygote and heterozygote), body size (large and small), and current velocity (low and moderate). More specifically, (1) size is not independent of current velocity ($G_{[1]} = 12.821; P < 0.001$), (2) genotypes are not distributed independently of current velocity ($G_{[1]} =$

13.502; $P < 0.001$), and (3) size is not independent of genotype ($G_{[1]} = 6.808$; $P < 0.01$). Observations (1) and (2) follow from the data in Table 1 and have been discussed earlier.

The third observation, that very large body size is genotype-related, is important because in animals capable of degrowth (shrinkage) as well as growth depending on nutritional conditions, maintenance of near-maximal body size indicates their ability to maintain a greater positive energy balance through time. This observation, and the fact that the heterozygotes in the low velocity area produce larger clone biomass (which likely requires substantial time to accumulate), emphasize the temporal component of success in these animals.

Because the three-way interaction among the terms examined in the G-test suggests that the factors may be non-orthogonal ($G_{[1]}= -3.848$), the possibility also remains that the near-maximal size of PGI heterozygotes is an artifact of their abundance in a flow regime favoring large size. However, when we consider only heterozygotes, there is not a significant difference in their tendency to be large (≥ 9.75 g) in the two flow areas ($G_{[1]} = 2.236$; $P < 0.20$). Of course, this implies that those anemones in moderate-flow areas that are large tend to be heterozygotes. Both current velocity and genotype contribute to individual size in *Metridium senile*.

Some unanswered questions

If it is particularly difficult for a small *Metridium* in the boundary layer of a low current velocity environment to capture food, how does the population become established there originally, if metamorphosing planulae find little to eat as juvenile anemones? Perhaps most of the juveniles do not survive, and the few that do are products of selection for high growth efficiency on what ration is available, since *Metridium* is not limited to planktonic food. Cnidarians are known to take up dissolved organic molecules (see Shick, 1975; Schlichter, 1978), and we have also observed small specimens of *Metridium* to feed on bottom

materials by sweeping the substrate with their tentacles. An additional possibility is that recruitment is accomplished by adult anemones, and we in fact observed 13 adult immigrants into the area within four months after we had cleared it.

If we accept the hypothesis that large individual size is maintained at the expense of asexual proliferation in low velocity habitats, how is it that some clones (*e.g.*, 11, 12, and 13) produce large individuals *and* large clones in such habitats? Perhaps once large size is achieved and food becomes increasingly available as a consequence, any surplus is *then* put into asexual propagules. Perhaps most of the resultant pedal lacerates do not survive, but some (PGI heterozygotes, among others) may have sufficiently high growth efficiencies to grow rapidly and to persist. Or perhaps migration of adults is the key.

The disproportionate representation of PGI heterzygotes in the low velocity area suggests a particularly intriguing question: Is their predominance a result of selection against the homozygotes, or is there an active choice of habitat, since anemones display rheotactic behavior (Pearse, 1974; R. Robbins, unpublished observations)? Although less planktonic food will be available per unit time in the low velocity environment, might this be offset by reduced competition in this less populated area, an area which is best exploited by large (tall) individuals?

In summary, we believe that we have demonstrated that the genotype of a clone contributes at least some measurable component of success. We wish to reiterate that we do not propose PGI genotype to be the only genetic factor determining this success, but that the metabolic flexibility afforded PGI heterozygotes may provide some advantage in this thermally variable environment. Tidal current velocity, likely operating through providing variable amounts of planktonic food, also contributes to individual body size and to the tendency of anemones to reproduce asexually, and this contribution is reflected in clone biomass and presumably in gamete production. Thus, there are both genotypically determined and environmentally in-

duced effects on measures of success in asexually reproducing sea anemones, so that these animals can provide quantitative insight into evolutionary processes. However, the environmental relationships may be quite complex, and there is also the possibility that current, acting to transport adult anemones, is to some extent responsible for the non-random distribution of large anemones in our study site. This could occur if already-large adult animals are deposited preferentially in the low velocity area while planulae or small anemones are carried beyond this area. Finally, we are well aware that this study represents but a single data point, spatially and temporally, in any verification of these several hypotheses, and we are following the course of repopulation of the site at Blue Hill Falls.

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