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Partitioning Phenotypic Variation: Genotypic, Environmental And Residual Components From Bryozoan Skeletal Morphology

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Abstract

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Partitioning phenotypic variation: genotypic, environmental and residual components from bryozoan skeletal morphology

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Using morphometric studies of colonial (clonal) organisms such as Bryozoa grown as replicates in controlled laboratory experiments, phenotypic variation (hard part morphology) can be partitioned into its genotypic and environmental (ecophenotypic) components. The interaction between these, i.e. different genotypic responses to the same environmental change, can also be recognized. Palaeobiological studies are inherently constrained by species concepts based on morphotypes (preserved morphological phenotype). Uncertainties associated with fossil species concepts restrict the deductive resolution potential of fossil taxa in discussions of the broader biological questions of species evolution, ecology, biogeography and phylogeny. The relationship between species-level morphological variation and genetic variation in modern taxa is central to evaluating the viability of fossil morphotypes as biological species. Results from a preliminary study of three genotypes of *Electra pilosa* L., grown as replicate colonies in tanks comprising different microenvironmental conditions, allow for direct evaluation of morphospecies concepts.

Numerical analyses (Cluster Analysis, Principal Component Analysis and Two-way ANOVA) of six morphometric characters demonstrated a strong Genotype control over zooid morphology and limited environmental (Tank) effects with minimal environmental differences among tanks. No significant Genotype by Tank (= environment) interactions were found for any characters. These results demonstrate that it is possible to quantify the extent to which a given trait is plastically expressed in different environments. The strong degree of correlation between morphology and genetics for this species is encouraging for the use of morphotypes as proxies for biological species.

KEYWORDS: Phenotype, genotype, environment, variation, morphology, species.

Introduction

Interpretation of morphological variation within and among populations is an implicit requirement for most whole-organism studies, whether they be systematic

or ecological. Whatever the focus of study, assumptions must be made about the intrinsic and extrinsic factors (components) that actually control morphological variation. In general terms, all morphological variation can be attributed to genetic and environmental differences within and among populations. Uncertainty about the degree of correlation between quantified genetic variation and quantified morphological variation among colonies has raised questions about the biological significance of morphotaxa (e.g. Joysey, 1952; Nevesskaya, 1967; Levinton, 1988). Whenever taxonomic concepts rely heavily on morphology, as do those for most fossil species, documentation of the relative importance of the genetic and environmental components of morphological variation is crucial. But only seldom (Jackson and Cheetham, 1990; Cheetham *et al.*, 1993; Hunter and Hughes, 1994, 1995; Whitehead *et al.*, 1994) have there been empirical tests of the significance of ecological and genetic variation in a contemporaneous study, due largely to logistical problems encountered with implementation of the experimental design. The latter should, however, be readily resolvable by the experimental generation of multiple populations of cloned organisms. There are many studies of environmentally induced variation in solitary organisms (e.g. Alexander, 1975; Malmgren and Kennett, 1976; Bergström and Levi-Setti, 1978; Johnson, 1981). However, among individual solitary organisms, the relative contribution of genetic differences (and independent genotype by environment interactions) cannot be known without exhaustive genetic typing of individuals or systematic production of clonal replicates.

Colonial organisms such as Bryozoa provide a study group particularly well suited to the partitioning of phenotypic variation into its genetic and environmental sources. As a single colony grows, individual bryozoan zooids (modules) generate their skeletal morphology in response to a developmental programme and the prevailing environmental conditions at their morphogenesis (e.g. Boardman *et al.*, 1970; Jebram and Rummert, 1978; Taylor and Furness, 1978; Jebram, 1980; Harvell, 1986; Okamura, 1987, 1992; Okamura and Bishop, 1988; Barnes and Clarke, 1994; Barnes, 1995). As a result, a series of asexually generated, individual zooids within each colony may provide a record of phenotypic variation induced within a single genotype.

Excluding possible somatic mutation or horizontal gene transfer, all individual zooids within a colony are genetically identical. Individuals (zooids) within a colony can be thought of as repeated, genetically identical modules. Therefore, an individual zooid within a single colony can be treated experimentally and statistically as a member of (datum representing) any level within the hierarchy of:

- a species or any higher taxonomic group,
- a biological population of multiple colonies,
- a colony (= single genotype), analogous to a unitary organism,
- a zone or region within a colony (e.g. single branch or cormidium),
- an individual zooid within any of the above higher categories.

Typically, sexual reproduction in bryozoans results in planktonic larvae that settle and metamorphose to establish new colonies; these grow by asexual budding within single colonies sharing tissue and, when present, hard skeletal material. These and other characteristics of bryozoans make them particularly well suited for studies in which phenotypic variation is partitioned into its component sources. Here we use the term *single* as the unit adjective for a colony (single genotype) and *individual* as the unit adjective for a zooid (module) within a colony. Under different contexts,

characteristics both of a single colony and an individual zooid can be compared to an individual solitary organism.

The goals of this study of the modern cheilostomatid bryozoan *Electra pilosa* (L.) are four-fold:

(1) to summarize briefly the existing models that account for sources of morphological variation within and among populations;
(2) to demonstrate, from a preliminary study, that phenotypic variation can be partitioned into its components of genotypic and environmental origin, using colonial Bryozoa;
(3) to discuss the importance of residual variance, and propose improved models, which account for these components of morphological variation; and
(4) to discuss the relevance of studies using morphotypes to the taxonomic, ecological and evolutionary interpretation of fossil species concepts.

Past studies have focused either on the genetic or environmental control of hard part morphology; in either case, one has largely been considered whilst ignoring the other. The present study was a preliminary exploration of the possible methods which might allow the broader simultaneous evaluation of both the environmental and genetic controls on hard part morphology. We considered that the next logical step in the study of the relationships between hard part morphology and heritable genetic variation would be to undertake similar studies on multiple populations of cloned colonies, maintained in controlled environments, as in the present laboratory study. This approach also offers the advantage of circumventing the constraint imposed by fossil specimens on the study of within- versus among-colony variation.

Historical background

Species concepts

Historically most bryozoan species, fossil or modern, have been recognized on the basis of their hard part morphology. Species concepts in Bryozoa are based primarily on zooid-level characteristics. Colony-level characteristics, such as growth habit and overall shape, either are of higher level taxonomic significance and/or are ecologically controlled (Boardman *et al.*, 1983). Bryozoan taxa have variously been treated as anything from nominal species-level morphotypes (of unknown genetic significance) to true biological species. Although early descriptions of bryozoan species often comprised quantitative descriptions or comparisons of morphological features (i.e. observed size range), it was not until the 1960s that rigorous statistical methods were applied to populations (Boardman, 1960; Perry and Horowitz, 1963; Tavener-Smith, 1965, 1966; Cuffey, 1967; Anstey and Perry, 1970). Multivariate approaches to bryozoan morphological and taxonomic analysis were presented in the late 1960s and 1970s (Cheetham, 1968; Ryland, 1975; Cheetham and Lorenz, 1976; Cook, 1977), but did not become widely applied until the late 1980s.

The application of multivariate morphometric methods to the development of species concepts in colonial organisms was pioneered by Cheetham (1968, 1986) for bryozoans and Budd (Foster 1984, 1985; Budd, 1990, 1993) for scleractinian corals (Knowlton and Budd, in press). Those methods exploit the features of colonial animals that allow for the establishment of testable hypotheses regarding the relationships of individual zooids or corallites within a (taxonomic) hierarchy, based on

multivariate morphological variance within colonies (shared genotype) versus among-colony variation for the same or putatively different species.

Snyder (1991) generated a multivariate data set based on 44 morphological characters collected from multiple colonies representing 39 species (13 genera) of Early Carboniferous bryozoans. Hageman (1991a, 1991b) employed univariate and multivariate statistical methods to evaluate Snyder's taxonomic classification, and all 39 species could indeed be recognized objectively using only the morphometric data. That is, the breaks in multivariate morphospace between the morphology of identified species were sufficiently discrete to allow for grouping or allocation of individual zooids to their *a priori* species using only hard part measurements. Nonetheless, even though Snyder provided for his 39 species one of the most comprehensive descriptions supported with quantitative arguments, his fossil species were based inevitably on his classification of morphotypes.

Concerns about fossil species concepts extend far beyond taxonomy. Fossils can provide a tangible record of the evolutionary succession through geological time, a perspective not available to students of modern ecology. However, even in strata with an almost complete time representation, the question of the degree to which morphological variation reflects genetic variation looms large in the application of fossil species to studies of fundamental microevolutionary patterns and processes (Hillis, 1987; Levinton, 1988; Larson, 1989; Shaffer *et al.*, 1991). Resolution of this problem for empirical studies of microevolution through stratigraphic successions has important implications for addressing both the origin and subsequent history of species. Faced with this uncertainty as criticism of their broader studies of evolutionary patterns, Jackson and Cheetham (1990) set out to test the relationship between morphology and genetics in 23 species of modern Caribbean bryozoans from three genera. Allocation of individual zooids to 23 species on the basis of the morphometric analysis of hard part morphology (Cheetham, 1986), and the classification of individual zooids into species using data from the same colonies on protein electrophoresis, were congruent for 99% of the cases (Jackson and Cheetham, 1990). Studies of scleractinian corals using similar techniques produced similar conclusions, documenting strong correlations between hard part morphology and genetic composition for the organisms studied (Potts *et al.*, 1993).

The clear correlation between quantified morphological variation and quantified genetic variation among colonies in Jackson and Cheetham's (1990) modern bryozoans and in the corals of Potts *et al.* (1993) suggests that the 39 discrete morphotypes of late Palaeozoic fenestrate bryozoans recognized by Snyder (1991) also represent true biological species. Using the methods of Cheetham (1986), Lidgard and Buckley (1994) also recognized discrete morphotypes within modern *Adeonellopsis*. Other studies of Late Carboniferous fenestellid and cryptostomatid Bryozoa support the recognition of discrete, species-level morphologies based on multivariate morphometric analysis of a large suite of characteristics (Holdener, 1994, 1998).

Any assertion of species recognition using morphometric criteria assumes that the important morphological patterns can be distinguished from subspecific morphological variation. Hageman (1994, 1995) and Holdener (1994, 1998) demonstrated that subspecific morphological variation correlated with geographic and environmental variation among time-averaged populations in a number of Late Palaeozoic bryozoan species (i.e. a distinct morphological difference among time-averaged populations, but an among-population difference of a magnitude far less than differences between their morphologically closest species).

Other studies of variation within large single colonies of bryozoans (Abbott, 1973; Taylor and Furness, 1978; Hageman 1995; Holdener and Hageman, 1998) have been undertaken in order to identify the range and distribution of morphological variation (1) within a colony (i.e. non-genetic); versus (2) variance among conspecific colonies (at least partially genetically controlled); versus (3) variance among colonies of different species. Ranges and limits of morphological variation within and among colonies, populations and species must be documented in order to establish strong species concepts, and so that potential non-genetic sources of variation can be recognized within a species (Abbott, 1973). Results suggested that zooids could be assigned not only to the single colonies from which they were measured, but to their original region within the colony based on their morphology alone (Hageman, 1995; Holdener and Hageman, 1998). In addition, although the separate colonies within the morphospace often overlap, the morphospace occupied by a single colony represents only a small portion of the morphospace occupied by all other conspecific colonies combined (i.e. morphospace of the species). These observations have important implications for strategies of morphometric data collection, when a limited number of measurements are intended to represent a single colony, whole population or even an entire species.

Although the collective work of Hageman and Holdener evaluated variation within and among (putative) conspecific specimens, the fact that specimens were from fossil fragments of unknown genetic affinity limits the broader application of inferred controls over morphological variation. By contrast, Cheetham *et al.* (1993, 1994, 1995) evaluated within- versus among-colony variation in three species of modern bryozoans from two genera grown in 'common garden' conditions. In assessing patterns of maternal inheritance of colonies reared through three generations, they applied quantitative genetic techniques to hard part morphological data (Falconer, 1981). Those data also strongly supported a correlation between variation of hard part morphology and genetic background and they were able to partition phenotypic variation into its genetic, environmental and approximated interaction components (Cheetham *et al.*, 1994). Moreover, they were able to differentiate between the heritable and non-heritable components of genetic variation (Cheetham *et al.*, 1995).

Paleoenvironmental analysis

Within- versus among-colony (conspecific) variation can be effectively partitioned by ANOVA (Brande and Bretsky, 1982). It has been argued that the within- to among-colony variation ratio in bryozoans is a proxy for environmental stability (Schopf, 1976; Schopf and Dutton, 1976; Pachut and Anstey, 1979; Pachut, 1982; Key, 1987; Pachut and Cuffey, 1991). Although intuitive at extremes of high environmental instability, the relationship between high within-colony variance and greater environmental instability is largely a circular argument. The only assumption that can be made *a priori* is that all of the genetic variation is attributable to the among-colony variance (assuming no somatic variation). The assumption made by all previous studies—that *all* of the among-colony variance is controlled by the genetic component (i.e. environmentally induced variation among colonies from a single sample site is zero)—is not warranted without independent testing (see below). In addition, as we show here, much of the within-colony variance can be independent of even microenvironmental influences, a factor not accounted for in most previous studies.

Partitioning morphological variation

As a precedent to the development of models concerning morphological variation, ideas related to the partitioning of phenotypic variation into its component sources are now well developed in quantitative genetics (e.g. Falconer, 1981; Futuyma, 1986). Briefly, an organism's phenotype is controlled by a combination of: (1) its genotype; (2) the environment in which it develops and grows; and (3) in many, but not all, cases an interaction between genotype and environment (i.e. each individual genotype responding independently). Following Falconer (1981) and Futuyma (1986), these relationships can be expressed as:

$$V_P = V_G + V_E + V_I$$

where V_P is the phenotypic variance, V_G the genetic variance, V_E the environmental variance and V_I is the genotype by environment (hereafter G × E) interaction. Note that we use the term 'environment' here rather than 'ecophenotypic'. In addition, we are considering here only the morphological aspects of the phenotype and not behaviour or physiological components of the overall phenotype.

Partitioning of the phenotype is illustrated in figure 1A, where the bar length represents the overall phenotype of a population, such as observed/measured morphologies. The bar (of arbitrary width) may represent the 'overall' phenotype, or the phenotypic expressions of a single measured characteristic. Although the bar can represent the relative proportion of genetic and environmental contribution to the phenotype of a single organism, phenotypic variation and its components (sub-segments) are most directly measured by assessing the variance of individuals within a population. Lengths of the bar's sub-segments represent the relative proportions

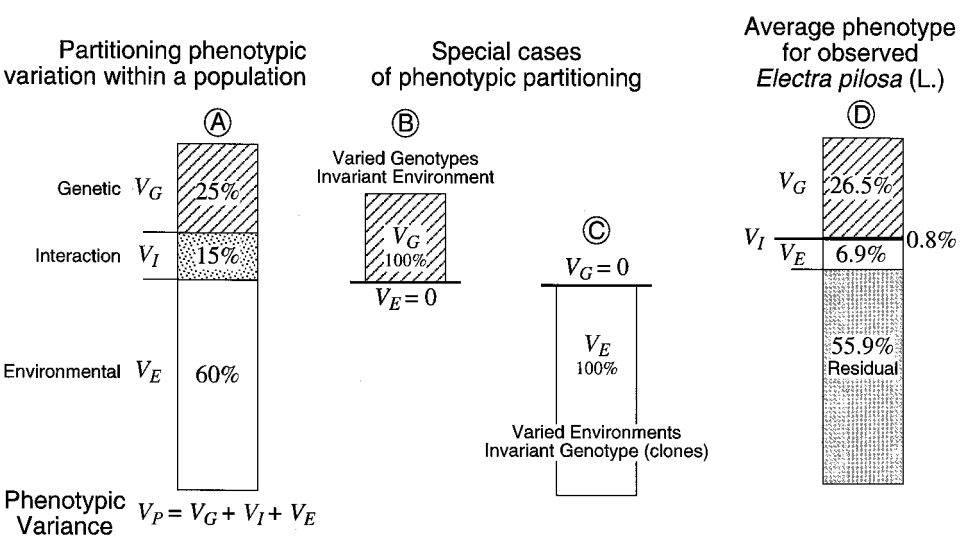


FIG. 1. Partitioning of the phenotype into genotypic, environmental and G × E interaction components: (A) hypothetical case of the percentage each component contributes to the overall phenotype of a population; (B) a hypothetical case with all members of a population experiencing an *identical* environment; (C) a hypothetical case with a population of genetically identical individuals experiencing varied environments; (D) the observed phenotype of six morphological characters of a small population (six colonies of three genotypes) of *Electra pilosa*; ANOVA model (genotype, environment and interaction) accounts for only 34.2% of variance.

of the total (phenotypic) variance controlled by each component. For example, in figure 1A, 25% of the morphological variation in the population is due to genetic variation among individuals; 60% is due to environmental differences among their life histories and 15% is due to G × E interactions (independent responses) between the genome(s) and environment(s).

Partitioning of the phenotype (figure 1A) may be more easily understood by comparing the results of three end-point scenarios. (1) If an entire population of genetically variable, conspecific organisms is grown under identical environmental conditions, there will be no environmental variance (V_E = zero; figure 1B). In such a case, all phenotypic variation will be accounted for by the genetic variance, V_G, among the individuals of the population. (2) If, however, the population is composed of a multiple clone (i.e. organisms with identical genotypes) that are grown in slightly variable environments, the genetic component V_G will be zero (figure 1C). In such a case, all phenotypic variance will be accounted for by the environmental differences, V_E, among the individuals. (3) Theoretically, if a clonal population of genetically identical individuals is grown under identical environmental conditions, there will be no variance among the individuals ($V_P = V_G = V_E = 0$; bar length = zero), assuming no non-environmental developmental 'noise' (i.e. ultramicroenvironmental variation intrinsic to the internal environment of an individual organism/colony).

The genetic component controlling a phenotype can be partitioned further into its additive (V_{GA}), dominance (V_{GD}) and epistatic (V_{GE}) components (Falconer, 1981; Futuyma, 1986). These factors deal primarily with allelic distributions and are important for determining the relative amount of the genetic component of variance that is heritable. Here, we have not partitioned V_G, but establishing the heritable versus non-heritable component of genetic variation was considered a prerequisite to future studies which may provide information on evolutionary processes and patterns (Futuyma, 1986). Partitioning of the genotypic effects can be achieved using the methods described here, albeit with an experimental design that tracks character traits through multiple generations of controlled breeding (e.g. Cheetham *et al.*, 1995).

Previous models have not partitioned the environmental component of phenotypic variation, V_E. However, the scale and degree of both (1) temporal environmental fluctuations and (2) environmental heterogeneity through spatial dimensions, both have important implications on how these factors affect morphogenesis in bryozoans. In theory, therefore, environmental factors could be further partitioned into small- to large-scale, temporal and spatial variation.

Materials and methods

Experimental organism

Electra pilosa (L.) probably is the most common and abundant encrusting marine bryozoan in British waters, and its distribution is assumed to be cosmopolitan (Ryland and Hayward, 1977). It extends to water depths of approximately 50 m, occupying a range of substrata, but it is most commonly epiphytic on macroalgae (especially *Fucus serratus* (L.) and *Laminaria* spp.) in the shallow sublittoral.

Like many other bryozoans, *Electra pilosa* has a remarkable potential for regeneration after injury, in that damaged colony parts usually are healed within days of disruption: zooid budding then proceeds as normal (Bayer *et al.*, 1994; Bayer and Todd, 1996). The present methodology exploits this capacity in allowing the physical

fragmentation of colonies into pieces that will then form autonomous replicates of the same genotype. A particularly striking feature of *E. pilosa* is the high consistency of growth rates and colony shape among replicates of single genotypes (see e.g. Bayer *et al.*, 1994, 1997; Bayer and Todd, 1996).

Laboratory culture

Young colonies of *Electra pilosa*, complete with ancestrulae and comprising approximately 10–100 zooids, were collected on *Fucus serratus* from the shores of Clachan Seil, Argyll, west Scotland, and St Andrews Bay, Fife, east Scotland, in January 1992. Colonies were transferred to the laboratory and maintained in a circular 6-l glass tank with filtered seawater. The water temperature was increased daily by 3°C until the optimal experimental growth temperature of 18°C had been attained.

Each young colony was excised, together with a surrounding piece of the algal thallus tissue, and clipped on to separate microscope slides by means of slit PVC tubing and a sandwich of two microscopic cover slips (figure 2). The uppermost cover slip had been prescored in strips by means of a diamond pencil. The top half of the algal thallus adjacent to the cover slip sandwich had been removed previously to provide a continuous, flush surface for the colony to grow on. Colonies then grew from the algal thallus on to the uppermost (prescored) cover slip. Once colonies were established on cover slips, the algal pieces were removed and the cover slips broken by means of a scalpel, thus providing numerous attached fragments of the same colony (= genotype). Fragments (= 'replication strips') then were clipped on to 5×6 cm glass plates by means of sheet glass strips held by slit PVC tubing, and colonies were allowed to grow on to the plates. Prior to the definitive experiment, the glass strips and replication strips were removed and the newly formed colony parts on the glass plate were reduced by manual trimming to starter groups of 12 zooids. During this first phase of the experiment, three starter colonies were maintained in a single 8-l circular glass trough containing 0.2 μm filtered seawater, with the glass plates held upright in randomized positions in a circular Perspex rack. The tank was situated in a waterbath ($18°C \pm 0.1°$).

After trimming each colony to the starter groups of 12 zooids, the plates were allocated to two 1-l tanks for the 48-day second phase of the experiment (both tanks aerated, but isolated from each other with tops covered and clear of the water bath surface). A subset (Secondary Growth zooids) of the zooids budded during that 48-day period was examined to provide the morphometric data for this study (figures 2 and 3). Colonies were again maintained upright in Perspex racks, with each facing the same way and spaced at equal distances. Throughout the experiment, the flagellate *Rhodomonas* sp. (100 cells μl^{-1}) was given as food. The water in the culture tanks was replaced every 24 h prior to feeding. At the end of the 48-day secondary growth period the colonies were removed, cleaned in freshwater and air-dried for subsequent examination and measurement.

Experimental design

The experimental design accounts for three possible sources of morphological variation. (1) Genotypic variation among zooids assignable to three replicated genotypes ($n = 6$ colonies) (figure 3; Genotypes 1 and 3 from St Andrews Bay, Genotype 2 from Clachan Seil). (2) Environmental variation between the two Tanks, B and C (figure 3), allows for recognition of minor environmental effects.

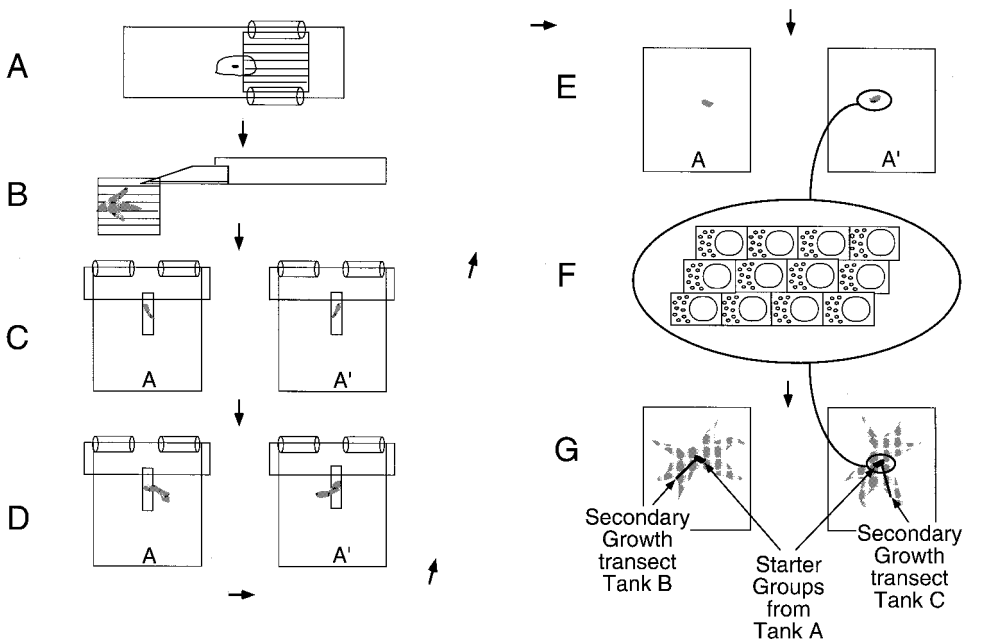


FIG. 2. Diagram of cloning methodology used (see main text for further details). (A) Ancestrula prepared for growth off algal substratum and on to prescored coverslip. (B) Young colony on coverslip prior to the fragmentation procedure. (C) Replication strips clipped to experimental glass plates. (D) Colonies growing on to experimental plates. (E) Clamps and replication strips are removed and colonies cut back to starter groups of 12 zooids each. (F) Starter group (= 'primary zooids'); note change in scale (group $\sim 0.9 \times 2.6$ mm). (G) Secondary growth of colonies in tanks B and C during the second phase of the experiment.

(3) Genotype by Environment (G × E) interaction can also be determined directly from these measurements. In addition, the relative degree of variation from other sources not accounted for in this model (Residual), can also be observed, but not subdivided here.

The experimental design was, therefore, of one colony of each Genotype (1, 2 and 3) allocated to each Tank (B and C) for a total of three colonies per tank. Note that, in contrast to the natural setting, environmental differences are minimal in this study because the two tanks were maintained in the one waterbath. The experimental design for the original, broader study (Bayer *et al.*, 1994), called for no tank effect (i.e. replicate tanks). However, in the present subset of the larger experiment, a Tank effect (possible freshwater contamination from the waterbath) did enter the study, which resulted in stunted growth of colonies in Tank B (table 1). In part, these data were selected for the present study because a Tank effect was actually observed for growth rate from visual inspection of colony development, and could be further explored using morphological characters. Therefore, environmental effects among replicate tanks would be expected to be much smaller in the remainder of the samples from the original study of Bayer *et al.* (1994).

In this initial study, no genetic analyses have been undertaken to quantify the degree of genetic difference among these three genotypes. Due to their separate sexual origins, however, it is certain that there would have been allelic differences

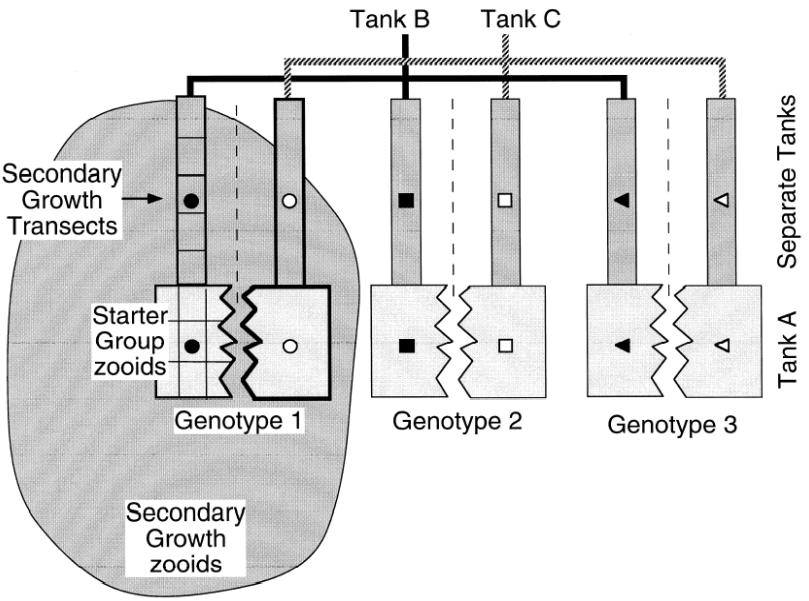


FIG. 3. Experimental design for analysis of phenotypic variation in *Electra pilosa*. Genotypes are designated by their experimental number (1, 2, 3). Starter colonies were grown in Tank A during the initial phase, whilst B and C refers to the same colonies, later split and grown separately in a second and third tank under near identical environmental conditions.

Table 1. Number of zooids in each replicate colony from lineal budding series after 48 days of growth.

	Tank B	Tank C
Genotype 1	11	25
Genotype 2	13	26
Genotype 3	10	15

between the three genotypes. It is also assumed that there are no genetic differences among the asexually produced zooids within each colony.

Morphometric measurements

For the present study we used six morphological characters for zooids that were reliably measurable at the available resolution (figure 4). These included: operculum width (OprW); operculum length (OprL); opesia width (OpsW); opesia length (OpsL); zooid width (ZW); zooid length (ZL) (all maximum measurements). Zooids were photographed using a video camera on a WILD M8 stereomicroscope, and measurements (to 0.1 μm) were made on the resulting images using image analysis software (analySIS 2.0, Soft-Imaging Software GmbH, Münster, Germany, 1994). Zooids in areas of Secondary Growth (i.e. those budded during the second phase of the experiment in Tanks B and C) were sampled in single-zooid column transects from the origin of secondary growth to the colony periphery (figures 2G and 3). Measurements were obtained for a total of 100 Secondary Growth zooids across

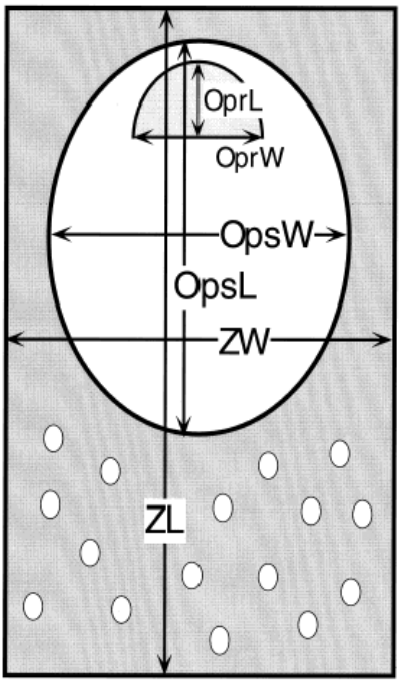


FIG. 4. Morphological characters measured from *Electra pilosa*. OprL = operculum length; OprW = operculum width; OpsL = opesia length; OpsW = opesia width; ZW = zooid width; ZL = zooid length. All characters were measured as maximum measurements.

the six colonies. Because a single, linear transect was sampled for each colony, the number of zooids in the colony sample (range 10–26 zooids; table 1) was a function of colony radius. Several zooids had become slightly damaged during the drying process and were omitted from the analysis.

Statistical methods

Multivariate morphological relationships among all zooids were explored for Secondary Growth zooids. Individual zooids were grouped into clusters using the average Euclidean distance for six standardized morphometric characters with JMP v. 3.0 statistical software (SAS Institute). Principal Components (Systat v. 5.2) also were calculated for zooids using these six characters, and scores for each zooid were plotted on the first two Principal Component axes.

Following Two-way ANOVA, in order to appraise the relative contribution of genotypic, environmental and G × E interaction effects on the overall variation for each character, we partitioned the overall (= phenotypic) variation into variance component estimates (Sokal and Rohlf, 1981, p. 216). Variance component estimates were calculated as $s^2_A = (MS_{Groups} - MS_{Within})/n_o$ and converted to percentages of all estimates summed (tables 2 and 3). Prior to analysis, all data were inspected for normality but no transformation was found to be necessary. In some cases, the MS_{Within} component exceeded the value of the MS_{Groups} component, resulting in a negative contributory term, which, accordingly, was assumed to be a zero contribution.

Table 2. Illustrative example of ANOVA, and estimation of variance components, for the character zooid width (ZW). The absolute values of the variance component estimates were calculated as $s^2_A = (MS_{Groups} - MS_{Within})/n_o$ (n_o is a function of average sample size within groups, see Sokal and Rohlf, 1981, p. 216) and are expressed also as percentages of all estimates summed. Negative variance components were assumed to be zero contributions to the overall variance.

Source	df	SS	MS	F	p	n_o	s^2_A	Var. %
Genotype	2	16 758.71	8379.36	19.15	0.001	32.79	242.20	33.66
Tank	1	191.91	191.91	0.44	0.510	44.86	Negative	0.00
Genotype× Tank	2	2161.32	1080.66	2.47	0.090	16.17	39.77	5.53
Residual	94	41 136.49	437.62	—	—		437.62	60.82
							719.59	100.00

Table 3. p Values and percentages of variance component estimates from Two-way ANOVA of zooidal characters.

	p Value	% Variance	p Value	% Variance
	Operculum width (OprW)		Operculum length (OprL)	
Genotype	0.546	0.00	< 0.001	48.73
Tank	< 0.001	33.32	0.002	8.39
Genotype× Tank	0.344	0.33	0.375	0.00
Residual		66.36		42.89
	Opesia width (OpsW)		Opesia length (OpsL)	
Genotype	< 0.001	37.32	< 0.001	16.75
Tank	0.994	0.00	< 0.001	26.23
Genotype× Tank	0.138	3.73	0.354	0.17
Residual		58.96		56.86
	Zooid width (ZW)		Zooid length (ZL)	
Genotype	< 0.001	33.66	< 0.001	49.05
Tank	0.510	0.00	0.093	2.05
Genotype× Tank	0.090	5.53	0.586	0.00
Residual		60.82		48.90

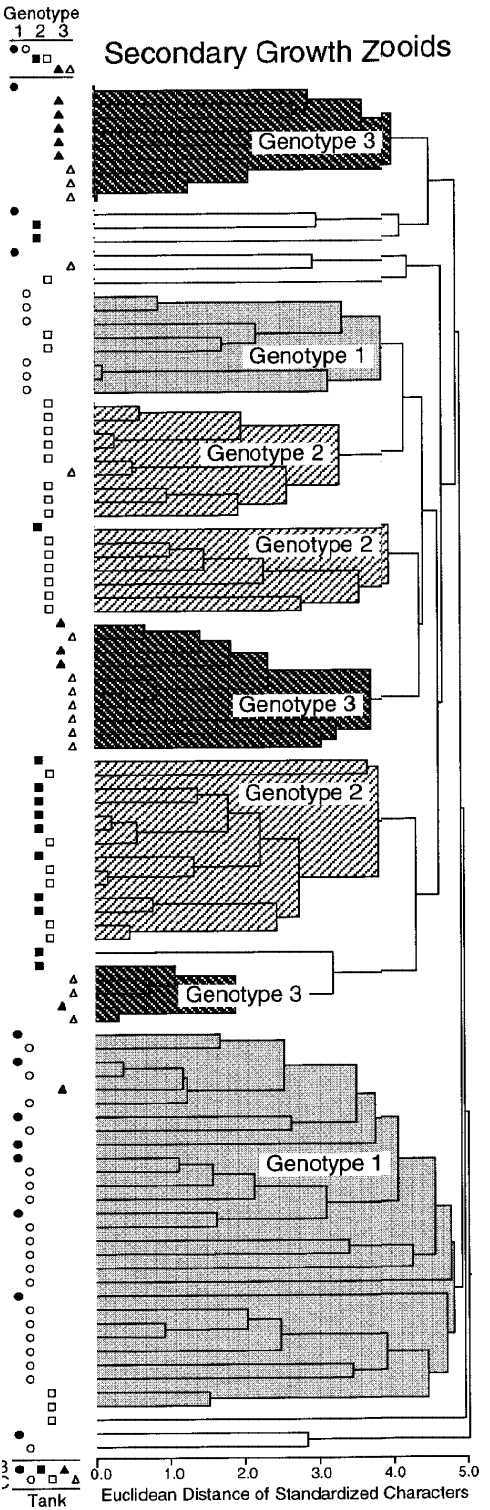
Results

Exploratory multivariate analyses

Cluster analysis for all Secondary Growth zooids revealed a strong tendency for zooids to form groupings on the basis of common genotypes (figure 5). Subgroups also tended to form within those larger groups according to Tank (figure 5; solid—Tank B versus open—Tank C symbols), and some clusters clearly reflected an overall Tank effect (figure 5; upper clusters of Genotype 2 are primarily from Tank C—open symbol).

When the Principal Component scores for Secondary Growth zooids were plotted for the first two axes (figure 6; 69.3% of the total variance), the differentiation of zooid morphology by genotype also was evident. Differentiation of Secondary

FIG. 5. Phenogram of Average Distance Cluster Analysis of Secondary zooids. The illustrative shaded regions of the phenogram were based on a predominance of a single genotype and not exclusivity. The circular, square and triangular symbols represent the three genotypes, 1, 2 and 3, respectively. Solid symbols represent specimens grown in Tank B and open symbols those from Tank C.


Genotype
1 2 3
Secondary Growth Zooids
Genotype 3
Genotype 1
Genotype 2
Genotype 2
Genotype 3
Genotype 2
Genotype 3
Genotype 1
Tank
0.0
1.0
2.0
3.0
4.0
5.0
Euclidean Distance of Standardized Characters

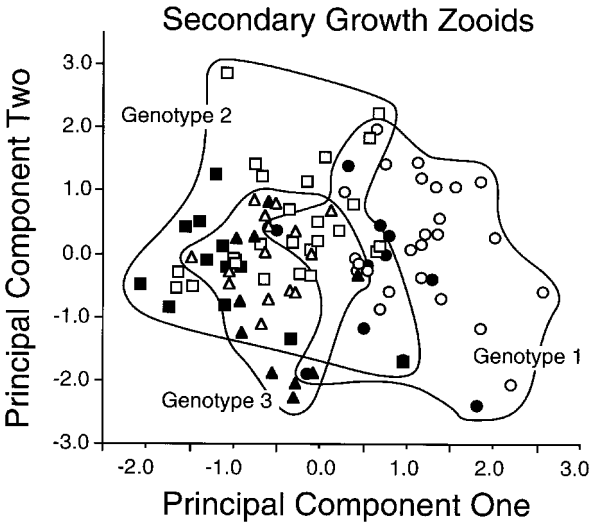


FIG. 6. Individual zooids from Secondary Growth plotted on Principal Component axes one and two, explaining 69.3% of total variation. The circular, square and triangular symbols represent the three genotypes, 1, 2 and 3, respectively. Solid symbols represent specimens grown in Tank B and open symbols those from Tank C.

Growth zooid morphology between tanks (figure 6; solid—Tank B versus open—Tank C, within each genotype) was apparent, albeit with much overlap, suggesting a Tank effect and possibly G × E interaction. Examination of the correlation matrix for these six characters (table 4) revealed, as expected, significant correlation between most variables (10 out of 15 cases).

Analysis of variance

The objective of Two-way ANOVA was to elucidate the relative importance of the different sources of variation. Genotype effects were significant for all characters except operculum width (OprW; tables 3 and 5); the relative contribution of Genotype to the overall variation in the data ranged from zero (operculum width) to 49.1% (zooid length, ZL). The mean variance component (across all variables) attributable to genotype was 26.53% (table 6). A significant Tank effect was observed only for opercular characters (operculum width (OprW) and length (OprL)) and opesia length (OpsL), with variance components ranging from zero contribution to 33.3%. The mean contribution of the Tank factor, at 6.87% (table 6; arcsine back-transformed), generally was small compared to that of the Genotype component.

Table 4. Pearson correlation coefficient for zooid morphological characters (all zooids, $n = 100$). Coefficients significant at the 5% level are asterisked.

	OprW	OprL	OpsW	OpsL	ZW
OpsL	0.51*				
OprW	0.19	− 0.17			
OprL	0.25*	0.27*	0.12		
ZW	0.21*	− 0.18	0.90*	0.15	
ZL	0.27*	0.47*	− 0.26*	0.27*	− 0.28*

Table 5. Values significant at the 5% level (*) from Two-way ANOVA of zooid morphological characters.

	Genotype	Tank	G × T interaction
Operculum width	NS	*	NS
Operculum length	*	*	NS
Opesia width	*	NS	NS
Opesia length	*	*	NS
Zooecia width	*	NS	NS
Zooecia length	*	NS	NS

NS, not significant.

Table 6. Mean and standard errors (averaged across all characters) of back-transformed arcsine percentage variance components (table 3). Due to the data transformation, means shown are not additive.

	Mean %	Neg. SE	Pos. SE
Genotype	26.53	9.85	11.21
Tank	6.87	4.43	6.46
Genotype× Tank	0.78	0.56	0.90
Residual	55.85	3.50	3.47

None of the characters showed significant G × E interaction, with a mean variance component of 0.78%. The largest component of the overall variation in the data was attributable to the residual variance (i.e. factors not accounted for by the present model), at 55.85% of the total variance (range 42.89–66.36%, table 3).

The average phenotype of the six measured characters for observed *Electra pilosa* is partitioned in figure 1D. The present model for partitioning (genotype, environment = tank, and G × E interaction) accounted for only 34.2% of the observed variance, whereas the residual accounted for 55.9%. Note that the arcsine transform is required to correct for the inherently non-normal distribution of values expressed as percentages—the resulting back-transformed values, therefore, do not sum to 100% (table 6, figure 1D).

Discussion

In a general sense, it is important to emphasize the relative lack of complexity of this particular species of bryozoan, the limited number of morphometric characters employed and the close relationship among the objects of interest (i.e. conspecific and congenetic zooids). Morphological patterns recognized here would likely become more robust with a more comprehensive data set from a morphologically more complex species. However, even for these restricted data, the patterns of genotypic, environmental effects and their interaction are clear.

Genotypic component

The analyses demonstrated a strong degree of genotypic control over hard part morphology in the available samples. These results are particularly striking when viewed in the context of among-species comparisons from other morphometric studies (e.g. Cheetham 1986; Hageman 1991a). Here we have detected much smaller scale genetic effects on hard part morphology (i.e. even greater resolution) than

pertain to previous studies, which themselves convincingly argue the significance of species-level distinctions. The strong correlation observed here between genotype and hard part morphology provides encouraging support for existing species concepts in Bryozoa and fossil species in general, although the degree is unknown to which larger environmental differences may ultimately swamp these distinct genetic signals.

One of the visually striking features of laboratory-grown replicate colonies of *Electra pilosa* is the remarkable degree of consistency of growth rate and colony shape within genotypes (Bayer and Todd, 1996). Figure 7, for example, shows five replicates each of five different genotypes of *E. pilosa* grown in a single tank under controlled laboratory conditions. Our observations of several hundred colonies propagated from actively budding peripheral zooids, and more central (astogenetically older) zooids, indicate that the colony growth rate and shape are independent of the original astogenetic position of starter groups and of size/age of the source colony. This remarkable within-genotype consistency leads us to be confident that bryozoans offer much potential to both palaeontologists and neontologists alike in the partitioning of morphological variance into its component sources and allowing an informed judgement of the distinction between species on the basis of morphology alone. Similar patterns of strong correlation between singular genotypes and colonial morphology observed in some corals (Willis and Ayre, 1985; Knowlton and Budd, in press) suggest that results may have broader applicability among marine invertebrates.

It should be noted that assumptions of independent genotypes among co-occurring, conspecific colonies cannot be made *a priori* in the field. Factors such

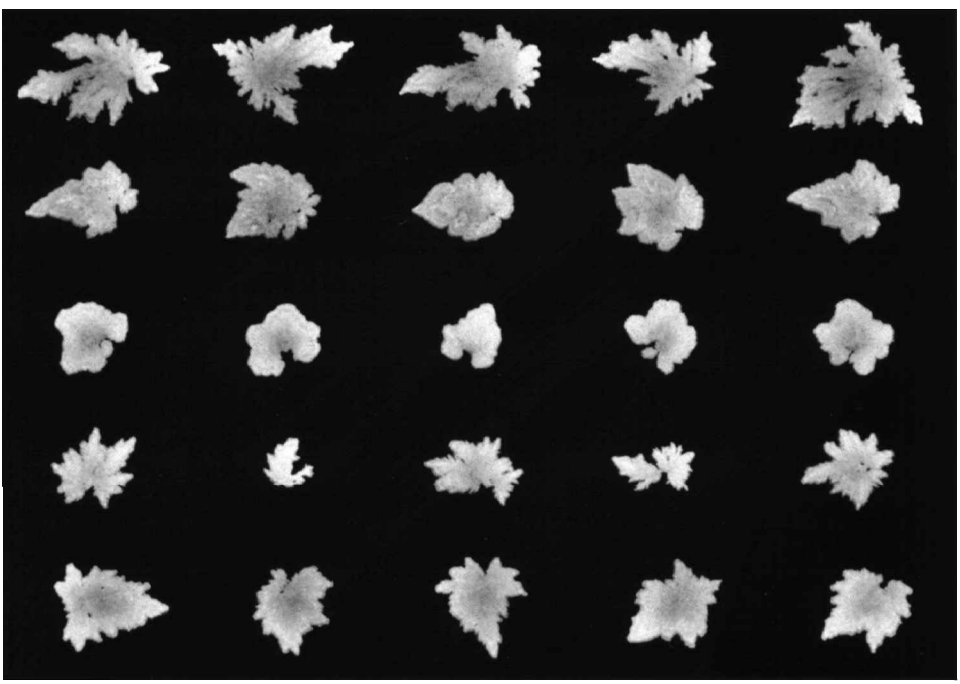


FIG. 7. Five replicates (in columns) of five distinct genotypes (in rows) of *Electra pilosa*, grown in a single tank under controlled environmental conditions in the laboratory; maximum dimension of larger colony 4 cm.

as asexual propagation of new colonies by fragmentation (Blake, 1976; Thomsen and Hakånsson, 1995) and fission (Jackson and Winston, 1981) must be accounted for. The degree to which self-fertilization plays a role in bryozoan reproduction also engenders uncertainty about the composition of the genotype of even some sexually produced colonies (J. B. C. Jackson, pers. comm.).

Environmental component

The environmental effects on morphological characteristics of *Electra pilosa* observed here are of minimal interpretative value because they were not specifically controlled for in the original experimental design. However, the fact that environmental effects were observed between two tanks in a common waterbath demonstrates that minimal environmental effects are detectable in these organisms with this experimental design. Previous variance partitioning studies of genotypic and environmental effects in Bryozoa have had less control of microenvironmental variation and have, in fact, assumed the latter to be zero (Farmer and Rowell, 1973; Pachut 1982; Key, 1987; Cheetham *et al.*, 1993, 1994, 1995).

Spatial and temporal scales may not correlate directly with the degrees of environmental effect on morphology. For example, previous laboratory experiments (Bayer *et al.*, 1997) demonstrated that environmental effects on the morphology of zooids from two conspecific bryozoan colonies located only centimetres apart, one abraded by algae the other not, can be greater than the differences in environmental effect expressed by two conspecific colonies in similar environments on shores a hundred kilometres apart, but neither of which are abraded by algae. By contrast, in other laboratory experiments, local variation in colony neighbours and flow regime apparently affect colonial morphology but not zooidal morphology in *E. pilosa* (Okamura, 1992). Classifications of the scales of environments and their morphological effects therefore require further investigation before methods of phenotypic partitioning can be broadly applied to the fossil record.

Genotype × Environment interaction

In our study, none of the characters exhibited significant G × E interaction effects for Secondary zooids, grown in the two tanks (table 5, figure 1D). These results are encouraging for our ability to develop predictive models for phenotypic partitioning in fossil material. If a significant amount of interaction was present, i.e. there was independence of response of genotypes to the same environmental conditions, then no conclusions could be derived about the relationship between the genetics and morphology of an organism without a full partitioning of variance in every case. Cheetham *et al.* (1995) reached the same conclusion about minimal G × E interaction effects in their studies, but their values were based on estimates rather than a direct partitioning of interaction effects as allowed for in this study. It should be noted, however, that the assumption of non-significant G × E interaction cannot be made too readily, if only because extreme morphological plasticity is a characteristic of many organisms, and differential responses (interaction effects) provide a heritable character upon which selection may act (Scheiner, 1993).

Residual components

The Residual (= within-colony/among-zooids) variance component includes all factors not accounted for in the ANOVA model. The contribution of the Residual component was relatively consistent across characters and averaged 56% (table 6;

figure 1). These values are closely comparable to the residual components observed by Jackson and Cheetham (1990), Cheetham *et al.* (1993, 1994) and Hunter and Hughes (1994) in their morphometric and genetic studies of several cheilostomatid bryozoan species (40–65%).

The Residual variance component in the present data set may include measurement error, covariances of variables with other characteristics not measured here, and a host of other effects. The latter include:

- non-deterministic fluctuations of the internal environment (biochemistry) of the organism during zooid development (developmental 'noise');
- within-colony morphological gradients controlled by ontogeny (e.g. progressive calcification as a function of zooid age) or astogeny (i.e. systematic, deterministic changes in zooid morphology as a function of their position within the colony). Evidence for intracolonial gradients in zooid size were noted here across all three genotypes.
- deterministic variation among aberrant, enigmatic 'monster' zooids and adjacent zooids (polymorphs in other taxa);
- localized effects of zooid arrangement (packing constraints) within colonies.
- sub-microenvironmental effects arising from environmental variation on a scale smaller than the size of the colony itself. A full discussion of these is beyond the scope of this study, but they are likely to include heterogeneities in substratum quality or hydrodynamic flow regime (= food availability), localized infection by pathogens or predation, or spatially discrete competitive interactions with other fouling organisms at various regions of the colony periphery.

Future studies

Using simple and repeatable experimental protocols, replicate colonies of numerous genotypes can be grown in replicate tanks and these allow manipulation of important microenvironmental and macroenvironmental parameters (e.g. temperature, salinity, oxygen tension, food availability; e.g. Jebram and Rummert, 1978; Okamura and Bishop, 1988; Bayer *et al.*, 1994). Moreover, even such ecologically potent factors as wave action and abiotic abrasion of colonies can be successfully mimicked in the laboratory (Bayer *et al.*, 1997).

Figure 1 provides a conceptual framework within which future studies could be formulated. The methodology presented here would allow the direct appraisal of questions such as:

(1) What is the minimum amount of variation, attributable to environmental sources, that can be produced within a single genotype? That is, how close to zero can V_E and V_G simultaneously approach in an ideal laboratory setting (cf. figures 1B and 1C)?

(2) What is the maximum amount of variation attributable to a specific environmental source that can be produced within a single genotype? Here, one is interested in how large V_E can become in response to a single environmental factor (e.g. seasonal temperature range) when all other environmental features are held constant (figure 1C). Effects of many different environmental factors (e.g. food availability, salinity fluctuations, competition for space, physical abrasion, and even predatory influences) can be tested for in independent controlled studies.

(3) What is the observed range of variation, attributable to genetic differences among conspecific individuals, that is produced within a notional invariant environment? This is equivalent to predicting the expected value of V_G within a single species (figure 1B).
(4) Under what conditions do sources of environmentally induced variation obscure genetically controlled variation (i.e. what are the actual proportions of the components in figure 1A)? Do extremes of environmentally induced variation ever obscure significant genetic signals?
(5) What is the prevalence and magnitude of sources of variation due to the interaction between genetic and environmental factors, and do interaction effects influence species concepts (V_I in figure 1A)?

Future experimental designs should attempt to account for factors that are presently included in our residual component. Analytical genetic studies which establish relative differences among genotypes, and that are employed in combination with the types of analysis presented here, will provide even more information about the interplay between environment, morphology and the genetic background. Further studies also should incorporate analyses of multiple generations of controlled populations in a multivariate approach, in order to further partition genotypic effects into their heritable and non-heritable components (Cheetham *et al.*, 1993, 1994, 1995).

Summary

In assessing and applying the morphospecies concept explicitly in relation to fossil material, palaeontologists need to have confidence in their ability to discriminate intraspecific, microenvironmentally induced, morphological variation within facies and on very small spatial scales within strata. Preliminary studies employing cloned populations of a bryozoan demonstrated that genetic and environmental morphological signals are present and interpretable. Our results show a strong correlation between individual genotypes and their resultant hard part morphology, and minimal genotype × environment interaction, thus supporting species concepts based on zooid-level morphotypes. However, our data also show that even on small spatial and temporal scales, subtle fluctuations or heterogeneities in environmental variables and factors can exert striking phenotypic effects within genotypes of a single known species. In addition, typically, more than 40% of the morphological variation was due to residual sources. These findings call for an improved model to account for the remaining components of variation.

The outcome of the present analyses suggests that by subjecting replicated genotypes to a sufficiently large range of different, controlled, environments and by partitioning variation into its genetic, environmental components and their interaction, it is possible to quantify the extent to which a given trait is plastically expressed in different environments. Such methods can also be extrapolated to studies of heritability of morphology and of those character traits important for distinctions among species.

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