Micro- and macroevolutionary change of colony-level traits in bryozoans

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Abstract: The evolution of trait variation among populations of animals is difficult to study due to the many overlapping genetic and environmental influences that control phenotypic expression. In a group of animals, bryozoans, it is possible to isolate genetic contributions to phenotypic variation, due to the modular nature of bryozoan colonies. Each bryozoan colony represents a snapshot of the phenotypes that correspond to a single genotype, which can be summarized as a phenotypic distribution. We test whether these phenotypic distributions are heritable across generations of colonies in two sister species of the bryozoan *Stylopoma*, grown and bred in a common garden breeding experiment. We find that components of phenotypic distributions, specifically median trait values of colony members, are heritable between generations of colonies. Furthermore, this heredity has macroevolutionary importance because it correlates with the morphological distance between these two species. Because parts of phenotypic distributions are heritable, and this heritability corresponds to evolutionary divergence between species, we infer that these distributions have the potential to evolve. The evolutionary potential of these phenotypic distributions may underpin the emergence of colony-level traits, like division of labor in colonies.

Introduction

Trait variation among groups of organisms is a feature intrinsic to living systems, from colonies of yeast cells (1) to populations of marsupials (2). The way this variation among populations evolves over time, if at all, is difficult to analyze due to the difficulty in isolating genetic and environmental factors (3). In a group of colonial animals called cheilostome bryozoans, colonies are composed of genetically identical individuals, termed zooids, that possess a wide range of phenotypic variation (4; 5). This variation is present in measurable morphometric variables, like zooid body length and width (6), so that colonies represent a snapshot of the phenotypic variation that stems from a single genotype (7). At its most extreme, this variation within colonies is expressed as differentiated body types, called polymorphs, which serve different functions in the colony. The origin of polymorphism in bryozoans remains a hotly contested issue (8), but it is impossible to resolve without first understanding whether phenotypic variation within colonies is actually evolvable across colony generations.

Cheilostome bryozoans have a complex life cycle with two distinct modes of reproduction (8; 4): asexual and sexual. Within a colony, zooids reproduce asexually by budding in discrete lineages, forming genetically identical offspring zooids that are either standard feeders, termed autozooids, or zooids that cannot feed and serve another purpose, termed polymorphs. Asexual reproduction allows for a single colony to grow larger by increasing the number of zooids in a colony. The sexual stage of the cheilostome bryozoan life cycle leads to the formation of new colonies, via the release of gametes from colony members (9; 4; 8). The gametes from a single colony will fuse with gametes released by other colonies, forming larvae that found new, genetically-distinct colonies upon maturation.

The structure of phenotypic variation present within a colony is neither the result of trait similarity between asexually-budded zooids (10) nor environmental change along axes of colony

growth (11). We propose that phenotypic variation present among zooids within colonies is instead a result of inherited phenotypic distributions across the sexual stage of reproduction. According to this model, trait variation within a colony stems from a distribution of phenotypic values that corresponds to each colony's genotype, and is passed between generations of colonies (Fig. 1). If these distributions are heritable between colonies, then they have evolutionary potential (10), allowing the phenotypes expressed in colonies to evolve across the sexual stage of reproduction. The evolution of distributions may underpin the emergence of division of labor in bryozoan colonies, as polymorph types are believed to be derived from preexisting body types within colonies. These body types are thought to diverge gradually, so that unimodal phenotypic distributions become bimodal (12).

In this study we assessed trait distributions of parent-offspring pairs in two Stylopoma species to test the hypothesis that trait variation in colonies is heritable and therefore potentially evolvable. We use linear measurements of taxonomically significant zooid traits to generate phenotypic distributions, and assess whether there is heritability of summary statistics of these distributions across colony generations.

Methods

Specimens

We used colonies from two species of the bryozoan genus *Stylopoma*. These specimens were originally used in several studies on skeletal morphology and the assessment of morphological difference between species of *Stylopoma* (13; 6; 14; 7). Colonies were grown in the 1980s from ancestrulae to adults in a common environment at the Smithsonian's field station in the San Blas islands in Panama. The experimental design was based on Maturo's protocol (15) to isolate maternal colonies with a clean substrate for their larvae to settle. Once offspring colonies were

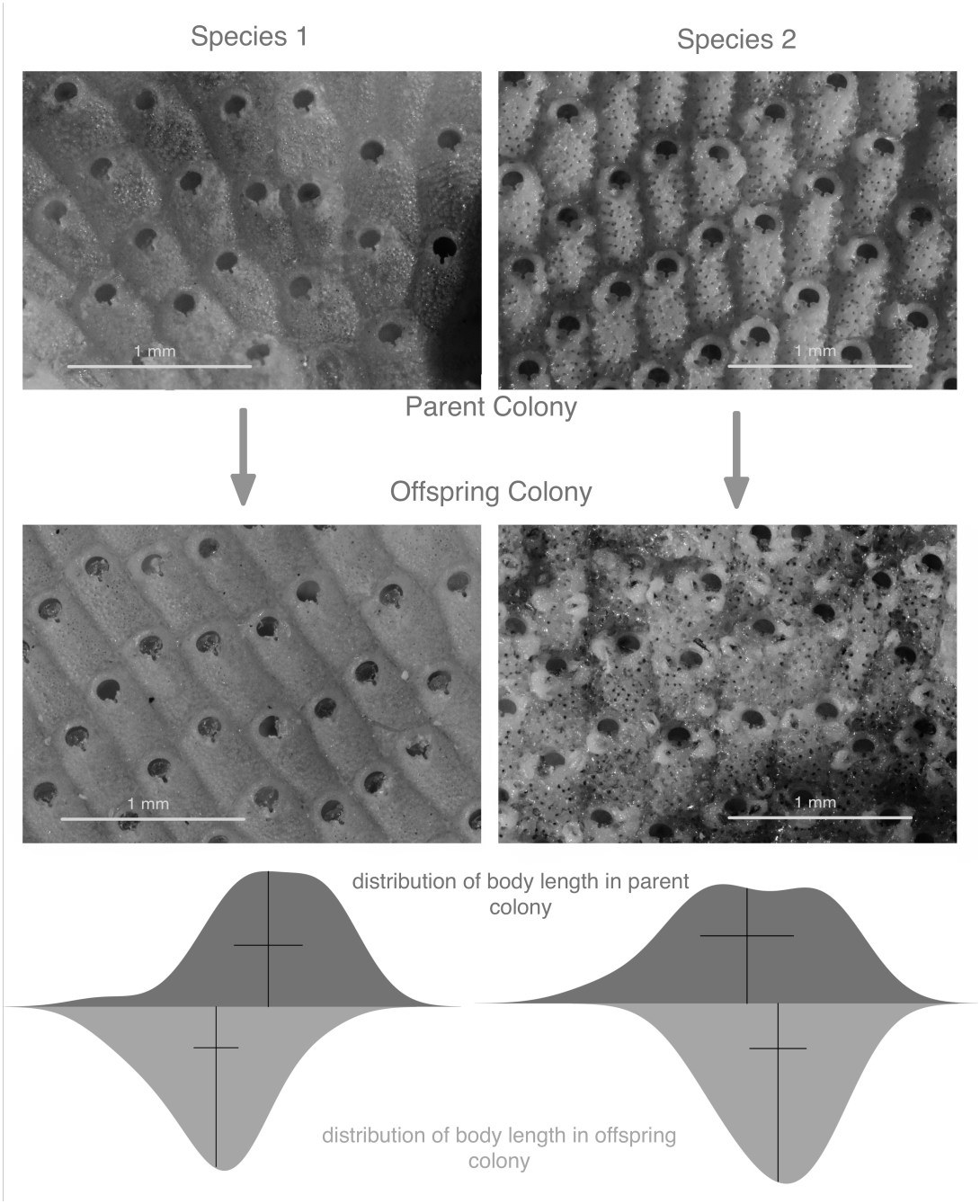

Figure 1: **An example of aggregate phenotypic similarity across a colony generation.** We show an example of two colony generations from two species of *Stylopoma*. The resulting distributions of length values are displayed below each maternal and its offspring colony. To quantify the distributions, we measure two aspects of these distributions: central tendency with the median (vertical black lines) and dispersion around the median with median absolute deviation (MAD, horizontal black lines). The analysis in this study measures similarity, or heritability, by comparing the medians and median absolute deviations of traits across colony generations.

established, they were reintroduced into a common environment. More details on the rearing of these specimens can be found in (13; 6; 10).

We used a total of 93 parent-offspring colony pairs of *Stylopoma sp 1* (42 pairs) and *Stylopoma sp 2* (53 pairs).

Quantifying phenotypic distributions

We measured the distributions of five autozooid traits in digitized mother and offspring colonies. Within each colony, we selected thirty autozooids at random and measured four of their skeletal traits: zooid length, zooid width, orifice length, orifice width. A fifth trait, frontal pore density, was quantified using a subset of five zooids. We selected these traits because they are taxonomically significant traits in *Stylopoma* (10; 13).

For every colony, we estimate two summary statistics to quantify its phenotypic distribution. For the first summary statistic, we quantified the central tendency using the median. For the second, we quantified dispersion in the data using the median absolute deviation (MAD). We chose these two summary statistics as proxies for the mean and variance because they are robust to small sample sizes (16; 17) and if the data are normally distributed, these values are estimators of the mean and variance. Given the large number of measurements ($> 25,000$) required for this study, we prioritized measuring more colonies over sampling more zooids within a colony. The use of the median and MAD values (18) ensures that central tendency and dispersion in the dataset are adequately captured. Of course, it is possible that evolution can occur at higher moments (skew and kurtosis) (19) of the distributions, but for this analysis we are focusing on first and second moments.

Measuring colony-level heredity

We use parent-offspring regression (20; 21; 19; 22; 23) in order to evaluate whether phenotypic distributions of zooids are similar between colonies. Phenotypic similarity between parents and offspring is a measure of heritability, but it is purely phenotypic. Phenotypic similarity between parents and offspring is correlated with heritability measures based on genetic similarity, additive genetic variance, and broad-sense heritability (22). We use this technique because it allows for the study of specimens that lack soft tissues, including skeletons and fossils (6; 14; 7; 10). Because the phenotypes are distributions of traits within a single colony, we want to clarify that we are evaluating the heredity of distributions across colony generations. It is easy to confuse this with the heritable variation that is measurable across generations of solitary animals. But heritable variation is shorthand for broad-sense heritability, which is the ratio of genetic variance to total phenotypic variance in a population of individuals (22). We are instead evaluating the heredity of colony-level variation. In this case, the distribution is the phenotype.

To estimate the heritability of these distributions, we regressed offspring median and MAD values (estimators of the center and dispersion of the phenotypic distributions, respectively) onto those of the parents. The resulting slope values are the heritability values between parents and their offspring colonies. Positive slope corresponds to similarity between colonies. Slope values of 0 indicate no similarity and negative values indicate phenotypic dissimilarity between parent and offspring colonies. The majority of our results are based on these slopes.

Additionally, we constructed a parent-offspring covariance matrix for each species using mean-centered and scaled measurements. In a multivariate world, the interaction between traits is important (24). A parent-offspring covariance matrix allowed for us to determine if traits can evolve even in the absence of direct heritability across generations (25).

Evaluating macroevolutionary impacts

Since these two species of *Stylopoma* are likely sisters (26), we have the opportunity to understand the macroevolutionary importance of colony-level heredity. We can estimate the Euclidean distance between mean trait values in each species from these sister species' implied common ancestor. The expectation of a Brownian motion model is that an ancestor will be centered between its two descendent species (27). We took the grand mean of the measured values pooled across both species to estimate this ancestral phenotype. To determine the deviation of trait values between these two sister species, we calculated the distance of the species-specific means from the grand mean. This estimates the deviation of each species from their inferred common ancestor, which we then compared to the pattern of inheritance for each trait.

Additionally, we estimated the within-species variation by calculating the interquartile ranges (IQRs) for each trait in each species. We compared the IQR values to the inheritance patterns of the MAD for each trait across a colony generation.

Results and discussion

The partial heritability of colony-level phenotypic distributions

We find that the median traits of colony members are heritable across generations of colonies (Figure 2). As a summary statistic of the phenotypic distribution, the median trait values of colony members are therefore evolvable colony-level traits. However, our results for MAD trait values of colony members are less conclusive, with our confidence intervals including similarity values of 0. While median trait values of zooids are passed between colony generations, MAD trait values do not follow the same pattern. This result indicates that different components of colony-level phenotypic distributions evolve differently. Nevertheless, the heritability of median

trait values across generations of colonies demonstrates the partial evolvability of phenotypic distributions of zooid traits.

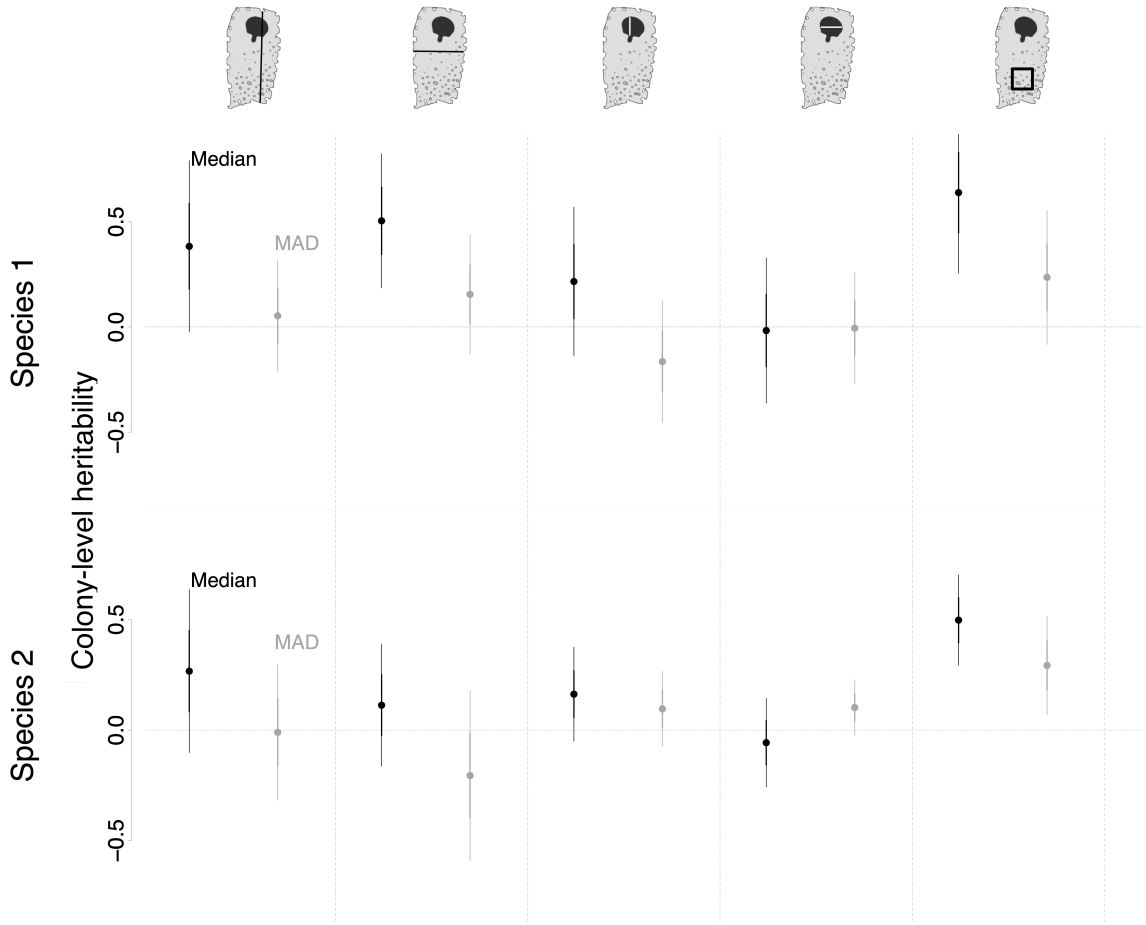

Figure 2: **Heritability of phenotypic distributions in five colony-level aggregate traits in two species of the bryozoan *Stylopoma*.** Heritability of median and median absolute deviation (MAD) values across a generation of colonies for five member-level traits. The colony-level heritability for each trait is shown in the columns. The heritability of median traits is shown in black and the heritability of MAD traits is shown in grey. Thin lines represent the 95% confidence interval of the mean slope value, and thick lines represent the standard error around each point.

Depending on the trait, the heritability of phenotypic distributions (the MAD and median

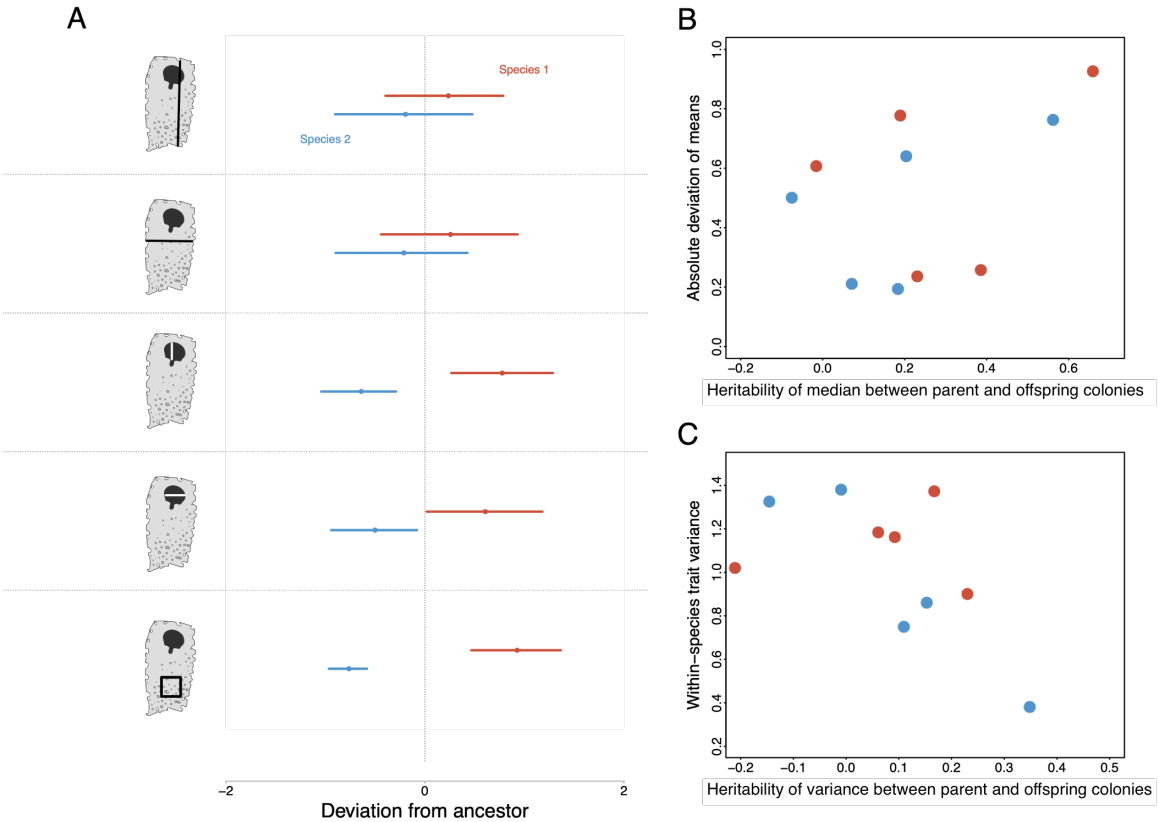

Figure 3: **Macroevolutionary impact of colony-level heritability. A.** Trait deviation from the ancestral state for five different colony-level aggregate traits. The points represent the mean trait values in each species, with red points corresponding to species 1 and blue points corresponding to species 2. The horizontal lines show the first and third quartiles of the species trait values. **B.** We compared the absolute distance of species means from an ancestor (from Panel A) with the mean heritability value of medians across a colony generation. The number of data points corresponds to the number of measured traits. The small sample size of data points limits the statistical power of any test, but we find a positive correlation (Spearman's ρ = 0.44, p≈0.2). **C.** We compared the interquartile range (IQR) values from Panel A with the mean heritability value of MADs across a colony generation. There is a negative correlation between IQR and heritability of MADs (Spearman's ρ = -0.52, p≈0.1).

trait values of colony members) between colonies varies. For example, the median trait value of orifice width is not heritable between colony generations in either species. However, the median orifice width value has a positive covariance with other traits across generations of colonies

(Tables 1 and 2). Heritabilities of median absolute deviation (MAD) values are much more variable than heritabilities of the medians. MAD values measure the spread of the distribution, which might be more difficult for the colony to control than measures of central tendency.

There are generally two types of colony-level traits that can be measured - aggregate traits and emergent traits (28; 29; 30). Emergent traits, like polymorphs in colonies, are known to evolve across colony generations (10). Our results confirm that aggregate traits of colonies, which are the summary statistics of traits in colony members, are also potentially evolvable. Because median traits are evolvable at the colony level, sexual reproduction among colonies allows colonies to maintain and evolve this part of their phenotypic distributions.

Table 1: **Parent-Offspring Covariance Matrix *Stylopoma sp 1*.** Covariance values for five trait medians across a colony generation. ol=orifice length, ow=orifice width, pd=pore density.

	parent length	parent width	parent ol	parent ow	parent pd
offspring length	0.231	0.343	0.0497	0.17	0.162
offspring width	0.147	0.387	0.132	0.178	0.25
offspring ol	0.1	0.161	0.19	0.176	0.185
offspring ow	-0.0075	0.0154	-0.106	-0.0154	0.0482
offspring pd	-0.0694	0.377	0.325	0.238	0.659

Table 2: **Parent-Offspring Covariance Matrix *Stylopoma sp 2*.** Covariance values for five trait medians across a colony generation. ol=orifice length, ow=orifice width, pd=pore density.

	parent length	parent width	parent ol	parent ow	parent pd
offspring length	0.184	-0.019	-0.106	-0.0521	0.086
offspring width	0.108	0.0715	0.0818	0.045	0.0287
offspring ol	0.0942	-0.137	0.204	-0.163	0.282
offspring ow	0.166	-0.159	0.229	-0.0751	0.164
offspring pd	0.0509	0.00156	0.0179	-0.0219	0.562

The macroevolutionary importance of evolvable distributions

In *Stylopoma*, the microevolutionary structure of heredity across colony generations is reflected on a macroevolutionary scale. Heritabilities of median trait values between parent and offspring colonies have a positive correlation with the distance between species (Spearman's ρ=0.44, p≈0.2) (Figure 3B). This result hints that evolutionary potential of phenotypic distributions at the microevolutionary level contributes to the macroevolutionary patterns observed among species. Our results comparing variation within species and heritabilities of variance between colonies are less straightforward. The heritability of MAD values across a generation of colonies negatively correlates with the variation of traits within species (Spearman's ρ = -0.52, p≈0.1) (Figure 3C). Traits with high MAD heritability across generations of colonies have lower variance at the species level, indicating that these colonies may be evolving lower levels of phenotypic variation over time.

The connection between the microevolution and macroevolution of phenotypic variation remains unclear (31). In light of this, it is interesting that *Stylopoma*, a taxon that experiences morphological stasis and punctuated equilibrium (26), has morphological differences between species that are predicted by microevolutionary processes (Figure 3). The vector of punctuated change between species correlates with heredity across generations of colonies.

Our measure of heritability between colonies and trait divergence between species complements the previously observed positive correlation between trait variance in a population and trait divergence between species, termed evolution along lines of least resistance (32). In fossil species of ostracods (33), in extant species of fiddler crabs (34), and in stickleback fish (32), macroevolutionary change occurs along directions of maximum phenotypic variance. The measure of parent-offspring heritability appears to also influence evolutionary change, since we observe that more divergent traits between species are those that exhibit the most inheritance between colonies. High values of heritability, like high values of phenotypic variance, contribute

to morphological divergence between species. Since trait variation (phenotypic variance) and heredity (parent-offspring similarity) are the two components of evolutionary potential (35), it is no surprise that macroevolution is governed by both.

The negative correlation of MAD heritability and within-species variance (Figure 3C) suggests that there is directional reduction in trait variance over time. Perhaps *Stylopoma* is evolving lower levels of phenotypic variance within colonies. However, given the unusual inheritance patterns for some trait MADs, it is not possible to form a conclusive interpretation. For heritable traits, response to selection typically follows the direction of selection (36). Some of our MAD values exhibit a negative heritability pattern across colony generations (Figure 2). Since our 95% confidence intervals for negative heritabilities include positive values, it is difficult to estimate whether this result is a reflection of *Stylopoma* biology. In this context, what negative heritability means is that parent colonies with large variances produce offspring colonies with small variances, and parent colonies with small variances produce offspring colonies with large variances. When subject to directional selection, negative trait heritability would result in a response to selection that opposes the direction of selection. In response to a selection favoring reduced phenotypic variation, a trait with negative heritability would increase its variance. This is consistent with our findings because negative MAD heritability correspond to traits with relatively high phenotypic variation (Figure 3C).

The role of evolving phenotypic distributions in the emergence of division of labor

George C. Williams, in his famous work *Adaptation and Natural Selection*, deemed modular invertebrates uninteresting compared to eusocial insects because he assumed the clonal nature of these colonies explained any division of labor that they possess (37):

> "To provide benefits to a genetically identical individual is to benefit oneself. There is no paradox in committing itself to a sterile somatic role in development if this development benefits a genetically identical cell in the germline."

This quote implies that the evolution of division of labor is trivial in modular animals. From this perspective, relatedness is omnipotent in its evolutionary potential. Yet, most species of group-living animals, whether they be social insects, mammals, or modular invertebrates, are monomorphic (38; 39; 40). Even the most differentiated societies or colonies exhibit much less division of labor than even the average multicellular individual (38; 41; 42; 43). The high diversity of monomorphic invertebrate colonies and the persistence of animal groups with few cell types, such as sponges, indicates that although relatedness may be necessary for differentiation, it is insufficient. In bryozoans, the heritability between asexually budded clones is essentially 0 (10), indicating that clonality prevents evolution from occurring.

The partially evolvable nature of colony-level phenotypic distributions underpins a hypothesis which proposes that polymorphs, or morphological division of labor in bryozoan colonies, are derived from preexisting body types within colonies as an expansion of that phenotypic distribution (12). These body types are thought to diverge gradually, so that unimodal phenotypic distributions become bimodal. If some components of the phenotypic distribution have evolutionary potential, it is possible that these distributions could diverge over time, leading to morphological polymorphism in colonies.

In both eusocial insects and modular animal, evolution occurs across the sexual stage of reproduction. This colony-level evolution is possible through reproductive skew. In eusocial insects, reproductive skew and relatedness are easy to conflate due to their coupled relationship. The fewer queens there are in an ant colony, the higher the relatedness between colony members (44). In contrast, reproductive skew and relatedness are decoupled in modular animals, which allows for the study of differentiation while controlling for relatedness. Division of labor is

observed only to evolve in groups with reproductive skew (38). Monomorphic colonies have maximal relatedness but no reproductive skew and therefore lack division of labor. By contrast, modular colonies with polymorphs have both high relatedness and reproductive skew (40; 38), and their macroevolution exhibits ever-increasing division of labor (45; 46; 47; 48; 39).

However, reproductive skew, like relatedness, does not explain how divergence in polymorph types first evolved. Our results show that components of phenotypic distributions are heritable and therefore evolvable at the colony level. As evolvable features, trait distributions can shift and change shape over time, leading to an array of phenotypic expressions within colonies. On a macroevolutionary timescale, the evolution of phenotypic distributions may underpin the initial emergence of division of labor within colonies. Division of labor, can then be understood as a partitioning of phenotypic variation into different castes or polymorph types (49; 12; 50) and an expansion of phenotypic expression. The key to understanding differentiation, and therefore division of labor, is to study how these distributions evolve.

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Author Contributions

S.L. and S.J-T. digitized all available specimens for analysis and collected measurement data. S.L. performed the data analysis. S.L. and C.S. wrote the manuscript.

Competing Interests Statement

The authors declare that they have no competing interests.

Data Accessibility

All data needed to evaluate the conclusions in the paper are present in the paper and/or Supplementary Materials. Additional data related to this paper may be requested from the authors.

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