

Supplementary Materials for

How colonial animals evolve

Carl Simpson*, Amalia Herrera-Cubilla, Jeremy B. C. Jackson

*Corresponding author. Email: carl.simpson@colorado.edu

Published 8 January 2020, *Sci. Adv.* **6**, eaaw9530 (2020)

DOI: 10.1126/sciadv.aaw9530

The PDF file includes:

Supplementary Materials and Methods

Fig. S1. A small *Stylopoma* colony consisting of many thousands of members.

Fig. S2. A closeup of a *Stylopoma* colony showing a clonal lineage of autozooids.

Fig. S3. Polymorphic zooids of *Stylopoma*.

Fig. S4. A closeup of a *Stylopoma* colony consisting autozooids, three types of avicularia, and an ovicell.

Fig. S5. Evolutionary potential as measured by the heritability of traits between parent and offspring zooids.

Fig. S6. Evolutionary potential as measured by the heritability of traits between parent and offspring colonies.

References (52–64)

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/2/eaaw9530/DC1)

Table S1 (Microsoft Excel format). Zooid measurements of maternal *Stylopoma* colonies.

Table S2 (Microsoft Excel format). Zooid measurements of offspring *Stylopoma* colonies.

Supplementary Materials and Methods

Bryozoan basics

Bryozoan colonies, like this *Stylopoma* colony, consist of large numbers of modular animals termed zooids. Zooids are physically connected and each is homologous to an individual solitary organism (39, 63).

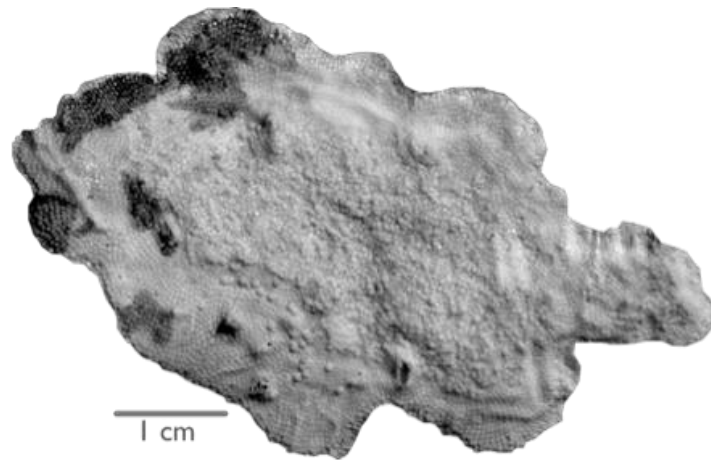


Fig. S1. A small *Stylopoma* colony consisting of many thousands of members.



Fig. S2. A closeup of a *Stylopoma* colony showing a clonal lineage of autozooids.

Colonies are often initiated by a sexually produced larva, that settles to form an ancestral zooid termed an ancestrula. The colony then grows from the ancestrula by clonal budding. Each new generation of zooids are clones of the previous generation. And depending on the growth form of the colony, the numbers of zooids can increase slowly, by one or two each generation if the colony forms a chain, or exponentially if the colony expands out as a sheet.

This close-up of a *Stylopoma* colony shows subset of a clonal lineage. The mother of zooid 1 is below the image. Zooid 1 gives rise to zooid 2, that then buds zooid 3, which in turn buds zooid 4. Parallel to this highlighted lineage are other clonal lineages. In colonies like these, zooids will occasionally bud two daughter, allowing the colony to grow quickly by increasing the total number of clonal lineages in the colony. These repeated rounds of asexual reproduction continues for many generations. Once colonies reach sufficient size, they may begin producing sexual larvae that are competent to form daughter colonies. The life cycles of bryozoans are characterized by a complicated dance between the sexually propagating colonies and the asexually propagating zooids. It is this co-occurrence of sexuality and asexuality that leads us to look for mechanisms for understanding how these animals mediate the evolutionary potential that results.

Many zooids in a colony are generic feeding zooids with a stereotyped form. These are termed autozooids. Autozooids have all the full compliment of bryozoan organs and organ systems and are connected to each other by pores. Autozooids are the sole feeding zooid in the colony. The polypide contains a tentacled feeding structure, the gut, and also sexual organs. The polypide emerges from the zooid by using muscles to deform the frontal wall and thus decrease the volume within the zooid. The polypide then is pushed out through a hatched operculum to feed. Autozooids are the evolutionarily basal condition for zooids (52–54, 64).

Located among the autozooids are other types of zooids that are morphologically distinct from autozooids, sometimes dramatically so. These differentiated zooids are termed heterozooids or polymorphs and

have a subset of the bryozoan organs and organ systems. They cannot feed and they are sterile. While the evolutionary origin of heterozooid types is unknown, there seems to be two classes of heterozooids that have independent evolutionary origins. One type of heterozooid, called avicularia (four examples are shown to the left), are homologous to and derived from autozooids and have a highly reduced polypides—often only retaining their musculature and nervous functions (56). Avicularia can be as large as autozooids and may take the place of an autozooid in the colony. These avicularia are asexually competent just like autozooids and often bud daughter autozooids. Other types of avicularia may be found peppered across the colony surface and most have lost their asexual ability. Kenozooids are another type of heterozooid that is essentially an autozooid reduced to only a diminutive skeletal box, yet can bud daughter autozooids asexually.

Integrated into many autozooids are spines, another class of heterozooids that are not homologous to avicularia. Spines are highly reduced consisting of only skeleton, epithelium, and a cuticular boundary that allows them to be identified as zooids rather than protuberances. In some cheilostomes, sets of spines are modified to form the frontal shield on autozooids and they form ovicells, a novel dome-shaped zooid type that functions to brood larvae. Ovicells are budded from the distal end of a parent autozooid, so that the orifice of an ovicell is within reach of the parental autozooid polypide tentacle crown. The position of ovicells relative to the surface of the colony is highly variable. In some species they are submerged below the colony surface within the parental autozooid. But in other species, the ovicells stand above the frontal surfaces of nearby autozooids adding significant texture to the surface of the colony. Although we do know the site of fertilization, unfertilized eggs or embryos are passed from the tentacles of the adjacent zooid to the ovicell where they brood until they are ready to disperse (17).

Here are the zooid types present in one species of *Stylopoma*. We are showing these zooid types dissected out of the colony and to scale to show you just how distinctive these zooids are. Each zooid shown here come from the same colony and are genetic clones of each other.

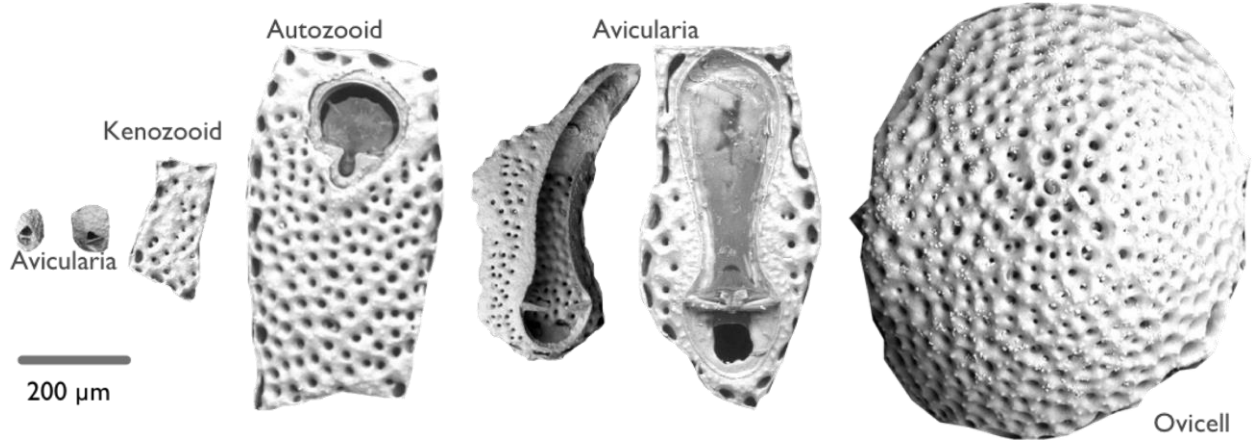


Fig. S3. Polymorphic zooids of *Stylopoma*. These images are removed from their colony context in order to highlight their differences.

Asexuality and sexuality in the life cycles of colonies

The amount of asexual activity within a colony can vastly dwarf the amount of sexual activity a colony engages in. As an illustration of this disparity, take a look at the close up of this *Stylopoma* colony surface

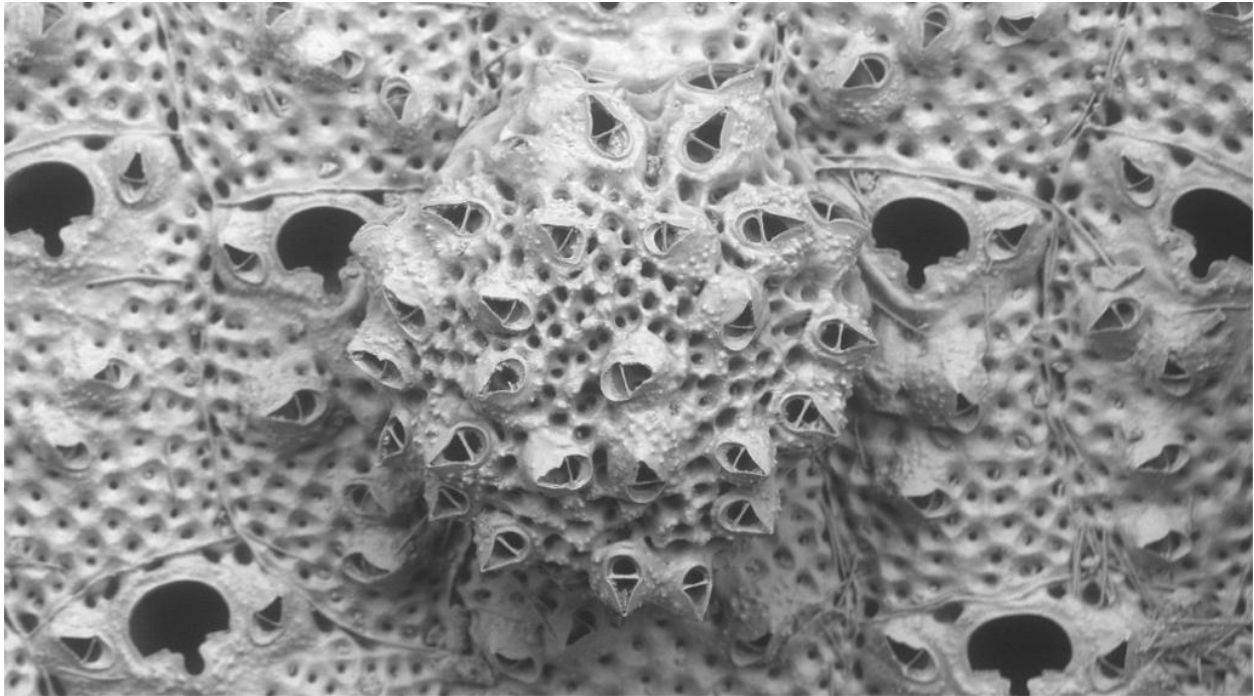


Fig. S4. A closeup of a *Stylopoma* colony consisting autozooids, three types of avicularia, and an ovicell. All zooids within this image are asexually produced. Sexually produced larva are brooded within the ovicell.

showing dozens of zooids:

Every zooid containing this 100 μ m-wide hatched orifice is an autozooid. Every surface of the colony is bristled with pointy avicularia. Every zooid in this image is asexually produced. Sexually produced larvae are brooded within the domed ovicell in the center of the image. In *Stylopoma*, ovicells are rare. In some colonies, ovicells make up fewer than 25 of every 5000 of zooids (20, 50). Nevertheless, these colonies can contain many millions of zooids (62) and so colony fecundity is not low by any measure—embryos have been observed at a density of 2000 per square meter in Rio Bravo, Jamaica (50).

One of the ways that *Stylopoma* gets ahead ecologically is that it uses its large ovicell to grow large larvae that begins asexual reproduction before settling. When its larva settle, the ancestrula already consists of six zooids (39).

Natural history of *Stylopoma*

The cheilostome bryozoan *Stylopoma* is a diverse genus first appearance about 17 million years ago (32). *Stylopoma* is common in the coral dominated tropical waters, especially in the Caribbean (32) and the Indo-West Pacific (33). *Stylopoma* predominately encrusts the undersurfaces of foliaceous corals (49) forming thin sheets and competing for this limited space with other encrusting bryozoans, ascidians, sponges, and crustose algae (37; 58). Their colonies can grow large, the maximum size observed for *Stylopoma spongites* colonies growing at 20 meters depth at Rio Bueno Jamaica was 500 square centimeters. An average square centimeter of that colony had 383 autozooids (50) and this species has at least one oral avicularia associated with each autozooid and common vicarious avicularia overgrowing the colony surface. As seen in the image above, *Stylopoma* has large ovicells, and as consequence it broods large embryos (about 0.3 mm in diameter for *Stylopoma spongites* Jackson and Wertheimer 1985). Sexual maturity tends to occur only after colonies grow sufficiently large. In Jamaica, the smallest *Stylopoma* to brood embryos was observed to be about 12 square cm in area which occurs after growing for about 18 months (32).

Stylopoma is one of the most abundant bryozoans on Panamanian reefs (37; 57, 60). But at small and large spatial scales, *Stylopoma* and other bryozoans have patchy distributions and variable abundances in coral reefs, because species vary in many factors related to their ability to compete for space and food (35; 50, 55, 59) and in their life-histories such as patterns of recruitment, reproduction, and dispersal that are important for occupying new space (57, 59). Variation in these life history attributes among several closely related species of *Stylopoma* permit their coexist along the Caribbean coast of Panama (49).

Experimental design

In this paper, we use colonies that were grown in a previous breeding experiment. The specimens were used previously to test if skeletons are enough to tell species apart (28), if selection or random change drove differentiation between species (29), and a phylogenetic estimate of the tempo of speciation (30). The scientific versatility of these specimens is due to the controlled way that they were grown and bred. From wild caught colonies, two generations of colonies with known maternity were born. Furthermore, offspring colonies grown in a common garden experiment, allowing generations of scientists to tease apart the complex processes involved in phenotypic macroevolution.

The experimental design is modified from one developed by F. J. S. Mauro (1973). This experiment was designed to strictly limit the number of possible paternal colonies that could fertilize maternal colonies. And then to use the limited dispersal ability of *Stylopoma* larvae to know the maternal colonies that gave rise to offspring colonies. The experiment was conducted at the Smithsonian field station just east of San Blas Point on the Caribbean coast of the Republic of Panamá. Using this field station allowed offspring colonies to be grown in a common garden that allows the quantification of the impact of the environment on phenotypic expression.

Bryozoans were collected from one to five sites, depending on species, between Holandes Cays and Isla Grande in depths of <1 to >40 meters and were maintained in running sea water for usually no more than one day before use. Maternal colonies of two *Stylopoma* species were collected from the localities shown on this map of localities. Isla Grande, Ulaksukan west and east, Palina West, Aguadargana southwest and northeast.

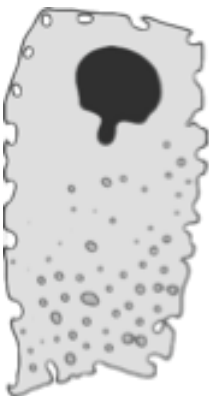
As described in Jackson and Cheetham 1990 and Cheetham, Jackson, and Hayek (1993), corals with *Stylopoma* colonies containing embryos were collected from these Caribbean localities. These coral substrata were cleaned of other organisms and isolated in brood chambers made from plastic food containers with sides cut open and replaced by plankton nets. Filtered sea water was run through the top of the chambers

and exited through the nets in the walls. A single maternal colony was kept in each container under a piece of bare coral for daughter colonies to settle. After five to ten days the coral substratum with newly settled F1 colonies were removed and attached to concrete blocks on a sandy bottom about -0.5 meters below low water at the Smithsonian field station just east of San Blas Point on the Caribbean coast of the Republic of Panamá. Every month the condition of the colonies was assessed and the colonies and substrata were cleaned of other organisms.

Character definitions

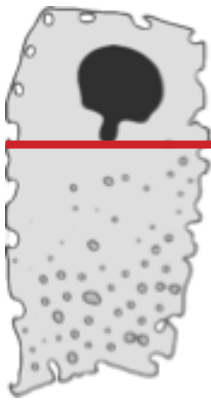
In this paper we analyze the evolutionary potential of morphometric traits using a multivariate quantitative genetics framework (described in the next section). Traits in *Stylopoma* colonies (and all other bryozoan species, for that matter) occur at two hierarchical levels of organization: the organismal and the colony-level. Organismal traits are easily measured on single zooids. Colony-level traits, on the other hand, must involve multiple zooids. For us, colony-level traits are strictly emergent and so we exclude traits that are aggregate summary statistics of individual zooid measurements. As such, we do not consider measures of central tendency and variability to be colony-level traits. The characters we use in the analysis are described here. The traits listed below are not exhaustive at either the organismal or the colony levels.

Organism-level traits



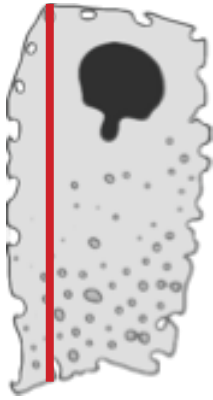
Many of the traits in this analysis are defined only at the organismal level. Primarily, traits are measured on autozooids, like the illustration on to the left. Each colony has generation after generation of asexually produced autozooids. And so these traits are

measured on many zooids per colony. We measured each trait from at least three zooids located near the growing edge of the colony. As zooids age they add skeletal material and in *Stylopoma* (and other species as well) zooids can frontally bud, which essentially adds a second story on top of previously existing zooids. Because both extra calcification and frontal budding may obscure these morphometric measurements, we avoid them. For orientation, the autozooid is dominated by the hatched orifice that the polypide emerges out of to feed. This orifice is located on the anterior end of the zooid and is closer to the direction of asexual growth. The other major feature is the frontal wall. This is located below the orifice in this image and is often pocked with small holes that allow sea water to fill the void the ascus makes below the frontal wall as the polypide emerges from within the skeletal zoecium.



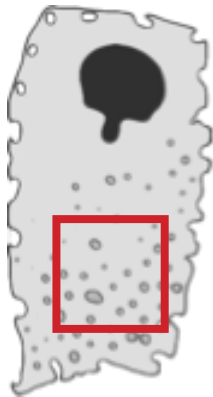
Zooid width is measured as the widest part of the zooid.
Metric trait, in millimeters.

ZOOID WIDTH



Zooid length is measured as the longest part of the zooid.
Metric trait, in millimeters.

ZOOID LENGTH



Frontal pore density is measured as the number of pores
within a 500 μ m square area.
Meristic trait.

FRONTAL PORE DENSITY

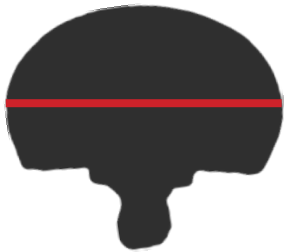


Zoom into the orifice specific traits

LENGTH OF ORIFICE



The length of the orifice is measured from the apex to the center of the hinge.
Metric trait, in millimeters.



The width of the orifice is measured across the widest part.
Metric trait, in millimeters.

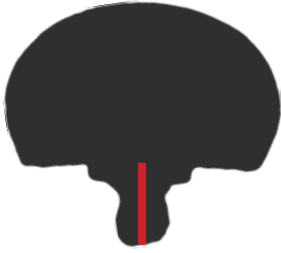
WIDTH OF ORIFICE

WIDTH OF SINUS



The width of the sinus is measured across the widest part.
Metric trait, in millimeters.

LENGTH OF SINUS

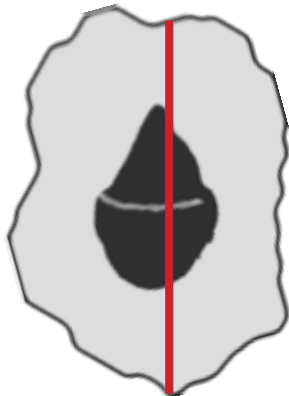


The length of the sinus is measured from the hinge to the apex.
Metric trait, in millimeters.



Many autozooids are associated with one or more avicularia, which are separate zooids in their own right. The remaining organismal-level character is measured exclusively on an avicularium.

LENGTH OF AVICULARIA






The length of the sinus is measured along the longest part of the blister that is the avicularium body.
Metric trait, in millimeters.

Colony-level traits



Colony-level traits are characteristics of zooid complexes. Each of these traits involves the measurements of the position and orientation of zooids with respect to each other.

These are traits that cannot be measured on individual zooids alone, nor are they statistics summarizing the same measurement on multiple zooids. As such, the traits we consider

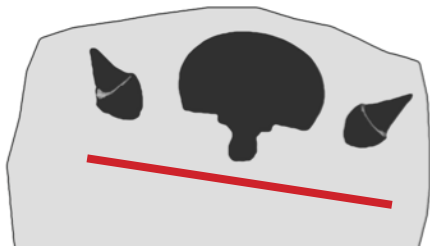
here are strongly emergent. In the illustration to the above, this shows a autozooid bounded by the grey frontal wall and indicated by its orifice . This particular zooid is associated with two oral avicularia  that flank the orifice and two frontal avicularia  that bud freely on the frontal surface of the autozooid.

NUMBER OF FRONTAL AVICULARIA



The number of frontal avicularia.
Meristic trait.

ORAL AVICULARIA POSITION



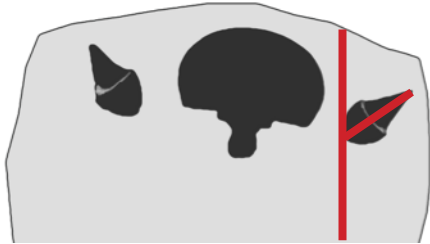
The position of oral avicularia relative to an imaginary line drawn through the hinge of the orifice.

1. Avicularia proximal to the orifice
2. Avicularia inline with orifice
3. Avicularia distal to center of orifice
4. Avicularia distal to the orifice



Ordinal trait, 4 states.

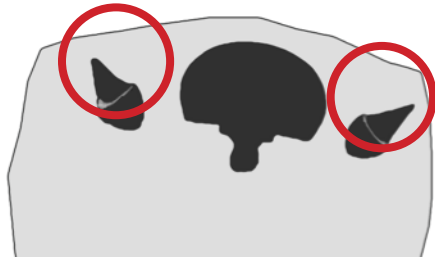
ORAL AVICULARIA ORIENTATION



The angle of oral avicularia relative to an imaginary line drawn parallel to the body axis of the autozooid.

1. Avicularia angled inward
2. Avicularia angled inline
3. Avicularia angled slightly outward
4. Avicularia angled outward

Ordinal trait, 4 states.



NUMBER OF ORAL AVICULARIA

The number of oral avicularia.
Meristic trait.

Quantifying evolutionary potential

If traits have the capability to respond to natural selection, then they have evolutionary potential. The trait may or may not experience selection, yet traits have the potential to evolve if they vary and if the trait is inherited with some fidelity from parent to offspring. The two components of variation and heritability together define the evolutionary potential. Without variation in a trait, there can be no evolution, because

without phenotypic variation there can be no variation in fitness. If heritability is zero, it means that there is no degree of similarity between parents and offspring. Then likewise, there can be no evolution by natural selection no matter the strength of selection or the amount of phenotypic variation. This is because, with zero heritability, offspring are free to take any form and therefore the change in frequencies of forms relative to their fitnesses is suppressed by the inability for forms to be propagated across generations. A trait with heritability over time is like throwing a snowball, it maintains its identity as it zooms through the air. A trait without heritability is more like throwing a handful of sand. As soon as it leaves your hand it spreads out and loses its coherence as it sprays out.

Multivariate Price's theorem

The evolutionary logic above forms the basis of quantitative genetics and breeding by artificial selection. These fields have given us powerful mathematical tools to understand just how evolution proceeds given patterns of selection, variation, and heritability (22–25). These tools derive part of their power because they are not reductionist, they deal with phenotypic evolution at the phenotypic level. There is no need to dig lower into genetic levels of explanation for them to work. And so the methods do not offer a complete understanding of the mechanism of evolutionary change at all levels, nevertheless their success in agriculture and evolutionary biology underline their utility. For our purposes, we want to know how hierarchically organized *Stylopoma* colonies evolve, given the simultaneous proliferation of asexually produced zooids and sexually produced.

To see why this works, let us use the formality of Price's theorem. Price's theorem describes the evolutionary response to selection of a trait or set of covarying traits, given the structure of variation and heritability of those traits. Price's theorem defines the evolutionary potential of traits in terms of two matrices: \mathbf{C} the heritability matrix, which measures the similarity of traits between parents and offspring. The diagonal values within the heritability matrix can be calculated as the variation in the offspring phenotype mul-

multiplied by the linear regression of parent phenotype onto offspring phenotype. The off-diagonal elements measures the co-heritability between two traits, for example how similar egg size in a bird is to the egg number that her chicks are able to produce. These interactions can be strong if, as in some birds, egg size and egg numbers have a strong inverse relationship that persists over generations. The phenotypic covariance matrix, \mathbf{P} , describes the amount of variation of all traits and the covariance between them. The product of \mathbf{C} and the inverse of \mathbf{P} defines the evolutionary potential of traits. Selection, $\beta_{w,\phi}$, is defined as the linear regression of fitness (w) on phenotypes (ϕ). The response to selection, is measured as the change in the average phenotype and denoted, $\Delta\bar{\phi}$, is determined by the product of evolutionary potential and selection (Rice 2004)

$$\Delta\bar{\phi} = \mathbf{C}\mathbf{P}^{-1}\beta_{w,\phi} \quad (1)$$

Because this equation is a simple product of three terms, if any of the three factors, selection ($\beta_{w,\phi}$), variation (\mathbf{P}), or heritability (\mathbf{C}) is equal to zero there will be no response to selection.

Hierarchical expansion

The equation above is very simple and it is another way to write the Breeder's equation. For solitary organisms such as cattle, chickens, or beans, it summarizes the evolutionary processes involved adequately. But it, as written, only gets half the story for colonial organisms like *Stylopoma*. It either partially describes colony-level evolution or it partially describes zooid level evolution. We want both. In the general form of Price's theorem, there is an additional term, $\bar{\delta}$, which represents the expected change in the mean phenotype due to processes within the parts

$$\Delta\bar{\phi} = \mathbf{C}\mathbf{P}^{-1}\beta_{w,\phi} + \bar{\delta} \quad (2)$$

It may help to use a paleontological example to think about this term (44). Species can evolve over time. New species may also change phenotypes during speciation so that they differ more or less from their ancestor. There is even a process of selection at the species level that acts by differential rates of extinction and speciation. The term, $\bar{\delta}$, is the average amount of evolution within all species—and in colonies, it is the change in phenotype due to biased changes among zooid members. Hamilton (1975) and Price (1972) were among the first to realize that Price’s theorem can be hierarchically expanded such that this last additive term is equivalent to a lower level of selection. The way this works is to notice that the change in the average traits within an entity has the same units as the change in the average traits among entities

$$\bar{\delta}_{whole} = \Delta\bar{\phi}_{parts} \quad (3)$$

If we rewrite Equation 2 with this recursive evolutionary level in mind we can combine the evolution of wholes with the evolution of constituent parts

$$\Delta\bar{\phi}_{whole} = \mathbf{C}_{whole}\mathbf{P}_{whole}^{-1}\beta_{\mathbf{w}_{whole},\phi_{whole}} + \mathbf{C}_{parts}\mathbf{P}_{parts}^{-1}\beta_{\mathbf{w}_{parts},\phi_{parts}} \quad (4)$$

This looks more complex than it is, because of the notation keeping track of whole colonies and their zooid parts. But in words, Equation 4 says that the evolution of whole colonies is due to the product of the evolutionary potential of traits and selection at the colony level, plus the product of the evolutionary potential of traits and selection at the zooid level.

Unlike previous hierarchical expansions of Price’s theorem (26, 46–48), we do not assume that fitness at the colony level is a direct function of fitness at the zooid level. The standard assumption would be that average fitness of zooids equals the average fitness of colonies. Yet we know, from observed life-history patterns of *Stylopoma* and other bryozoans, that colony fitness is not a simple function of zooid fitness. As discussed in the context of the natural history of *Stylopoma*, the rate of production of ovicells in a colony is not related to the growth rate of the colony. Ovicells can be rare in fast growing large colonies and

common in small slow-growing colonies just as often as ovicells can be common in fast growing colonies (20, 50, 51).

Therefore, selection may occur at both the colony and the zooid level as colonies beget colonies sexually and zooids beget zooids asexually. Given the importance of sexual and asexual modes of reproduction in these colonies, we should assume that selection is rampant at both levels. This fact brings the evolutionary potential at each level into stark focus. What is the pattern of and cause of evolutionary potential at both the colony and the zooid level? If colony traits are variable and heritable, then they can respond to colony-level selection. Likewise, if zooid traits are variable and heritable, then they too have the potential to evolve by natural selection. There may be a conflict between these two levels of selection or they may be aligned.

Quantifying evolutionary potential in *Stylopoma*

As noted above, covariances can be calculated as the variance of a trait multiplied by the linear regression of the trait on itself. This even works in the case of a single trait, because its variance can be multiplied by the linear regression of the trait on itself. Because a linear regression of a trait with itself will always equal 1, the product of a variance value multiplied by 1 is equal to the variance value.

Heritability is of special interest because it is the product of the variance of a trait and its change over generations. This means that the heritability of traits are an efficient feature to investigate because they automatically incorporate a measure of the variance of traits. And so we can understand the evolutionary potential of a trait by only looking at its heritability. This is true because there are two ways that heritability can be equal to zero. First, if there is no variation, then heritability will be equal to zero and as a consequence the equivalent element of the **P** matrix will also be equal to zero. The second way heritability will be equal to zero is if the linear regression of parent and offspring phenotypes is equal to zero.

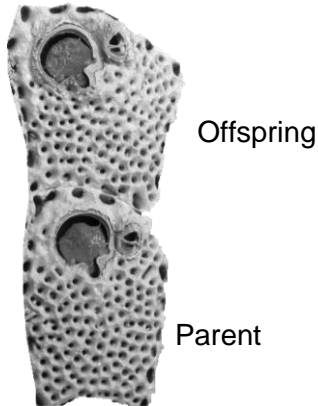
We take advantage of this algebraic shortcut to measure the evolutionary potential of *Stylopoma* traits because of the morphometric data was originally collected for a different purpose (28; 29). Given the results presented in the main article, we now know that there is interesting science to be found by expanding the traits we measure and also from measuring a greater population of zooids. The results we get from this algebraic shortcut are important and justify future work. We discuss some interesting open questions in a section at the end of this document, such as the insights we would get from a fully resolved quantitative genetics understanding, insights we can get from tracking evolutionary potential over time and phylogeny through the fossil record, and the potential of long term experimental evolution.

Measuring heritability

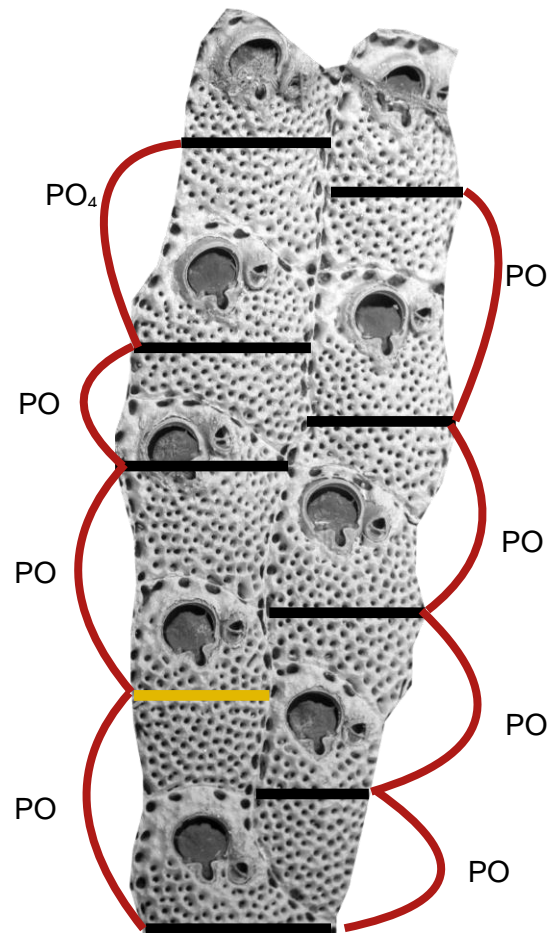
Figure 2 in the main text presents a matrix of heritability values for traits and pairwise combinations. Heritability is the phenotypic covariance between parent and offspring. Using the algebraic shortcut, we breakdown that covariance into a variance and a linear regression. So, for a single trait, the heritability (h) between parent (ϕ_p) and offspring (ϕ_o) is equal to

$$h = \text{var}(\phi_o)\beta_{\phi_o,\phi_p} \tag{5}$$

In *Stylopoma*, and all other bryozoans, clonal lineages are aligned in a chain. The distal end of a parent is where the offspring buds out and forms. At the growing margin of the colony, there will be many clonal lineages each contributing a new generation of zooid. For our analysis, we compare phenotypes of zooids along individual clonal chains of parents and offspring.



For our heritability measure, we compare parents to offspring. As a consequence, the offspring in one generation will be the parent in the next. And so, the phenotype of many individual zooids will be used twice in the calculation of heritability. For example, the width measurement of the zooid shown in gold contributes to both PO_1 and PO_2 , first as an offspring, and second as a parent. Each comparison, PO_i , represents a coordinate on a scatter plot comparing parent to offspring phenotypes, as shown below. It is from this scatter plot that the heritability is calculated. Below, we show the pattern of inheritance from parent to offspring zooids within three exemplar colonies of *Stylopoma* sp. 1 (the full analysis uses many more colonies). The inset image depicts the information that we use to generate the points within this plot. By building up individual



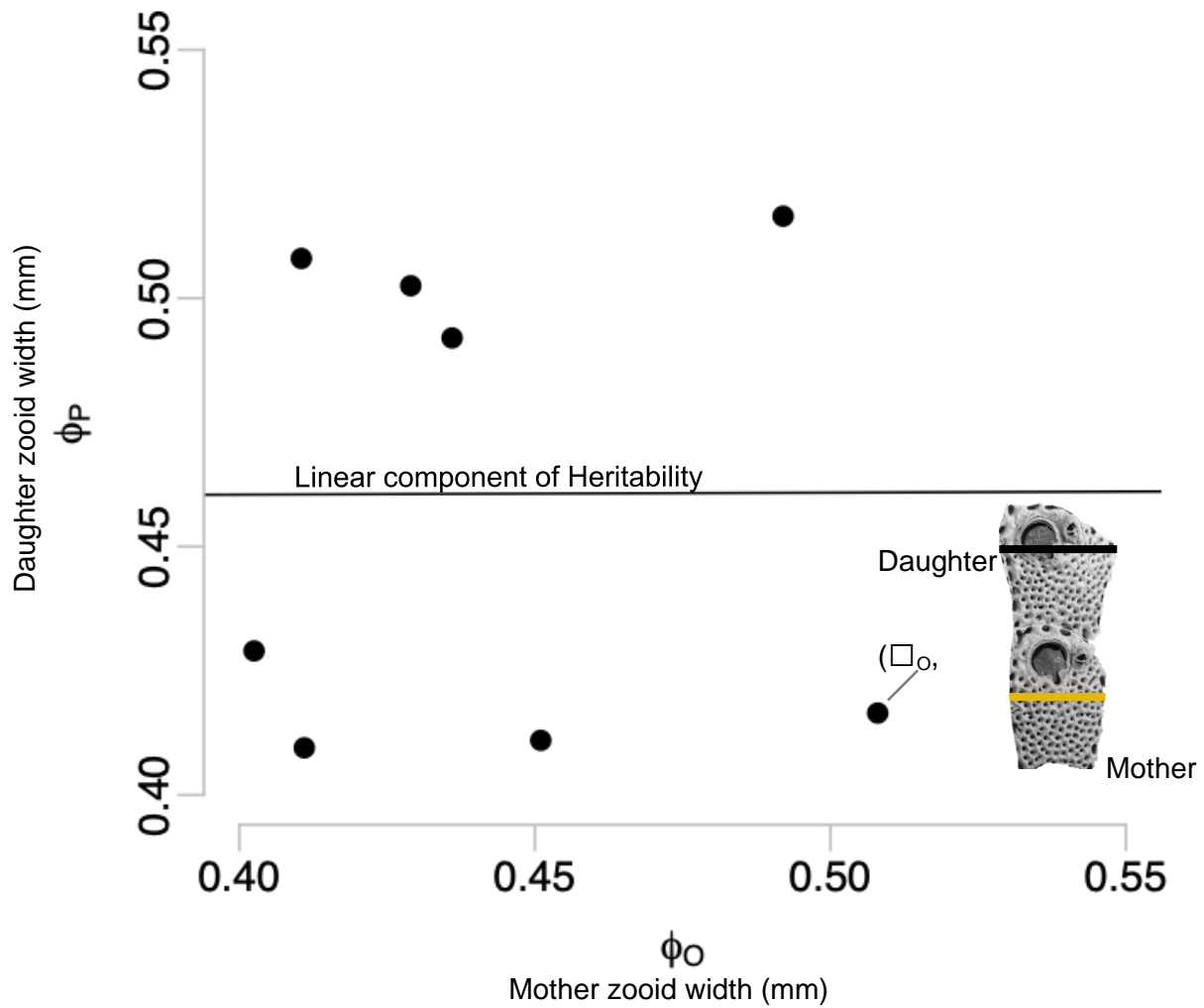


Fig. S5. Evolutionary potential as measured by the heritability of traits between parent and offspring zooids.

parent-offspring comparisons, we can then estimate the heritability of each trait.

We measure the heritability using the linear regression of parent on offspring phenotypes. In the plot above (fig. S5), this linear component of heritability is shown by the solid regression line. Heritability for

colony level traits (fig. S6) are calculated for parent-offspring pairs.

