

Changing allometric relationships among fossil and Recent populations in two colonial species

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Allometry is vital for understanding the mechanisms underlying phenotypic evolution. Despite a large body of literature on allometry, studies based on fossil time series are limited for solitary organisms and nonexistent for colonial organisms. Allometric relationships have been found to be relatively constant across Recent populations of the same species, separated by space, but variable among fossil populations separated by thousands of years. How stable are allometric relationships at the module level for colonial organisms? We address this question using two extant species of the cheilostome bryozoan *Microporella* with fossil records spanning the Pleistocene of New Zealand. We investigate size covariation between feeding modules and three traits with separate functions (reproductive, resource uptake, and defense). We found that within-population (static) allometry can change on timescales of at least 0.1 million years. These within-population relationships do not consistently predict overintraspecific evolutionary allometry, which in turn does not predict those estimated at the genus level. Different functional traits are constrained to different extents by module size with defensive traits being the least constrained and most evolvable, compared with reproductive and resource uptake traits. Our study highlights the potential of colonial organisms in understanding the constraints and drivers of long-term phenotypic change.

KEY WORDS: Bryozoa, colonial animals, evolutionary allometry, paleobiology, static allometry.

Morphological allometry, as described by Huxley (1932), reflects the size relationship between traits controlled by the same underlying growth parameters. Allometric relationships studied across multiple levels of the biological hierarchy (e.g., populations, species, and even genera) have the potential to illuminate evolutionary constraints and selective processes. A stable allometric relationship among populations and related species has been taken as evidence of constraint on phenotypic evolution and a lack thereof as evidence of selection (Pelabon et al. 2014). As a concept, it also has the potential of bridging gaps between disparate disciplines such as quantitative genetics (Cheverud 1982) and paleontology (Gould 1966). Phenotypic traits from breeding data have been used to estimate genetic and phenotypic (co)variances (Cheetham et al. 1993), and estimates and insights

were then used to understand morphological changes on geological timescales across species (Cheetham et al. 1994). However, even with many decades of empirical and theoretical research, clarity in the processes underlying allometric relationships is still wanting (Pelabon et al. 2014). Static allometries (allometric relationships measured within populations of a given species) can be highly similar across different populations of the same species (Voje et al. 2014). There is good evidence that allometric relationships across populations are constrained by pleiotropic effects (Houle et al. 2019), and that the limited variation observed is mainly due to phenotypic plasticity, as seen in selection experiments (Shingleton et al. 2009). Yet, such allometries can vary substantially across closely related species (Voje and Hansen 2013).

Variation in allometric relationships has been studied within and among natural populations and across related species in many different groups of organisms, yet studies of fossilized temporal populations of the same species, each separated by substantial evolutionary time, are still very rare (Wei 1994; Firmat et al. 2014; Brombacher et al. 2017; Yamaguchi et al. 2017; Voje et al. 2022). Studies based on fossilized material, where each sample typically consists of time-averaged individuals sampled across thousands to hundreds of thousand years (Hunt 2004a,b), have the advantage of offering a direct window into deep time. They also provide an opportunity to explore intermediate timescales of evolution difficult to capture using phylogenetic comparative methods or space-for-time studies based on Recent populations. In studies of phenotypic evolution, fossil populations allow us to more directly access differing selective regimes provided by past environments.

Most, if not all, morphological allometric studies have focused on solitary organisms with determinate growth. Here, some quantitative measure or proxy of the adult body mass is typically used as the response variable in morphological allometric studies, given its correlation with life-history, physiological, and ecological traits (Peters 1983). On the other hand, organisms with indeterminate growth, such as many clonal and colonial organisms, have been relatively neglected in the study of morphological and other types of allometry (Burgess et al. 2017). Colonial, modular organisms are thought to have isometric metabolic allometry (measured at the level of the colony) because metabolism primarily operates at the level of the individual module (Hughes and Hughes 1986). Less is known about morphological allometry at the module level. Modules display determinate growth, where a given module stops growing when fully developed, although the colony may continue to bud indefinitely. In this contribution, we aim to alleviate the lack of empirical knowledge with regard to morphological allometry at the module level using cheilostome bryozoans, a group of colonial, modular, marine organisms with a good fossil record.

In evolutionary terms, the modules of colonial organisms are homologous with the individuals of unitary organisms (Ryland 1970; Mackie et al. 1986), which justifies the use of the cheilostome module (i.e., autozoid) as the unit of body size. In addition, colonial modular organisms allow us to quantify environmental plasticity more easily than in solitary organisms. This is because the modular units in a given colony, although genetically identical, display plastic variation due to the environment under which they develop (Hageman et al. 1999). In other words, we can make replicate measurements of the same structures, built multiple times by the same genetic individual, with respect to its “body size” (i.e., autozoid size).

We estimate the allometric relationships through time for three morphological structures, ovicell, orifice, and avicularium,

with respect to feeding modules (autozooids). Each structure has a distinct function within the colony. An ovicell is a reproductive structure in which larvae are brooded (Fig. 1). Ovicell size is thought to directly mirror larval/offspring size (Jackson and Herrera-Cubilla, 2000). An orifice, the opening through which the lophophore (the suspension feeding apparatus) is extruded from an autozoid (Fig. 1), has a resource uptake function. It has been demonstrated that larger orifices correspond to a larger tentacle crown, which in turn translates into a higher feeding rate per zoid (Winston 1977). Avicularia are structures with defensive and/or cleaning functions (Fig. 1), entirely lacking a polypide for feeding. Each of these structures have determinate growth, like the autozooids that produce them, in contrast to the colony, which in many species is ultimately limited by the substrate on which it grows and/or “obstacles” such as other encrusters competing for the same space (Taylor 2020).

As alluded to above, static allometry commonly refers to within-population allometry, whereas evolutionary allometry usually refers to the among-population allometry in related species (Voje et al. 2014) or more rarely, the among-temporal-populations allometry for the same species (Firmat et al. 2014). We will be studying allometry at three different levels, namely, population (fossil and Recent), species, and genus, and hence use the terms static allometry, intraspecific evolutionary allometry, and genus evolutionary allometry for these levels, respectively, for clarity (Fig. 2).

Using two species of the cheilostome genus *Microporella*, richly preserved in the Pleistocene outcrops of the Wanganui Basin in New Zealand and still living today off the coast of northern New Zealand, we answer the following questions: (1) Have static allometric relationships within fossil and Recent populations remained the same through hundreds of thousands to millions of years so that we can predict intraspecific evolutionary allometries for different functional traits (ovicell, orifice, and avicularium)? (2) How far does the autozoid constrain each of these traits and (3) how much population standing variation, here, used as proxy for evolvability, do these different traits have? (4) Lastly, including four additional species of *Microporella* from New Zealand (Recent and fossil), we ask if intraspecific evolutionary allometries predict genus evolutionary allometries estimated using globally distributed species of *Microporella*.

Based on recent allometry literature (e.g., Firmat et al. 2014; Voje et al. 2014), we expect to see stable static allometries through the Pleistocene within the two species of *Microporella*. In other words, we do not expect the allometric slope (Fig. 2) to be detectably different from one time interval to the next for any of the three traits in either of the species. Although there might be selection for larger ovicells to accommodate larger embryos (and consequently produce larger and fitter larvae) and for larger avicularia to perform a better defense, we expect the changes in

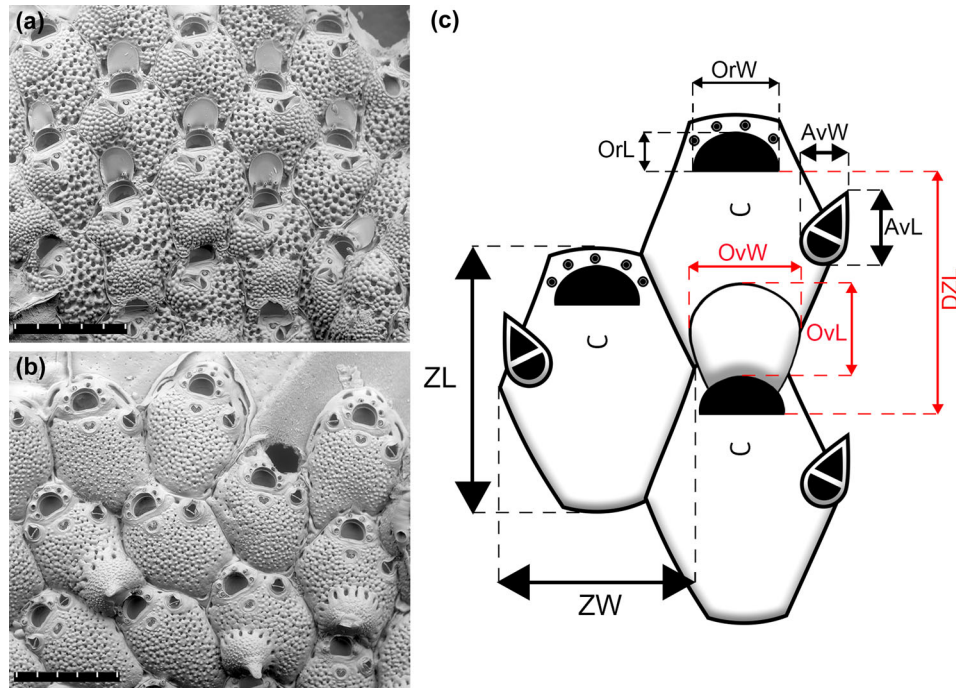


Figure 1. *Microporella agonistes* and *Microporella speculum* and the modular structures measured. Panel (a) shows part of a colony of *M. agonistes* (Recent, Cook Strait KAH1204.04 shell 1 colony 23; SEM number: edm8185) and (b) part of *M. speculum* (Recent, Cook Strait KAH1206.20 shell 3 colony 5; SEM number: edm8152). Scale bars are 500 μm . Panel (c) shows the linear traits measured where ZL = autozooid length, ZW = autozooid width, OvL = ovicell length, OvW = ovicell width, OrL = orifice length, OrW = orifice width, AvL = avicularia length, AvW = avicularia width, DZL = distal autozooid (producing ovicell) length.

ovicell and avicularium sizes to be due to changes in autozooid sizes (i.e., changes in allometric intercepts), rather than a change in the allometric slope, given the expected low evolvability of such slopes (Pelabon et al. 2014; Voje et al. 2014). However, we hypothesize that structures for resource uptake (orifice) are more strongly constrained than defensive (avicularium) and reproductive (ovicell) structures because the orifice is developmentally and functionally more tightly coupled with the autozooid (Taylor 2020). We also expect the latter two traits to be more evolvable than the orifice, as these traits can typically vary a lot among cheilostome species that possess them (Schack et al. 2018). Intraspecific evolutionary allometries are hypothesized to be able to deviate from genus evolutionary allometries, as allometries are thought to evolve on million-year timescales, the timescale separating species within genera (Voje and Hansen 2013).

Material and Methods

MATERIAL

We studied colonies of *Microporella* species, common encrusters of hard substrates (mainly bivalves), in Pleistocene shellbeds of the Wanganui Basin (North Island, New Zealand), and still living today off the coast of New Zealand. Fossil colonies were collected from transgressive system tract shellbeds (see

Table 1), that is, deposits that accumulate during marine transgressions. This is done to maximize the volume of potentially fossilized material collectable, and to minimize environmental differences among both fossil and Recent populations (henceforth “populations”). Recent analogues of fossil samples consist of dredge samples from the nearby Cook Strait, South Taranaki Bight, and the Northland Region (Fig. S1; Tables 1 and S1). Colonies were digitized using a Hitachi TM4000plus tabletop scanning electron microscope (SEM) before measurements of morphological traits. We focused on two species, *Microporella agonistes* and *Microporella speculum* (Fig. 1; Table 1), as they are found in most of the shellbeds we examined, but we also studied four other New Zealand species, namely, *Microporella discors*, *Microporella intermedia*, *Microporella ordo*, and *Microporella rusti*, that are co-observed with the at different times (Fig. S2 and Table S1).

For each colony, we measured the maximum length and maximum width of three randomly selected autozooids, orifices, avicularia, and ovicells, from a single or multiple SEM images (but see below for selection). These traits are homologous across *Microporella* species. For each ovicell, the length and width of the next distal zooid producing the ovicell were also measured. Given that the proximal margin of the distal zooid is in most cases hidden by the ovicell, zooid

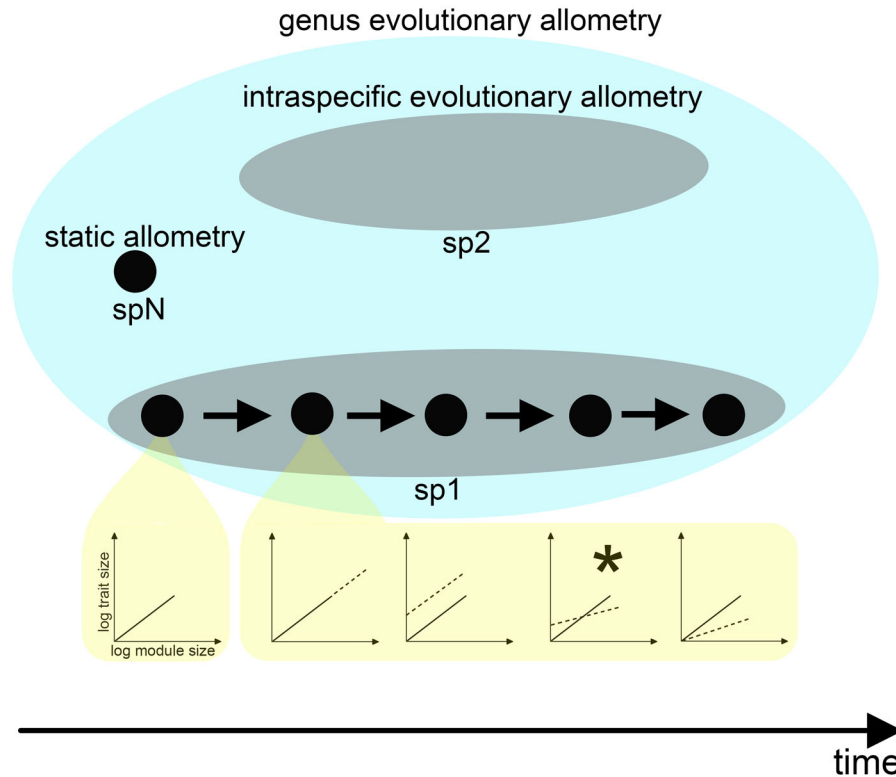


Figure 2. Schematic diagram showing the hierarchical levels of allometric relationships measured. Solid black circles refer to fossil and Recent populations in which static allometries are estimated. Gray ellipses indicate species (sp1 and sp2) with multiple temporal populations where we estimated intraspecific evolutionary allometry. Note that for a simplified representation, black circles were omitted in sp2 ellipse. The species spN is an example of species represented only by one population. Genus evolutionary allometry (light blue ellipse) is estimated using all species of *Microporella* for which we have data ($N = 151$). Note that vertical space in the main schematic diagram has no meaning. Arrows indicate the transitions between adjacent temporal populations. Using the first transition of species 1, we show how allometric relationships (slope and intercept) can change (dashed lines) from an ancestral state (solid black line) (adapted from Pelabon et al. 2014). The asterisk indicates the scenario that we generally find (i.e., both slopes and intercepts can evolve).

length was measured from orifice to orifice (see Fig. 1c, DLZ). Note that the maternal zooid bearing the ovicell is not measured, not only because it did not produce the ovicell it bears, but also because its distal margin is hidden by the ovicell (Fig. 1c). Only structures that show clearly defined boundaries, were undamaged, and nontilted with respect to the frontal plane were measured. Measurements were taken using ImageJ2 (Rueden et al. 2017). A selection of previously measured zooids was measured again on different occasions to quantify measurement error (see Fig. S3). We estimated areas for each of the four structures (autozooid, ovicell, orifice, and avicularium) by multiplying their maximum length and width (measured in microns) as this is a good proxy for areas measured using outlines, as shown for diverse cheilostome species (Liow and Taylor 2019).

To complement the static allometric (Fig. 2, black circles) and intraspecific evolutionary allometric relationships (Fig. 2, grey ellipses), genus evolutionary allometric relationships (Fig. 2, light blue ellipse) were also estimated using ovicell and autozooid length and width of 151 modern and fossil species of *Microp-*

orella taken from Di Martino and Liow (2021) and Liow and Taylor (2019). Orifice and avicularium length and width of the same colonies/species were measured in this study using the same SEM images used in those two earlier publications. All data and metadata required to reproduce analyses are provided in the Supporting Information.

ESTIMATION

We use Ordinary Least-Square (OLS) regression models to estimate morphological allometries where the logged colony median area of each of the three traits (X) was used as a response variable, and the logged colony median area of autozooids (AZ) was used as a predictor variable, as recommended (Hansen and Bartoszek 2012). By using colony medians for each trait, we capture some of the variation that is due solely to environmental variability, as autozooids and the other traits we measured within a colony are produced by the same genetic individual.

The estimated slope b is thus the allometric relationship between the given trait and autozooid size. We mean scale

Table 1. Summary of formations and Recent populations investigated. Populations used in this study, with name abbreviations (used throughout the text), age ranges in millions of years ago (Ma), and number of zooids and colonies (in parentheses) measured for the two main species, *Microporella agonistes* and *Microporella speculum*. Note that all the fossil samples come from transgressive system track shellbeds and the Recent material are dredged from sites where shells accumulate (i.e., modern analogues of shellbeds).

Formations or Populations	Abbreviations	Age Range (Ma)	<i>M. agonistes</i>	<i>M. speculum</i>
Nukumarū Limestone	NKLS	2.29–2.08	241 (35)	0
Nukumarū Brown Sand	NKBS	2.03–1.97	274 (42)	0
Upper Kai-iwi Shellbed	UKSB	0.68–0.62	280 (44)	242 (40)
Lower Castlecliff Shellbed	LCSB	0.58–0.52	254 (48)	227 (47)
Shakespeare Cliff Sand Basal Shellbed	SCBSSB	0.43–0.40	160 (24)	265 (55)
Upper Castlecliff Shellbed	UCSB	0.40–0.38	0	116 (25)
Cook Strait	CS	0	158 (23)	328 (43)
South Taranaki	ST	0	239 (33)	269 (38)

AZ before applying OLS such that, rather than estimating the intercept a , that is, the value of the trait X when autozooid size is zero, we estimate and report a_m , that is, the value of the trait X at population mean of autozooid size (Pelabon et al. 2014). With each model applied to data from different populations, we visually examined the residuals of the model and checked for outliers, correcting those with blatant measurement errors but retaining those data that represented real variation, and performed Shapiro tests to verify normality of the residuals. All model estimates reported are associated with residuals that conform to normality. There is very high replicability in our measurements (Fig. S3) such that measurement error can be disregarded.

In addition to reporting slopes b and mean-centered intercepts a_m and their 95% confidence intervals (CIs) for different functional traits from separate temporal populations of the same species, we also report adjusted R -squares for each of these linear models (a measure of how constrained each trait is by the autozooid) and present variances and standard deviations for all traits. We used the mean-standardized variance of each of the traits as a measure of their evolvability (Houle 1992).

To test if temporal populations (Fig. 2, black circles) have detectably different allometries, we used the Akaike information criterion (AIC) to compare modeled allometric relationships with (additively, i.e., population slopes differ, or multiplicatively, i.e., population slopes and intercepts differ) and without populations as an explanatory factor (i.e., including or excluding populations as factors). For traits in which a model with populations (formations or Recent p as a factor) was preferred by AIC, we also made pairwise comparisons of the per population estimates using least square means, with Bonferroni corrections, implemented in the R package emmeans (Lenth 2021).

To estimate evolutionary allometric relationships for each of the traits, we similarly fit linear models to species averages for genus-level evolutionary allometry, and colony medi-

ans for species-level allometry for all six New Zealand *Microporella* species. To test if species have detectably different allometries from the genus, we used AIC to compare modeled allometric relationships with (additively and multiplicatively) and without taxon (i.e., either a New Zealand species or all of the non-New Zealand *Microporella* data as “genus”) as a factor. For traits in which a model with taxon as a factor is preferred by AIC, we also made pairwise comparisons of the species estimates using least square means to highlight any species-specific differences.

All analyses are done in R (R Core Team, 2021) Version 4.1.0 (2021-05-18). Code, data, and metadata for the population samples necessary for replicating the study are deposited at Zenodo in its entirety.

Results

STATIC ALLOMETRY CAN CHANGE THROUGH TIME

We present the fit of OLS models for each separate combination of species, trait, and formation, summarized in Figures 3 and 4 and detailed in Figures S4–S9 and Tables S2–S7. It is apparent that estimates of static allometric slopes (b) and mean-centered intercepts (a_m) from some populations can be very different for the same species and trait (Figs. 3, 4), for example, the estimated slopes of two temporally adjacent *M. speculum* avicularia do not have overlapping CIs around 0.4 million years ago. The static allometric slopes and mean-centered intercepts are also often outside of the 95% CI of the intraspecific evolutionary allometric slope (solid black horizontal lines in Figs. 3, 4), for example, four out of six *M. speculum* populations fall outside of the intraspecific evolutionary allometric intercept for the orifice. Comparing models for a given combination of species and trait, we find that models where populations are included multiplicatively as a factor are often preferred over those without

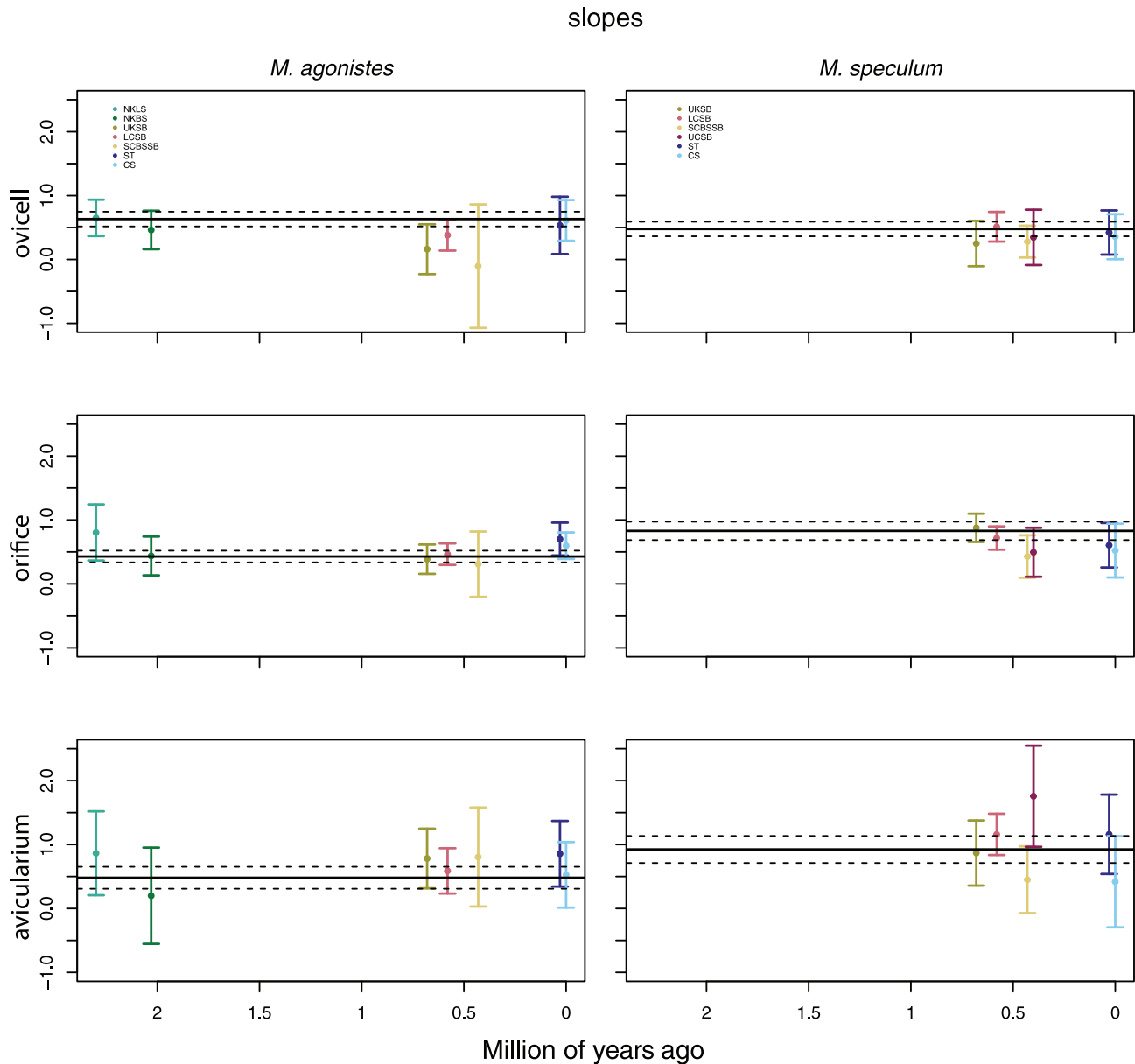


Figure 3. Allometric slopes for ovicell, orifice, and avicularia over time for *Microporella agonistes* and *Microporella speculum*. Each panel shows estimates for slopes (b) for each formation/population for ovicell (first row), orifice (second row), and avicularia (third row) for the two species (columns). The populations are color coded and named in the panels in the first row (see Table 1 for full names and age ranges). Each dot shows the estimate and bar shows 95% confidence interval (CI). The black solid horizontal line in each panel is the grand estimate, and the dotted lines their 95% CI. See Tables S2–S7 for further details.

(see next paragraph) for both *Microporella* species studied (*M. agonistes* and *M. speculum*) (Table S8). These results indicate that static allometries are measurably different from each other and as a consequence, we cannot predict intraspecific evolutionary allometries from them.

Pairwise comparisons identify which of the temporal populations of *M. agonistes* and *M. speculum* are different in their allometric relationships (Table S9). For example, in *M. speculum*, the allometric slope between ovicell and auto-

zoid is significantly different between the populations LCSB and SCBSSB (see Table 1 for full names and estimated age ranges), where their estimated difference is 0.4 (SE = 0.12, P -value = 0.013). In *M. agonistes*, the ovicell allometric slopes are significantly different at the $P = 0.05$ level between UKSB versus NKBS, CS or ST (see Table S9 for estimates of differences), where the slope of UKSB is lower than for the other three populations (Fig. 3). For the orifice, static allometric slopes (b) do not differ significantly for either species (Figs. 3, S5, S8),

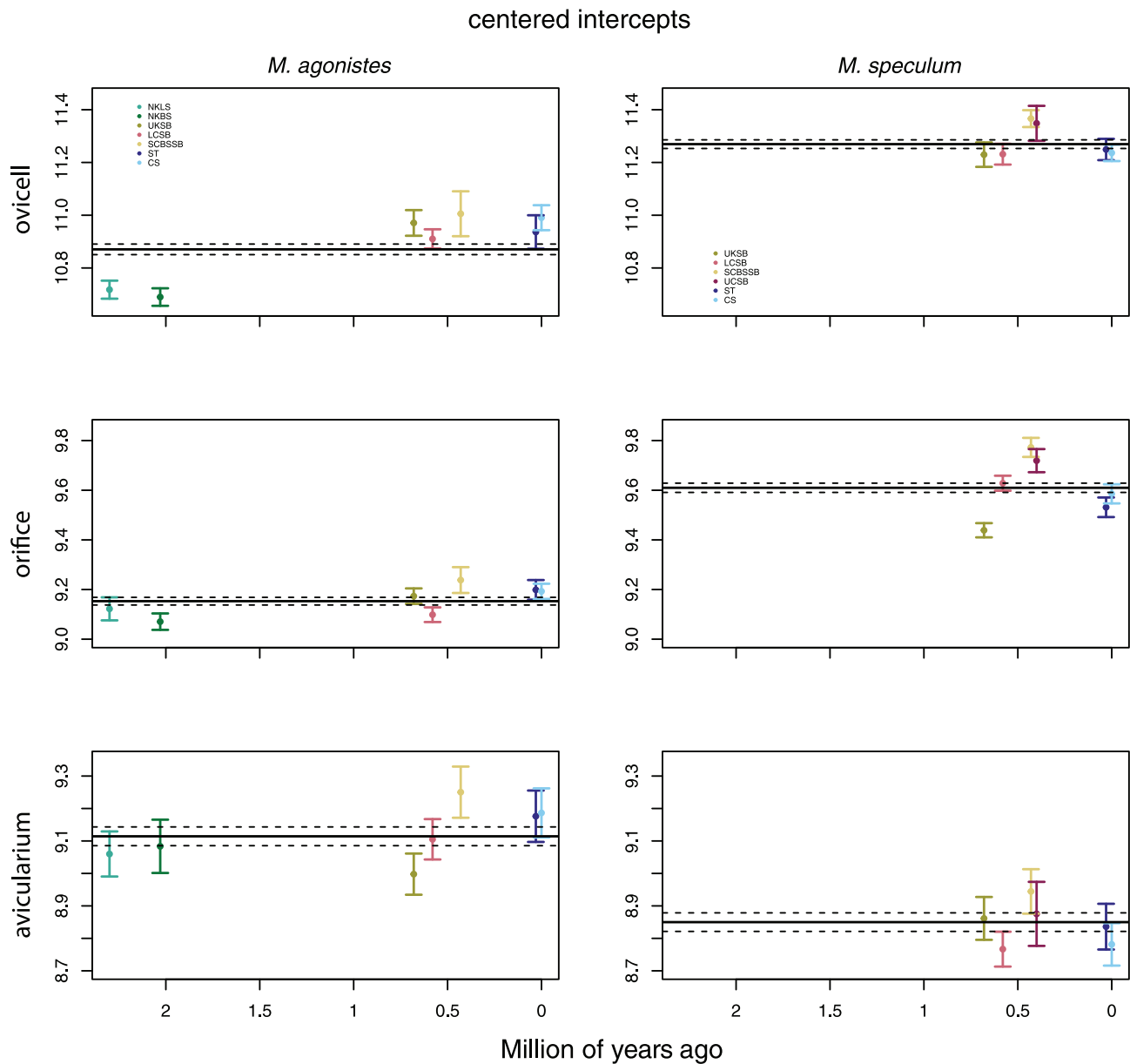


Figure 4. Mean-centered trait estimates for ovicell, orifice, and avicularia over time for *Microporella agonistes* and *Microporella speculum*. Each panel shows estimates for mean-centered intercepts for each formation/population for ovicell (first row), orifice (second row), and avicularia (third row) for the two species (columns). Each dot shows the estimate and bar shows 95% confidence interval (CI). The black solid horizontal line in each panel is the grand estimate, and the dotted lines their 95% CI. The populations are color coded as in Figure 2 and named in the panels in the first row (see Table 1 for full names and age ranges). See Tables S2–S7 for further details.

as for the avicularia allometry for *M. agonistes* (Figs. 3, S6). In contrast, the allometric slopes between avicularia and autozoid are significantly different at the $P = 0.05$ level between UCSB and SCBSSB, CS and ST, and also LCSB and SCBSSB (see also Table S9 and Fig. 3) for *M. speculum*. In general, allometric intercepts differ among more populations for any given species-trait combination than for their corresponding slopes with the only exception being *M. speculum* avicularia (Table S9).

Here, only one formation pair is detectably different in its allometric intercept but four population pairs are different in their slopes.

DIFFERENT FUNCTIONAL TRAITS ARE CONSTRAINED TO DIFFERENT EXTENTS BY THE AUTOZOID

In the static allometric models, adjusted r -squared (a.rsq for “All” in Tables S2–S7) values for *M. agonistes* are highest for ovicells

(0.38) and lowest for avicularia (0.13), whereas orifices are intermediate (0.30). Although orifices are most constrained in *M. speculum* ($a.rsq = 0.44$), ovicells and avicularia have similar constraints ($a.rsq = 0.26$ and 0.29 , respectively).

When using direct modules instead of colony median values, this general picture remains ($a.rsq$ [module] for “All” in Tables S2–27), although the r -squared values are consistently lower. These results suggest that different traits are constrained to different degrees by the size of the primary module (autozoid). The comparison of r -squared values at the colony and module levels suggests that there is more flexibility at the module level than at the colony level.

DIFFERENT FUNCTIONAL TRAITS ARE EVOLVABLE TO DIFFERENT EXTENTS

The mean-standardized variances ($stdvar$ in Tables S10 and S11) are between 0.0012 and 0.0016 for autozooids, orifices, and ovicells for both species. However, the mean-standardized variances are double to quadruple (0.0052–0.006) for avicularia compared with other traits for both species, suggesting that the avicularium is a more evolvable trait.

INTRASPECIFIC AND GENUS EVOLUTIONARY ALLOMETRIES CAN BE DIFFERENT

Superimposing intraspecific and genus allometric data on the same figure suggests that orifice allometry has less of a spread of values than that for the ovicells and avicularia (Fig. 5). The r -squared values for the orifice are also higher than those estimated for the ovicells and avicularia, regardless of the specific models being compared for a given trait (Table S12). Comparing models using data for a given trait (Fig. 5), we find that models where taxon is included as a factor, multiplicatively, are preferred over those without, suggesting that intraspecific and genus evolutionary allometries can be distinguished (Table S12). Pairwise comparisons within such models show that some estimated intraspecific evolutionary slopes for the New Zealand species are significantly different from the genus evolutionary allometric slopes and some are different from each other for the ovicell and the orifice, but not for the avicularium, where slopes are not different (Table S13). Many of the mean-centered intercepts for species are significantly different from the genus evolutionary allometric intercepts and also different from each other (Table S13). The mean-centered intercepts and r -squared values of the allometries are given in Table S14.

Discussion

Studies based on Recent natural populations find little variation in allometric slopes in the same species (Pelabon et al. 2014). On the other hand, allometric parameters have sometimes been

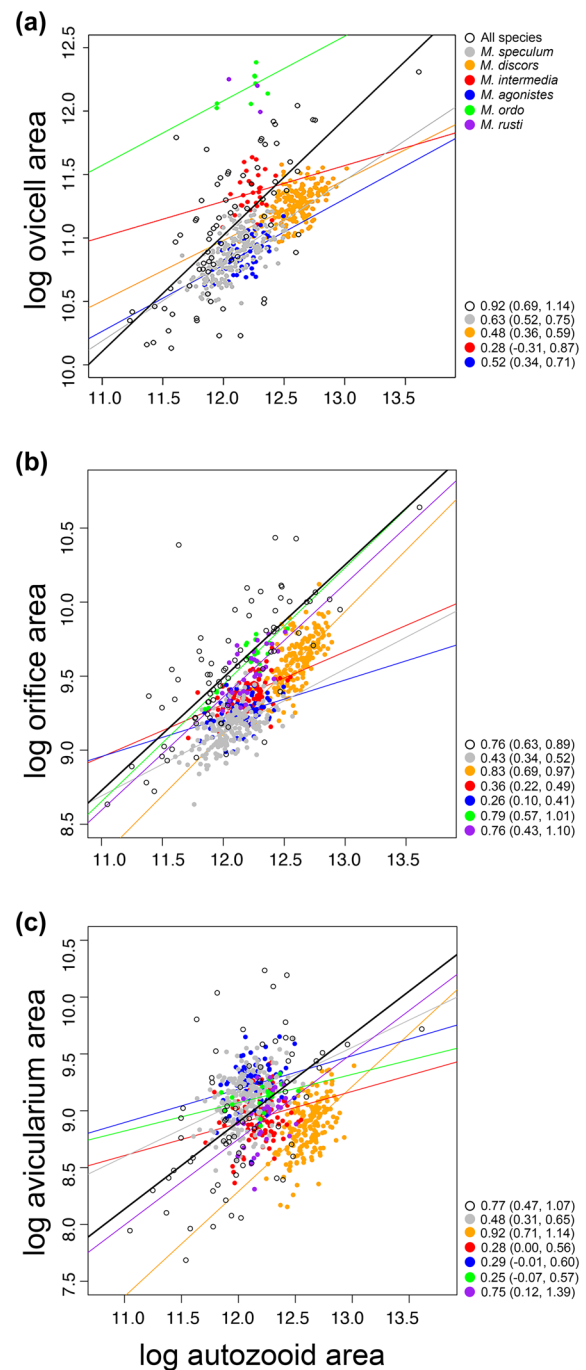


Figure 5. Genus- and species-level evolutionary allometric relationships for (a) ovicell, (b) orifice, and (c) avicularium. Each filled dot shows colony medians for traits and autozoid areas for New Zealand species, whose separately estimated allometries are shown with colored lines. Note that *Microporella rusti* is plotted but its ovicell allometry not estimated. The open circles are species medians, whose estimated evolutionary allometry is shown as a thick black line. In the bottom right of the plot, slope estimates are shown, together with their 95% confidence interval (CI) in parenthesis for the genus and for each New Zealand species with ≥ 20 data points. See Table S14 for r -squared values and mean-centered intercept (a_m) with 95% CI.

found to be different for the same species whose populations are separated by thousands of years (Voje et al. 2022) but other times not (Firmat et al. 2014). When exactly do allometric relationships change? Do they happen only at rare and large climatic upheavals within species lineages (Brombacher et al. 2017), such that most studies using fossil data will not detect them even over the timescales of the fossil record (Firmat et al. 2014; Yamaguchi et al. 2017)? Do they tend to happen at speciation rather than during anagenesis within the same species lineage (Hunt 2013)? Using temporal populations that go beyond decadal and centenary scales of the same species, our results suggest that static allometric slopes can change on timescales of at least 0.1 million years (the approximate temporal distances among our fossil populations; see Table 1) within species and, despite constraints, do differ appreciably among closely related species. In addition, the mean-centered intercepts also changed in our example (Fig. 2, asterisked scenario). However, our data do not span an easily delimited climatic shift and do not yet allow us to pinpoint the timing of speciation events.

This is one of the first systematic studies of allometric relationships among morphological structures in colonial organisms, as far as we are aware. With little prior research to base our expectations on, we only supposed that smaller autozooids cannot produce very large polymorphs due to resource acquisition and structural limits. However, we do not have much basis for having prior ideas as to the magnitude of constraints on modular traits by the producing unit (the autozooid). Similarly, large autozooids are likely to require larger orifices to accommodate larger feeding lophophores to meet their energetic needs, although they could produce smaller ovicells and avicularia. The r -squared values of the allometric relationship of any trait-species combination were all relatively low (around 0.2–0.3, with the highest at only 0.5) in this study. Interestingly, autozooids had a less tight correlation with the structures they directly produced, than that for colony averages, suggesting that the control of growth is greater by the colony rather than individual modules. We have shown that our low r -squared values are not due to measurement error, but we cannot rule out sampling error (due to relatively small sample sizes) and time averaging (a universal phenomenon for fossil samples). Despite these caveats, the lower r -squared values seem to be at odds with higher values seen in typical allometric studies, most of which are based on Recent and solitary organisms, and with whole body mass or volume as variable in the x -axis (e.g., see Voje et al. 2014, Pelabon et al. 2014, and references therein). Our estimated values are more reminiscent of other studies on fossilized populations (e.g., sticklebacks [Voje et al. 2022] and rodents [Firmat et al. 2014]), which could not rule out measurement error contributions to low r -squares. Alternatively, but not mutually exclusively so, our results suggest that colonial organisms are less constrained genetically and/or developmentally

than solitary organisms and/or have more room to respond to differential selective pressures. This ability to respond to selective pressure is in line with Houle et al. (2019), whose results from controlled, laboratory experiments involving *Drosophila* suggest that allometric slopes are held in place by natural selection rather than genetic constraints. The changes in autozooid size over evolutionary time within species can be interpreted as a change in the adaptive landscape (Figs. S10, S11), which might have to do with the shifting of environmental conditions (Di Martino and Liow 2021): these autozooid size changes are generally followed (direction-wise) by size changes in other traits (with the exception of avicularia, see below).

Variation is the ingredient for evolution, but the observed variation for a given trait can be due to plastic variation, genetic variation, or constraints between co-evolving traits, as already mentioned. In our data, it is clear that within-colony plastic variation and within- and among-population variation for any given trait is high (Figs. S10, S11), but the amount of trait covariance and the stability of allometric relationship vary for different traits. As we hypothesized, structures linked to resource uptake (orifice) were found to be more constrained by autozooid size than defensive polymorphs (avicularia) and reproductive structures (ovicells), regardless of whether data stem from a single temporal population, are at the species level (combining temporal populations) or at the genus level (using species averages). Our hypothesis is based in part on function but also on evolutionary developmental processes. In *Microporella*, the formation of the orifice can be attributed entirely to the autozooid to which it belongs as part of its frontal shield, whereas ovicells are formed as an outgrowth of the distal neighbor zooid, and avicularia are budded by kenozooids surrounding the autozooid in question.

Sexually selected traits are often contrasted with nonsexual traits in allometry studies, where there is some evidence that such traits demonstrate positive allometry (i.e., allometric slope > 1). Our hypothesis that ovicell size might demonstrate positive allometry due to selection for larger larvae with higher survival, and hence recruitment, was rejected. Intraspecific slopes are all shallower than the genus-level slope (Fig. 5; Table S13), suggesting that larger ovicells are produced by larger autozooids, even though such slopes can evolve, and despite ovicell size not being very highly constrained by autozooid size. Among the New Zealand species, the ovicell allometric relationship of *M. ordo* is the most dissimilar from the genus-level allometry (Fig. 5a). Although only a few data points are available for this species, the giant size of the ovicell (Fig. S2c) suggests that this difference might be confirmed if the sample size could be boosted. The same is valid for *M. rusti* (Fig. S2d), for which the allometric slope was not estimated owing to the finding of only three fertile colonies among our samples.

On the other hand, the intraspecific allometric relationship of avicularia cannot be distinguished from that at the genus level (Table S13). This is likely in part due to avicularia size not being limited by autozooid size as evident from the low r -squared values for the best model when compared with the ovicell and orifice (Table S12). The idea of the avicularium as a more evolvable trait, compared to orifice and ovicell, is supported by its mean-standardized variance being the double to quadruple of that of the other traits (Tables S10 and S11). Vestigial structures have long been thought to have high variability (Guthrie 1965), and it is through this vestigialization that avicularia have gained novel functions (Carter et al. 2011). Here, we show that this variability is also apparent at population level, compared with other structures not formed by vestigialization (the orifice and the ovicell).

In the context of the *Microporella* genus, which is monophyletic (Orr et al., 2022), evolutionary allometries, just as in the case of species, are not very tight, although they are in general higher than within species and are the highest for orifice and lowest for avicularia (r -squared values are 0.64 and 0.27, respectively; see Table S14). Similarly, the clade in New Zealand, whose six members we studied here, is monophyletic within *Microporella* (Orr et al. 2021). These New Zealand *Microporella* have been evolving separately for at least 1–5 million years, and in some cases as long as about 20 million years (Brown 1952; Ramsfjell et al. 2022). Again, the orifice is the most “constrained” among the three traits, whereas the avicularia is the least so and the ovicell intermediate (Table S14).

Despite our efforts in maximizing sampling and data collection, some combinations of populations and species have less data than we would have wished. But the amount of variance across formations spanning different amounts of time (Fig. S12) did not give us reason to worry that the trait variation measured is biased due to the amount of time potentially represented. Ideally, to be able to tease apart constraints and selection, some measure of fitness such as fecundity (Di Martino and Liow 2021) would have given extra information, but this was not available for this study. We have also not used a phylogenetic comparative framework (Hunt 2007) in this study as we lack phylogenetic (sequence or morphological) data for key species. Although we have discussed our material as if each *Microporella* species could be taken as an independent evolutionary unit, more nuanced inferences may be made when accounting for their relatedness.

Conclusions

The study system introduced here has untapped potential for bridging gaps between disparate disciplines such as microevolutionary and macroevolutionary biology and the timescale they usually represent. Fossil populations of the same species, such as those we used here, can complement spatially separated Recent

populations, both of which are separated by varying amounts of evolutionary time. Using cheilostome bryozoans, we can begin to separate plastic variation using their modular, colonial structure from genetic variation that must have arisen through the vast temporal separation of the populations. A vast number of these organisms could easily be collected from fossil outcrops, which can then be subjected to automated phenotyping (Lürig et al. 2021) allowing much speedier data collection. A total-evidence cheilostome phylogeny is estimable given the ground work already done (Orr et al. 2021, 2022), and quantitative genetics experiments are also viable as some of these species are extant. Using our model system, we now know that empirical allometric relationships can evolve on shorter timescales than seen before in natural systems (e.g., Voje and Hansen 2013), and that some traits (measured on the same genetic individual) are more subject to constraints than others. In particular, resource acquisition traits are more constrained than reproductive and defense traits. Quantifying the relative contributions of natural selection, constraints, and plasticity to phenotypic change in natural populations across the biological hierarchy is within reach.

AUTHOR CONTRIBUTIONS

EDM and LHL came up with the study. EDM collected the bulk of the data. LHL did the analyses. EDM and LHL wrote and revised the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

Data and code are deposited at Zenodo <https://doi.org/10.5281/zenodo.6725567>. All measured specimens are housed in and available from the Natural History Museum University of Oslo, Norway.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

- Figure S1.** Map of New Zealand showing the location of sampling localities.
- Figure S2.** SEM micrographs showing part of a colony of the four species of New Zealand *Microporella* used in this study, in addition to *M. agonistes* and *M. speculum* figured in the main text.
- Figure S3.** Reliability of measurements.
- Figure S4.** Static allometry for ovicell size through time for *Microporella agonistes*.
- Figure S5.** Static allometry for orifice size through time for *Microporella agonistes*.
- Figure S6.** Static allometry for avicularium size through time for *Microporella agonistes*.
- Figure S7.** Static allometry for ovicell size through time for *Microporella speculum*.
- Figure S8.** Static allometry for orifice size through time for *Microporella speculum*.
- Figure S9.** Static allometry for avicularium size through time for *Microporella speculum*.
- Figure S10.** Trait variation in colonies of *M. speculum*.
- Figure S11.** Traits through time.
- Figure S12.** Amount of time represented and trait variation.
- Table S1.** Colonies and zooids measured per formation/population.
- Figure S12.** Amount of time represented and trait variation.
- Table S1.** Colonies and zooids measured per formation/population.
- Table S2.** Allometric relationship between ovicell (Ov) and autozooid (AZ) size for *M. agonistes*.
- Table S3.** Allometric relationship between orifice (Or) and autozooid size for *M. agonistes*.
- Table S4.** Allometric relationship between avicularium (Av) and autozooid size for *M. agonistes*.
- Table S5.** Allometric relationship between ovicell (Ov) and autozooid (AZ) size for *M. speculum*.
- Table S6.** Allometric relationship between orifice (Or) and autozooid size for *M. speculum*.
- Table S7.** Allometric relationship between avicularium (Av) and autozooid size for *M. speculum*.
- Table S8.** Model comparison for temporally different allometries for *M. agonistes* and *M. speculum*.
- Table S9.** Pairwise comparisons of allometric slopes and intercepts for formations/populations.
- Table S10.** Summary of data for *M. agonistes*.
- Table S11.** Summary of data for *M. speculum*.
- Table S12.** Model comparison for genus-level evolutionary allometry.
- Table S13.** Pairwise comparisons of evolutionary and intraspecific evolutionary allometric slopes and intercepts.
- Table S14.** Values of mean-centered intercept (am) with 95% CI, and R-squares (r.sq) for the genus and the New Zealand *Microporella* species.