Main points:

* P substitutes for G, so can use the fossil record
  + Why we use bryozoans
  + Use random skewers to see how well correlate
  + Look at size (eigen vectors) to see what proportion of P G is
* Want to know if and how much G changes over time
  + Look at angle difference of Gmax over time
* Want to know if even as G changes if P changes in same direction
  + Look at angle difference of Gmax and Pmax over time
* Want to know drivers: estimate E; temperature proxy

Discussion:

* Changing G over time hints at constraints in evolution
* Variation is also changing across time (time effects) but also in Upper Kai-Iwi, suggesting a lot of change is happening and we can’t capture it

Sensitivity:

* Look at my measurement error compared to Steginator
* Look at degree of time averaging compared to variation over time

Terms:

* G matrix = genetic variance-covariance matrix, estimated by MCMC
* P matrix = phenotypic variance-covariance matrix, linear measurements
* Gmax =
* Pmax =
* E matrix = environmental variance-covariance matrix; E = P-G

**Introduction**

1. problem: fill a gap; within lineage and over time and include contemporary; and test independently if fossil data behave similar or not to recent; seeing if population; maybe 2 paragraph

2. system

3. background

4. what we do

My take on the problem/narrative: Adaptation depends on only to factors, natural selection and genetic variance. Selection’s role in adapting species to their environment is well-established, but to what extent trait variance can also predict evolution is more controversial. Interestingly, several studies have found that phenotypic divergence among taxa are often biased towards directions with greater amounts of genetic variance. This is a puzzle, as the amount of genetic variation has been considered a “weak” constraint on phenotypic diversification beyond microevolutionary timescales, i.e. the idea has been that genetic variance will at some point turn up through mutations so that selection can proceed in the direction it tries to move the population. So, why, then, is evolution mainly found to proceed in directions with high variability? The two main hypotheses are 1) that selection shapes the population variance to align with the direction of phenotypic change, or that 2) phenotypic diversification is constrained to mainly happen along directions of a lot of variance.

Comparing G estimated for extant populations belonging to the same species or extant species belonging to the same genus suggest G does change, albeit usually not a lot. Also, in the majority of cases, G in extant taxa predicts how populations and species have diversified phenotypically. However, such differences in G between living taxa has limited capacity to say something about the tempo and mode of change in G and if these changes in G affects trait evolution. In short, we know next to nothing about if and how G is changing across time within a lineage and how these changes affect trait evolution. These are key questions if we want to differentiate between the two main hypotheses mentioned in the first paragraph.

Estimating G from a time series of population data would enable an assessment of how much G changes across time and if G seems to systematically bias change within a lineage towards directions with large amounts of variance. This has not been done however, as estimating G demands large amounts of measured individuals with known pedigree. Bryozoa offers a unique possibility to tackle these two challenges as the combination of their very rich fossil record and their clonal nature allows decomposing the observed phenotypic variance into a genetic component and an environmental component.

This study capitalizes on the unique properties of bryozoans and estimate several G matrices belonging to the same lineage, spanning about 2.3 million years. We use this data to ask the following questions:

1. Do G change across the time interval of 2.3 million years?
2. Does past change (i.e. selection) affect G?
3. Is phenotypic change happening in directions with above-average variability?

Using quantitative genetics (QG) on fossil data is to some extent controversial in the sense that QG is a theoretical framework built to understand microevolutionary change. The time averaging issue in fossil data is for example violating the assumption of estimating QG parameters from a population at a single time point. Also, very few fossil taxa allow estimating G, while P is a more tractable matrix to estimate for fossil taxa. In this study, we are therefore also assessing the following questions to investigate the usability of QG in the fossil record:

1. How similar are the G matrices estimated from fossil samples compared to a G matrix estimated from a recent population? I.e., is time averaging a big issue?
2. How good of an approximation is the total phenotypic variance covariance (P) matrix of G? I.e., would be obtain the same results if we switched P with G in our methods?

Inherent in understanding the tempo and mode of evolution is understanding the variation being acted upon and the selection gradient. It is known that selection can direct evolution (obvs. Darwin), and has been suggested that variation – and covariation – can shift the direction of evolution away from the selection gradient (hunt study, Nei). Estimating the selection gradient is not an easy task, especially when investigating deeper time. As a result, there has been interest in understanding the role of variation, called evolvability, in predicting the direction of evolution. Much of this work has investigated evolvability in extant species, sometimes including fossil specimens; no work to date has investigated evolvability over time.

The variation of interest is often the genetic variation, or the variation in phenotypic traits from parent-offspring relationships, as this is the fodder for selection to act upon. The shape and size of the genetic variance-covariance matrix (G matrix) is important in determining the phenotypic space a lineage can explore (cite Schluter, others), with evolvability relating to the axes of variation within this space. If selection is low or constraints, defined as the covariation in the G matrix, are important, then phenotypic change should occur along the vector in multivariate space along the most variation, Gmax, or around Gmax (i.e., above average evolvability). Indeed, majority of studies investigating the role of variation on phenotypic evolution show that evolution often occurs along axes of above-average evolvability (i.e., variation) (cite Voje, etc.). While some of these studies (e.g., CITE) have used fossil data, none have been able to investigate how the G matrix changes over time.

Given the potential for the G matrix to evolve rapidly under micro-evolutionary time scales, it remains unclear if the G matrix is stable enough over macro-evolutionary time scales to provide insights into multivariate evolution. Here, we apply a quantitative genetic framework to the fossil record. We first estimate G across time. We then examine how well the P matrix can substitute for the G matrix, testing Cheverud’s hypothesis. We also ask if the G matrix changes through time, and thus if the phenotypic space available changes through time. Finally, we test if, based on the directions of the G matrix, if phenotypic change is occurring in directions of above-average evolvability. Our approach attempts to apply evolutionary quantitative genetics to fossil data to investigate how the G matrix changes within a single lineage over millions of years.

Paleontologists, or practitioners of biology in the fossil record, are often limited to estimating the phenotypic variance-covariance matrix (P matrix) as parent-offspring relationships are often unknown. The P matrix is naturally “noisier” than the G matrix, as it represents the amount of genetic variation and environmental variation to produce the observed phenotypic variation (cite). Cheverud (1988) proposed that a P matrix could substitute for a G matrix at sufficient effect sample sizes. This theoretical framework has been used as reason to pool phenotypic change over time and estimate a G matrix (e.g., Cheetham; Voje; Houle). There is reason to think P can substitute for G not just in the near time, but in the deep time too: the amount of phenotypic variation represented by fossil, time-averaged populations is only ~1% larger than those of modern populations (Hunt 2004). While there have been studies showing the P matrix is a good substitute for the G matrix in some groups, this has not been evaluated over deeper time by using fossil data.

Clonal organisms with a fossil record, such as bryozoans, provide a unique opportunity to branch micro- to macro-evolutionary studies by disentangling the P matrix from the G matrix and examine changes in the G matrix over longer time scales. Each colony is made up of genetically identical zooids. Because of their shared genetics, we can estimate the G matrix, calculate a P matrix, and extract the environmental variance-covariance matrix (E matrix). *Steginoporella magnifica* is a lineage of Bryozoa with few zooid polymorphisms and a well-preserved fossil record spanning 2.3 mya.

Here, we use the fossil record to employ quantitative genetic tools to ask: 1) does the G matrix change over time?; 2) does phenotypic evolution occur in directions of above-average evolvability?; and 3) is the P matrix a good substitute for the G matrix in the fossil record? Answering these outstanding questions may facilitate research of evolvability in the fossil record.

**Materials & Methods**

*Geologic setting*

Fossil and modern specimens were collected from the Wanganui Basin, North Island, New Zealand during DATE. The Wanganui Basin is well studied for its geology and invertebrate fossils (cite Naish, Seward, Liow, Carter, Abbott). Bryozoans were collected from seven formations that span 2.3 MY (SI Table Formations).

*Specimen processing*

Shell substrates were cleaned using diluted bleach and water, catalogued, SEMed, and stored. Multiple, non-overlapping images of each colony were taken. The SEMS are all taken at x30 magnification, thus standardizing the scale for all the images.

*Image processing / extraction of phenotypic traits*

We applied machine learning to the SEM images to detect individual zooids and place landmarks. We piped together DeepBryo (Di Martino et al.) to detect zooids and ML-Morph (Porto & Voje) to place 23 landmarks along the zooid (see SI Fig Landmarks & Measurements) to create Steginator (<https://github.com/agporto/Steginator>). Discuss individual box id?

We only retained images of *Steginoporella maginifica* with a magnification of x30. We also deselected zooids with image distortion or errorneous landmarking (see SI for more detail?). We retained colonies with at least 5 zooids measured. Our final dataset includes X images from Y colonies (Table Sampling).

We extracted 8 phenotypic traits (see SI Fig Landmarks & Measurements). These traits were calculated as the distance between landmarks using the Pythagorean theorem (see exploratoryAnalysis.R and outputs traits.csv <- get name right). We converted pixels to um and all trait measurements were log-transformed.

Q. why outputMetadata.R?? what is in the metadata file that is useful?

*Analyses*

All post-processing of the ML pipeline and subsequent analyses were done in R programming. We used the following packages (LIST THEM). All scripts are available here (github) and all images are available here (repo).

Sensitivity

We tested for normality of each trait (see SI Fig Traits & SI Table Normality?). Include this?? We don’t seem to care…

Compared ML measurements to my own (TBD).

* Checked for quality of ML measurements compared to my own
  + 10 images from each formation
  + At least 5 colonies (i.e., all images of the same colony)
  + Placed landmarks
  + Measured self, measured using code
  + Did this three times

Rarefaction…

P & G matrix

We calculate the P matrix by first creating a variance-covariance matrix for each formation for all 8 traits. We retain the first 5 dimensions given the PC analyses (SI Fig P & G PC).

We then use the P variance-covariance matrix as the input for the G matrix prior. We then ran an MCMC glmm with traits as fixed effects and colony as a random effect to account for variation within colonies (? Rcov measurement error) to estimate G matrices for each formation. We iterated this 1.5 million times, and retained every 1000th run after .5 million runs and onward. As with the P matrix, we retained the first 5 dimensions based upon eth PC analyses (SI Fig P & G PC).

P & G correlation

We calculated random skewers following Marroig (DATE) (Figure P & G Correlation).

Change in G over time

We calculated the angle change in Gmax between time points. We get the direction of Gmax from the first eigen vector and normalize it. We get difference between Gmax as the dot product of the two matrices and covert to degrees.

? conditional evolvability…

Directions of phenotypic change

We asked if Pmax and Gmax aligned. We did this in the similar to calculating change in G over time, but rather than comparing time points compared the Gmax of time point 1 to the direction of phenotypic change seen in time point 2.

We quantified whether phenotypic change occurred in directions of above-average evolvability. To do that, we first calculate the amount of phenotypic change between each adjacent formation and normalize the vector. We then calculate the amount of observed evolvability as amount of phenotypic change based on the amount of variation-covariation in the oldest time point (t1). We then generate 10000 selection gradients in random directions to calculate the minimum, mean, and maximum evolvability for each formation, excluding the last. We compare the range of observed evolability between time points to the min, mean, and max evolvability given by the time point prior.

**Results**

P & G correlation

Change in G over time

Directions of phenotypic change

**Discussion**

**EXTRA TEXT**

If genetic constraints (i.e., covariance structures) are important, it is hypothesized that phenotypic change should occur along axes of above-average evolvability (i.e., variance).

The fossil record provides a history of how the G matrix has changed over time in relation to the observed – phenotypic variance-covariance matrix (P matrix) – changes.

Bryozoans are colonial organisms with a fossil record and are a potential system to apply evolutionary quantitative genetics to deep time.

Paleontologists are restricted to estimating the P (phenotypic variance-covariance) matrix when assessing the effects of evolvability and constraints on phenotypic change within and across lineages in the fossil record.

Evolutionary quantitative geneticists estimate the G matrix, which is the amount of variance and covariance of phenotypic traits based on parent-sibling pairs, to evaluate evolutionary potential and genetic constraints on the evolution of populations and species over micro-evolutionary time scales. Quantifying the G matrix is time consuming and data intensive (Cheverud; Porto), and these studies are limited in temporal (cite examples including Houle 2017).

**Figures**

Figure P & G Correlation

Figure G over time

Figure Gmax Pmax

Figure above-average evolvability

**Tables**

**Supplemental**

SI Table Formations. Abbr = abbreviation; Ref = reference.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Formation | Abbr. | Min  Age  (MY) | Max  Age  (MY) | Isotope  Stage  Start | Isotope  Age  End | Ref. |
| Shakespeare  Shell Bed | SHCSBSB | 0.478 | 0.424 | 12 | 12 | 1 |
| Tainui |  | 0.533 | 0.478 | 13 | 13 | 1 |
| Upper Kai-Iwi |  | 0.676 | 0.621 | 16 | 16 | 2 |
| Waipuru |  | 1.8325 | 1.826 | 67 | 67 | 3 |
| Tewkesbury |  | 1.875 | 1.8325 | 71 | 67 | 4 |
| Nukamuru  Brown Sand | NKBS | 2.017 | 1.915 | 77 | 73 | 4 |
| Nukamuru  Limestone | NKLS | 2.291 | 2.088 | 89 | 79 | 4 |

SI Table Sampling. Abbr = abbreviation; N = number.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Formation | Abbr. | N  colonies | N  Zooids  (avg. per colony) | N  Images |
| Shakespeare  Shell Bed | SHCSBSB | 50 | 400  (8) | 102 |
| Tainui |  | 19 | 155  (9) | 41 |
| Upper Kai-Iwi |  | 21 | 170  (9) | 47 |
| Waipuru |  | 15 | 156  (9) | 33 |
| Tewkesbury |  | 107 | 1050  (11) | 217 |
| Nukamuru  Brown Sand | NKBS | 263 | 2934  (12) | 525 |
| Nukamuru  Limestone | NKLS | 66 | 615  (10) | 126 |
| **Total** |  | **541** | **5480**  **(11)** | **1091** |

SI Fig Landmarks & Measurements

* Calculated the following traits based on landmarks (Figure linear):
  + Zooid height (zh) from 4 to 12
    - Similar to LZ in Voje et al. 2019
  + Median process width at the base (mpw.b) from 5 to 6
  + Cryptocyst width at midline (cw.m) from 10 to 11
  + Cryptocyst width at distal end (cw.d) from 8 to 7
  + Operculum width at midline (ow.m) from 19 to 0
    - Similar to WO in Voje et al. 2019

SI Fig Traits

SI Fig P & G PC

*Errorenous landmarking?*

**SI References:**

1. Carter, R.M. and Naish, T.R., 1998. A review of Wanganui Basin, New Zealand: global reference section for shallow marine, Plio–Pleistocene (2.5–0 Ma) cyclostratigraphy. Sedimentary Geology, 122(1-4), pp.37-52; Rust thesis Appendix IIIC
2. Rust thesis Appendix IIIC
3. Naish, T., Kamp, P.J., Alloway, B.V., Pillans, B., Wilson, G.S. and Westgate, J.A., 1996. Integrated tephrochronology and magnetostratigraphy for cyclothemic marine strata, Wanganui Basin: implications for the Pliocene-Pleistocene boundary in New Zealand. Quaternary International, 34, pp.29-48.
4. Abbott, S.T., Naish, T.R., Carter, R.M. and Pillans, B.J., 2005. Sequence stratigraphy of the Nukumaruan Stratotype (Pliocene‐Pleistocene, c. 2.08–1.63 Ma), Wanganui Basin, New Zealand. Journal of the Royal Society of New Zealand, 35(1-2), pp.123-150; Rust thesis Appendix IIIC