**Sampling**

|  |  |  |
| --- | --- | --- |
| Formation | N colonies | N zooids  (avg per colony) |
| NKLS | 66 | 615 (10) |
| NKBS | 269 | 3012 (12) |
| Tewkesbury | 107 | 1050 (10) |
| Waipuru | 11 | 111 (11) |
| Upper Kai-Iwi | 18 | 130 (8) |
| Tainui | 19 | 155 (9) |
| SHCSBSB | 50 | 400 (8) |
| Modern | 17 | 214 (13) |

* Three formations with high sample sizes, and should trust those
* May want to deem those with under 20 as too low
* May need to increase modern sample size

**Mean by formation.** Means of each trait by formation. All measurements in (µm). Standard deviations are in parentheses and ranges are given in brackets.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | zooid height (zh) | Mean median process width (mpw.b) | Mean Cryptocyst width at the midline (cw.m) | Mean Cryptocyst width at the distal end (cw.d) | Mean Operculum width at the midline (ow.m) | Mean Operculum height (oh) | Mean Operculum side length (o.side) | Mean Cryptocyst side length (c.side)  (sd) |
| NKLS | 797.4 (1.13) [450.45 - 1701.34] | 171.17 (1.17) [107.26 - 265.18] | 386.94 (1.19) [189.04 - 635.59] | 430.36 (1.14) [262.98 - 607.39] | 428.83 (1.1) [310.39 - 626.58] | 0.11 (1.1) [0.08 - 0.15] | 310.92 (1.13) [199.59 - 665.71] | 351.01 (1.18) [193.08 - 745.39] |
| NKBS | 772.26 (1.12) [494 - 1495.07] | 172.75 (1.18) [91.36 - 321.51] | 390.2 (1.2) [203.14 - 764.38] | 429.88 (1.15) [224.48 - 804.12] | 427.34 (1.11) [251.74 - 787.47] | 0.11 (1.1) [0.07 - 0.17] | 304.18 (1.14) [164.06 - 638.15] | 335.58 (1.18) [172.77 - 626.08] |
| Tewkesbury | 774.42 (1.14) [330.57 - 1668.34] | 172.58 (1.17) [69.62 - 299.23] | 373.67 (1.21) [187.72 - 675.06] | 419.5 (1.16) [197.43 - 741.19] | 426.88 (1.12) [196.73 - 780.87] | 0.11 (1.11) [0.07 - 0.21] | 301.25 (1.14) [129.77 - 684.87] | 338.52 (1.19) [179.99 - 683.85] |
| Waipuru | 812.19 (1.12) [544.62 - 1361.01] | 190.4 (1.13) [140.53 - 252.52] | 395.32 (1.16) [263.7 - 543.11] | 424.4 (1.11) [308.88 - 538.15] | 419.24 (1.07) [354.84 - 501.98] | 0.11 (1.07) [0.09 - 0.13] | 305.79 (1.16) [212.59 - 629.21] | 369.68 (1.16) [222.25 - 560.03] |
| Upper Kai-Iwi | 909.57 (1.12) [632.01 - 1362.13] | 226.92 (1.17) [117.27 - 356.81] | 510.03 (1.17) [318.79 - 693.07] | 535.59 (1.14) [319.11 - 721.33] | 498.54 (1.1) [312.02 - 705.16] | 0.1 (1.1) [0.08 - 0.13] | 334.03 (1.11) [252.57 - 523.75] | 434.13 (1.16) [283.01 - 658.01] |
| Tainui | 942.07 (1.11) [592.39 - 1203.19] | 241.55 (1.16) [121.59 - 354.95] | 509.39 (1.16) [330.03 - 735.99] | 538.88 (1.12) [342.63 - 736.64] | 509.81 (1.09) [347.75 - 727] | 0.1 (1.08) [0.08 - 0.12] | 353.78 (1.1) [264.65 - 458.03] | 434.36 (1.17) [248.34 - 645.93] |
| SHCSBSB | 927.75 (1.11) [418.48 - 1333.87] | 214.81 (1.16) [122.21 - 307.88] | 499.68 (1.18) [278.33 - 726.26] | 533.93 (1.12) [298.84 - 694.84] | 497.2 (1.09) [329.08 - 652.12] | 0.09 (1.08) [0.08 - 0.15] | 337.03 (1.11) [174.82 - 538.21] | 444.44 (1.15) [199.95 - 674.34] |
| Modern | 875.75 (1.12) [653.02 - 1213.61] | 240.69 (1.18) [153 - 325.82] | 492.72 (1.18) [287.83 - 724.57] | 523.87 (1.13) [362.94 - 716.91] | 504.64 (1.09) [391.98 - 641.2] | 0.1 (1.09) [0.08 - 0.12] | 346.05 (1.13) [231.29 - 482.6] | 394.65 (1.15) [248.66 - 584.12] |

**Normality tests for each trait.** All fail the shapiro test (i.e., significantly different from normal)

|  |  |
| --- | --- |
| Trait | Shapiro p-value |
| LN zooid height (zh) | < 0.001 |
| LN median process width at base (mpw.b) | < 0.001 |
| LN cryptocyst width at midline (cw.m) | 0.0067 |
| LN cryptocyst distal width (cw.d) | 0.0959 |
| LN operculum width at midline (ow.m) | < 0.001 |
| LN operculum height (oh) | < 0.001 |
| LN cryptocyst side length (c.side) | < 0.001 |
| LN operculum side length (o.side) | < 0.001 |

**Correlation of P to G matrices**

|  |  |  |
| --- | --- | --- |
| Formation | P corr G matrix | P corr Global G |
| NKLS | 0.96 | 0.95 |
| NKBS | 0.96 | 0.96 |
| Tewkesbury | 0.98 | 0.96 |
| Waipuru | 0.90 | 0.91 |
| Upper Kai-Iwi | 0.97 | 0.97 |
| Tainui | 0.96 | 0.94 |
| SHCSBSB | 0.95 | 0.95 |
| Modern | 0.91 | 0.91 |

* Convincing that P is a substitute for G; P and G are correlated
* This is the shape

**Angle (in degrees) difference between max vectors**

|  |  |  |
| --- | --- | --- |
| Comparison | GMAX  Angle (˚ ) | PMAX  Angle (˚ ) |
| NKLS to NKBS | 7.79 | 7.07 |
| NKBS to Tewkesbury | 6.54 | 3.81 |
| Tewkesbury to Waipuru | 97.61 | 23.94 |
| Waipuru to Upper Kai-Iwi | 87.24 | 52.08 |
| Upper Kai-Iwi to Tainui | 31.89 | 27.13 |
| Tainui to SHCSBSB | 28.98 | 6.02 |
| SHCSBSB to modern | 64.57 | 23.44 |

* Can’t be more than 90, so 180-angle
* Big changes between Tewkesbury to UKI
  + The shape of G changes a lot (so the shape of P is also changing a lot too)
* Correlation between changes in G and sample sizes
  + Low sample sizes mean large change in direction of G max (esp for those under 20)
* When estimate p and g with larger sample sizes, the deviation ebtwen g and p is smaller too

**Evolvability of observed change and estimated change from individual G matrices. Estimated using random skewers.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | e.mean | e.min | e.max | observed\_e | above  average? |
| NKLS to NKBS | 0.00699369 | 0.00085056 | 0.0245855 | 0.01598211 | yes |
| NKBS to Tewkesbury | 0.00705572 | 0.00140311 | 0.02578052 | 0.01639705 | yes |
| Tewkesbury to Waipuru | 0.00743297 | 0.00169207 | 0.02556657 | 0.0106823 | yes |
| Waipuru to  Upper Kai-Iwi | 0.00756309 | 0.00258222 | 0.02220537 | 0.01250959 | yes |
| Upper Kai-Iwi to Tainui | 0.01033604 | 0.00263522 | 0.03852449 | 0.03192097 | yes |
| Tainui to SHCSBSB | 0.00756977 | 0.00220687 | 0.02163449 | 0.01755475 | yes |
| SHCSBSB to modern | 0.00574056 | 0.00119437 | 0.01919024 | 0.00669307 | yes |

* Almost always one order of mag higher than mean
* Often closer to max than mean
  + Exception is going to modern which is closer to mean than max

**Evolvability of observed change and estimated change from global G matrix. Estimated using random skewers.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | e.mean | e.min | e.max | observed\_e | above  average? |
| NKLS to NKBS | 0.00660373 | 0.001168614 | 0.02382359 | 0.02244720 | Yes |
| NKBS to Tewkesbury | 0.00660373 | 0.001168614 | 0.02382359 | 0.01545886 | Yes |
| Tewkesbury to Waipuru | 0.00660373 | 0.001168614 | 0.02382359 | 0.01151155 | Yes |
| Waipuru to  Upper Kai-Iwi | 0.00660373 | 0.001168614 | 0.02382359 | 0.02392323 | **Yes [higher]** |
| Upper Kai-Iwi to Tainui | 0.00660373 | 0.001168614 | 0.02382359 | 0.01345431 | Yes |
| Tainui to SHCSBSB | 0.00660373 | 0.001168614 | 0.02382359 | 0.01294711 | yes |
| SHCSBSB to modern | 0.00660373 | 0.001168614 | 0.02382359 | 0.00712744 | yes |

**Angle (in degrees) difference between max vector of matrices and ∆z.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Comparison | G matrix | P matrix | Global G matrix | E matrix |
| NKLS to NKBS | 80.91 | 103.50 | 156.00 [big sample size, if there is a diff will pick it up and do] | 153.75 |
| NKBS to Tewkesbury | 43.14 | 39.40 | 43.32 | 40.61 |
| Tewkesbury to Waipuru | 122.18 | 118.61 | 126.43 | 116.55 |
| Waipuru to  Upper Kai-Iwi | 93.2 | 127.68 | 165.78 [global G as close to G max as can be, while individual G is almost as far as can be] | 135.99 |
| Upper Kai-Iwi to Tainui | 145.77 | 134.40 | 128.73 | 120.91 |
| Tainui to SHCSBSB | 40.82 | 54.32 | 49.56 | 61.80 |
| SHCSBSB to modern | 88.29 | 99.51 | 95.33 | 100.53 |

* Could be because can’t estimate WAI and UKI because low sample sizes
  + Also UKI has most variation

Evolution in general happening in directions of above avg evolvability, but doesn’t aligned with G or P max; also P and G don’t always align or change in the same way

* Evol is not biased to G or Pmax, and does not refute constraint hypothesis
* Can predict dir, but not in max dir

Similar shape but not similar size; so expect to move in similar ways and ∆z to be different in similar ways

* Compatible with constraint

Max is the line of greatest variation; shape determined by covariation

Can we predict P and G based on c

**NOTES**

Observed evolvability:

Waipuru to upper kai iwi has a higher observed evolvability that global g max evolvability

0.0238 v 0.0239; estimate mean using 10000 draws and not using eigen vectors, so sampling matrix and so not getting the true max

Global g and gmax should be aligned, but it’s not

Gs are different but not so diff base don sampling size that they’re that diff which indicates a global g

Ematrix PC has negatives…

Global G does well if sufficient sample sizes

Rarefaction: not more different that sample size indicate, but sample size too low to estimate properly

Only estimate G matrix for formations with 50 + colonies

Undersampled formations:  
- Waipuru: only got rid of one image which isn’t a great one

- Upper Kai-Iwi: got rid of 8 images, 3 could be redone (1001\_CC, 1012\_CV, 1024\_CC) and 2 are ok (1020\_CV, 1021\_CV)

- modern: got rid of 2, both of which could be redone (1212\_CC, 1213\_CC)

- Tainui: got rid of 8 images, 2 of which could be redone (812\_CC, 838\_CV) and 2 which are ok (830\_CV, 837\_CV)

None would get it up to the sampling needed, so do need more samples

**Comparison to temperature.** Slope = 0.04, p-value = 0.52, r2 = 0.

**A graph with a blue line

Description automatically generated**

Three formations with smaller sizes:

NKBS

Waipuru

Upper Kai-Iwi

O’Dea & Okamura 1999, Amui-Vedel et al 2007, and DiMartino & Liow 2021 find zooid size varies with temperature.

Amui-Vedel et al 2007 find longer zooids in July than January (i.e., in warmer than colder) in nature, but in the laboratory had longer and sider zooids in cooler (14˚C) than wamer (18˚C) temperatures

O’Dea & Okamura 1999 found zooid length, width, and area are temperature-dependent, where zooids were longer, wider, and more area in cooler temperatures

DiMartino & Liow 2021 found larger zooids at higher ∂O18 values

Span from 3.4 to 4.7 ∂O and a mean size change from 11.1 to 11.6 mm log zooid size

**Rarefaction**. Red dot is similarity between modern and Upper Kai-Iwi.

A graph with numbers and dots

Description automatically generated with medium confidence

**Evolvability**

A graph of different shapes

Description automatically generated with medium confidence

**Global evolvability**

A black screen with white text

Description automatically generated

**Zooid height through time**

A colorful lines with text

Description automatically generated with medium confidence

A group of graphs showing different sizes of data

Description automatically generated with medium confidence

A black screen with white text

Description automatically generated

**Size (diagonal) of P matrices with E matrices.** Slope = 0.70, p-value = 0.005655, r2 = 0.70

A screenshot of a computer

Description automatically generated

**Size (diagonal) of P matrices with G matrices.** Slope = -0.077, p-value = 0.93, r2 = 0

If know G, can’t predict the variance, can have high or low P with the same G variance. The shape is highly correlated, but the shape isn’t. Shape is proportional.

A screen shot of a graph

Description automatically generated