

bold and PC2 seem to be size-independent

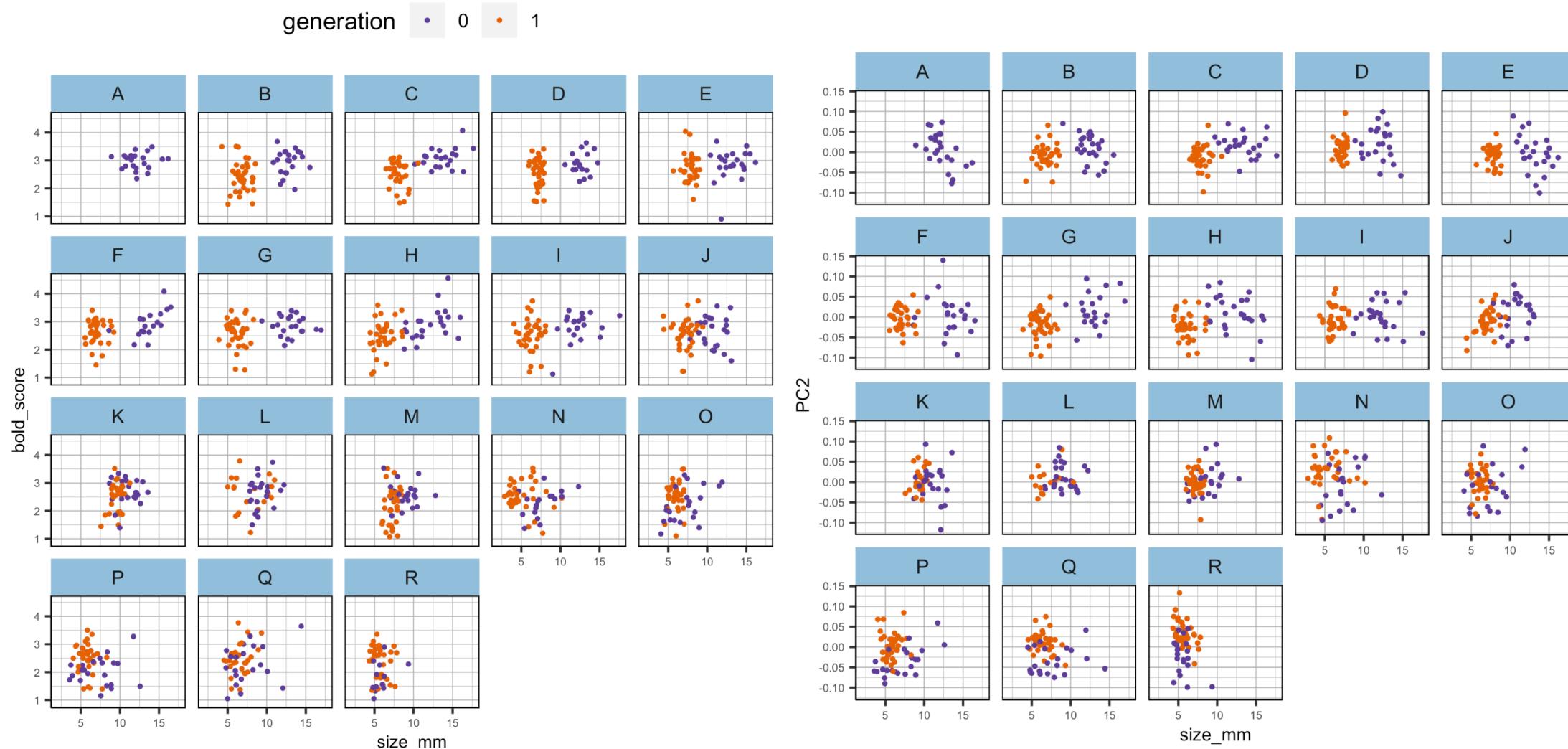
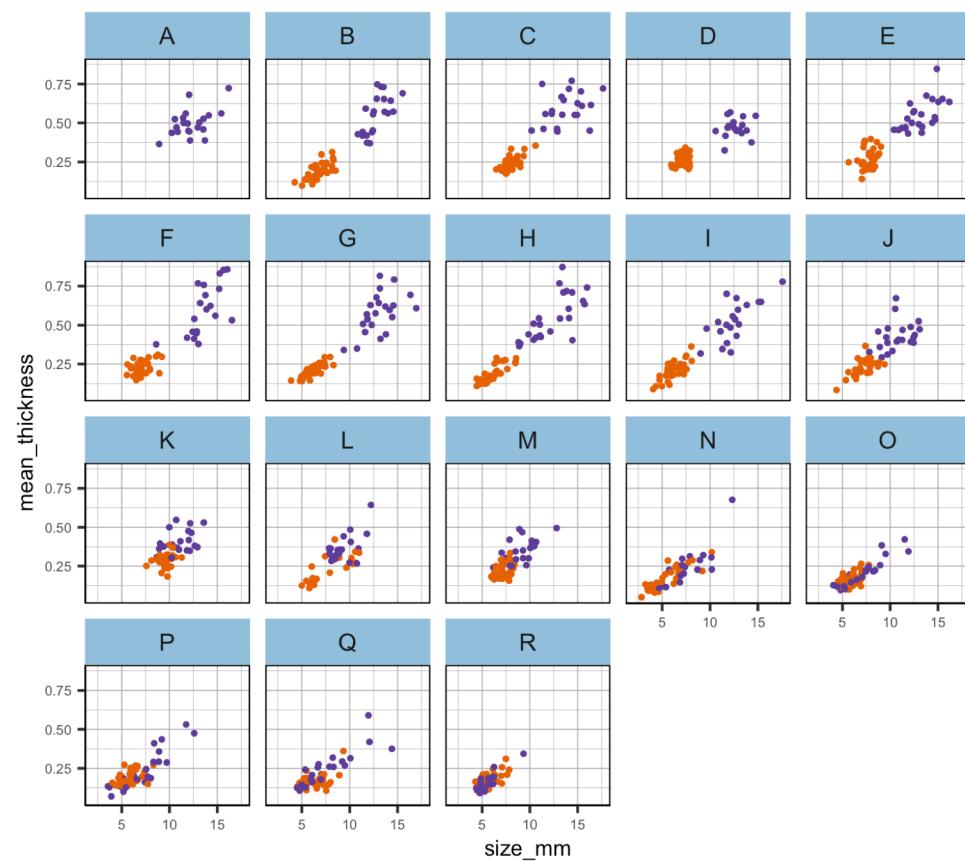


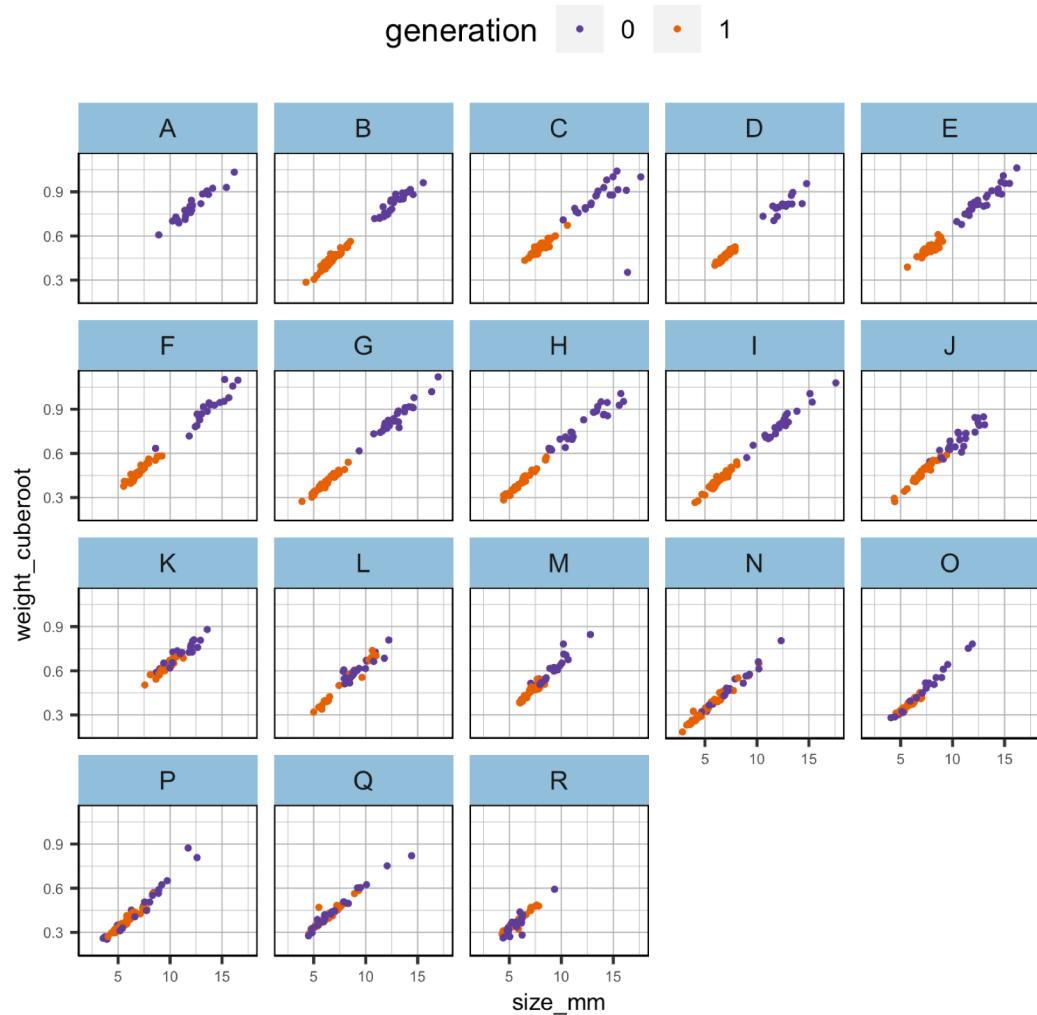
Figure 1ab. Scatter plots of the relationship between size and the other phenotypes per population in each generation separately. Phenotypic values have not been scaled.

generation • 0 • 1



thickness is clearly size-dependent. There is some scatter, which might mean that there is some interesting size-independent thickness variation but it might also just mean that the thickness measurements are noisy!

Figure 1c. Scatter plots of the relationship between size and the other phenotypes per population in each generation separately. Phenotypic values have not been scaled.

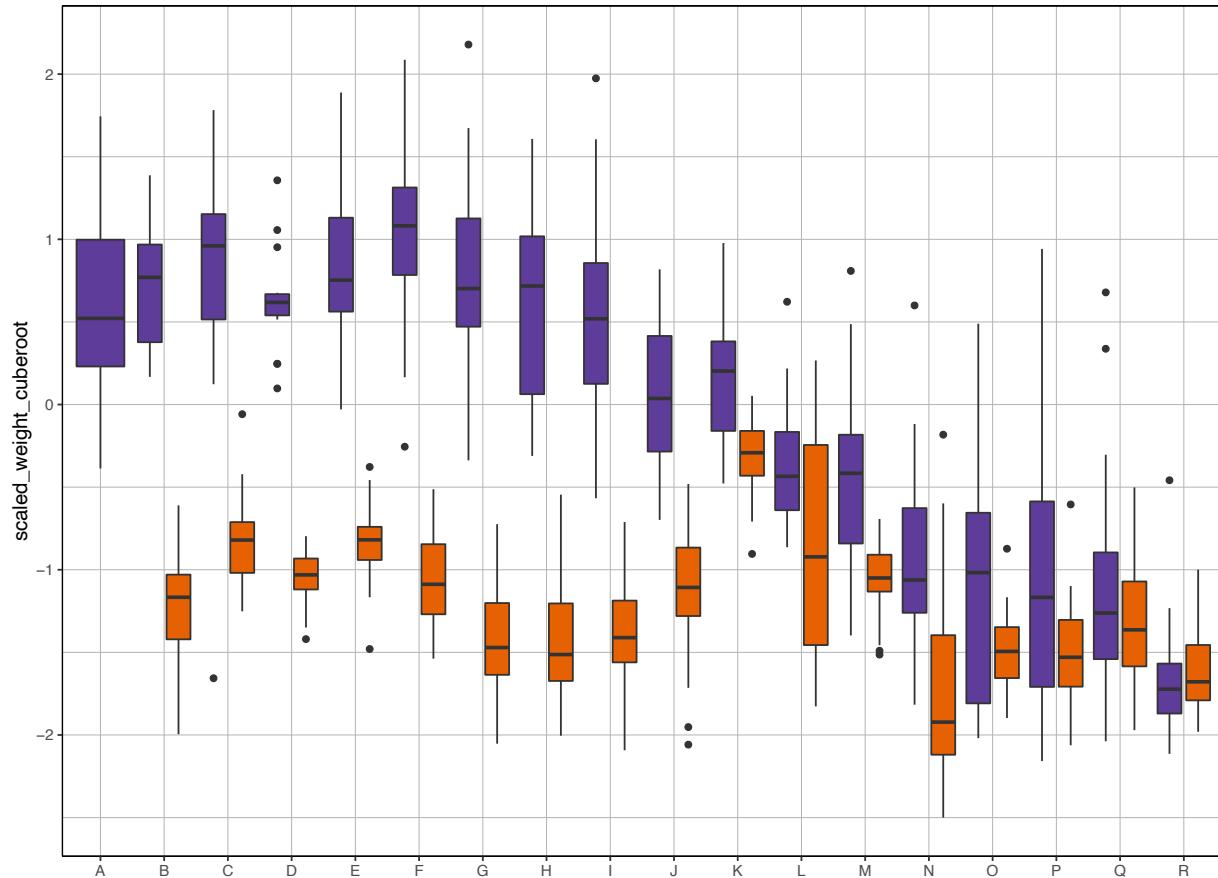


cube_root_weight looks almost perfectly correlated with size! Also, the relationship looks linear across the whole size range and very similar in all populations. It may be that there is no size-independent variation in weight.

Figure 1d. Scatter plots of the relationship between size and the other phenotypes per population in each generation separately. Phenotypic values have not been scaled.

WEIGHT

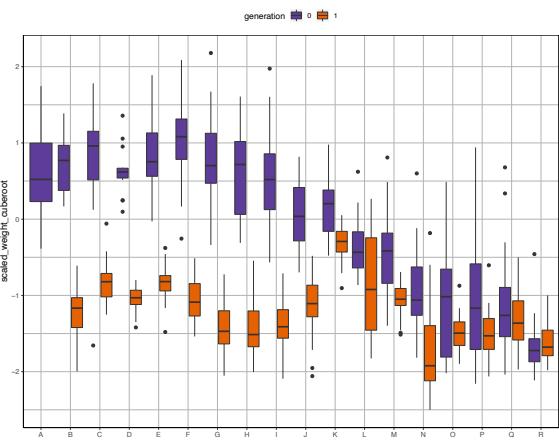
generation 0 1



Gen0 is the expected pattern. Gen1 is flat. This is a bit odd.

There are two obvious alternative explanations (not mutually exclusive): sampling was timed for when the size reached a certain point, or size is very plastic. We might separate these effects (partly) by correcting for sampling time (although larger snails were taken at each time point, I guess). Ultimately, this sampling time issue might make it hard to say anything about plasticity for size (or weight).

Figure 2. Boxplot of the scaled cube root of weight per generation and population. Phenotypic values have been scaled.

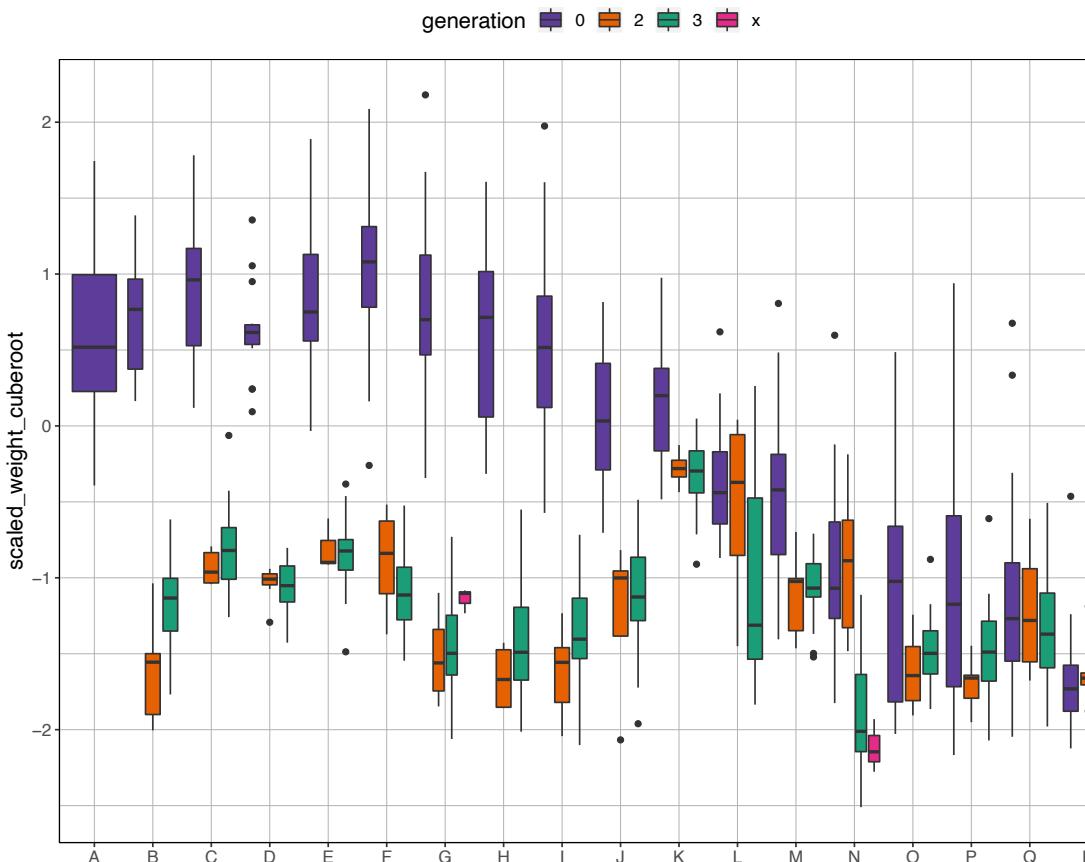


WEIGHT

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Figure 2. Boxplot of the scaled cube root of weight per generation and population. Phenotypic values have been scaled.



The lack of effect of sampling time on weight suggests that larger individuals were chosen on each occasion. I think this means that we really cannot say anything useful about plasticity for size. It is better to concentrate on (a) using size to adjust weight and thickness, and (b) trying to analyze size at maturity.

Figure tmp2. Boxplot of the scaled cube root of weight per generation and population. Generation 1 was further divided into two groups (2 in orange was sampled in June and 3 in green was sampled in August; group x in violet is for unknown samples and contains only six individuals). Phenotypic values have been scaled.

generation 0 – lowest AIC: weight ~ size x population

WEIGHT

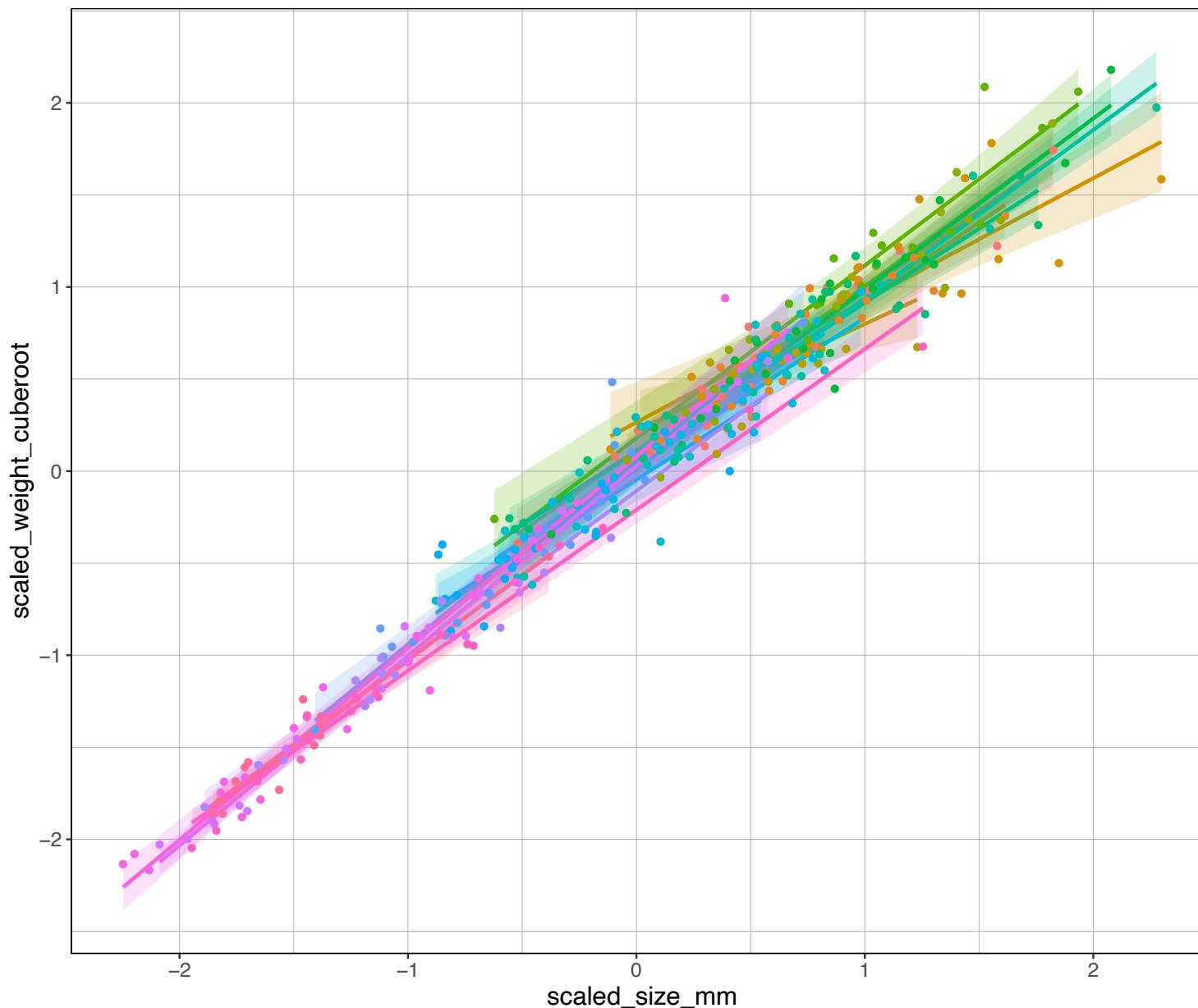


Table 1a. AIC of the models fitted to the observed data of generation 0 parents after scaling.

Formula	AIC
weight ~ size	-228.53
weight ~ size + maturity	-241.47
weight ~ size x maturity	-243.26
weight ~ size + population	-249.62
weight ~ size x maturity x population	-256.74
weight ~ size + maturity + population	-264.43
weight ~ size x population	-267.51

Figure 3a. Best model fit to the observed data of generation 0 parents after scaling.

generation 1 – lowest AIC: weight ~ size x population

WEIGHT

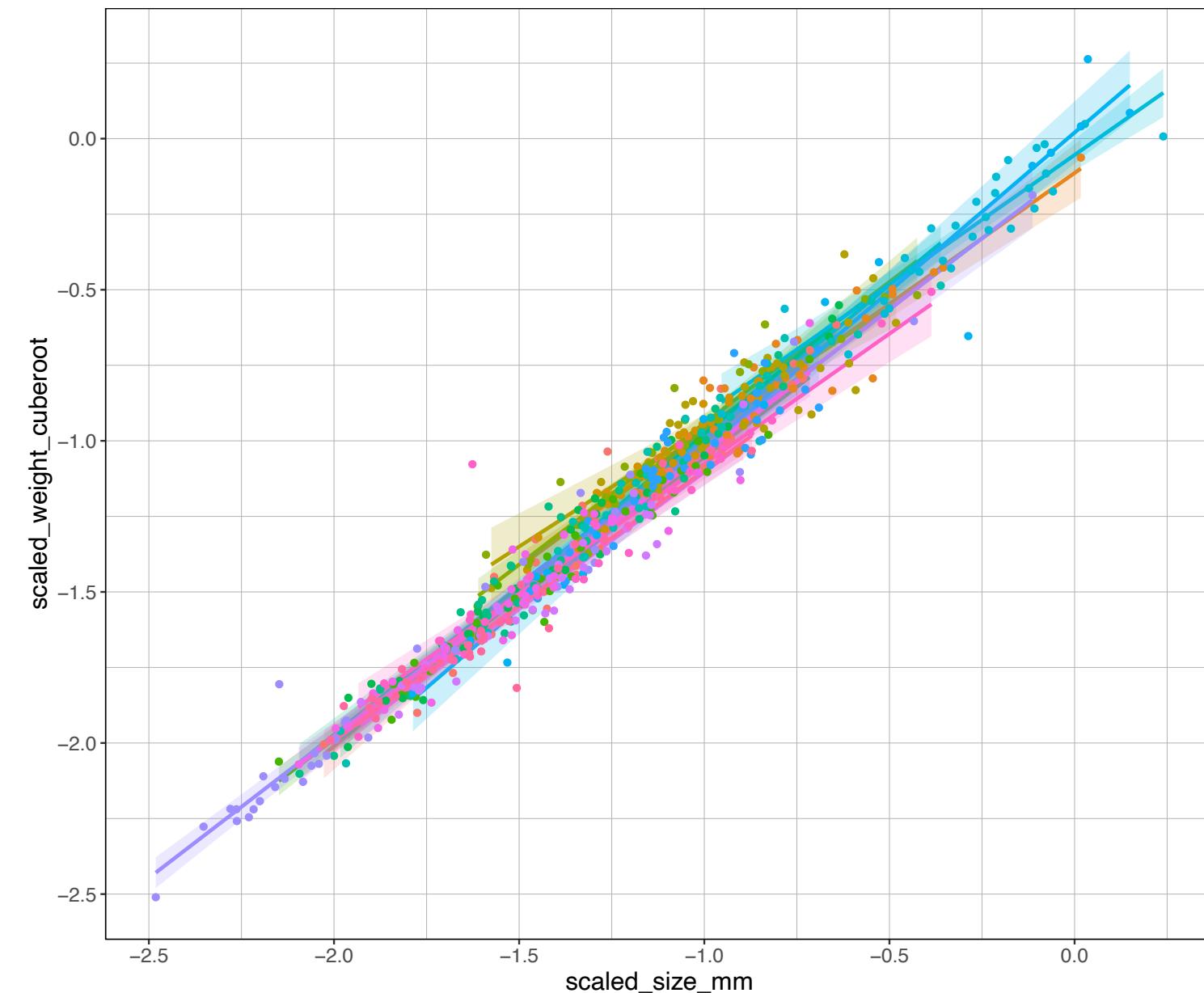


Table 1b. AIC of the models fitted to the observed data of generation 1 offspring after scaling.

Formula	AIC
weight ~ size x maturity	-1149.83
weight ~ size + maturity	-1151.78
weight ~ size	-1152.26
weight ~ size x maturity x population	-1179.71
weight ~ size + population	-1207.49
weight ~ size + maturity + population	-1211.52
weight ~ size x population	-1216.25

Figure 3b. Best model fit to the observed data of generation 1 offspring after scaling.

WEIGHT

Weights were adjusted to the overall mean size using the regression for each tank.

Adjusted weight for population B generation 1 offspring (there is no population A) was

$$\widehat{\text{weight}} = \beta_0 + \beta_1 \times \overline{\text{size}_T}$$

Where $\overline{\text{size}_T}$ is the overall mean snail size of the experiment after scaling.

And for population C generation 1 offspring was

$$\widehat{\text{weight}} = \beta_0 + \beta_1 \times \overline{\text{size}_T} + \beta_2 + \beta_3 \times \overline{\text{size}_T}$$

where β_2 is the offset from β_0 for population C and β_3 is the regression coefficient for the interaction between population C and the overall mean size.

WEIGHT

However, where slopes differ, it is not really appropriate just to adjust all weights to the overall mean but, given that slope variation is not very great, we used these adjusted values (Fig. 4) for the phenotypic analysis and also compared the slopes between gen0 and gen1, effectively as another trait (Fig. 3c).

Table 1c. AIC of the models fitted to the observed data with all samples combined after scaling.

Formula	AIC
weight ~ size x population x generation	-1304.886
weight ~ size x population	-1231.196
weight ~ size x generation	-1178.288

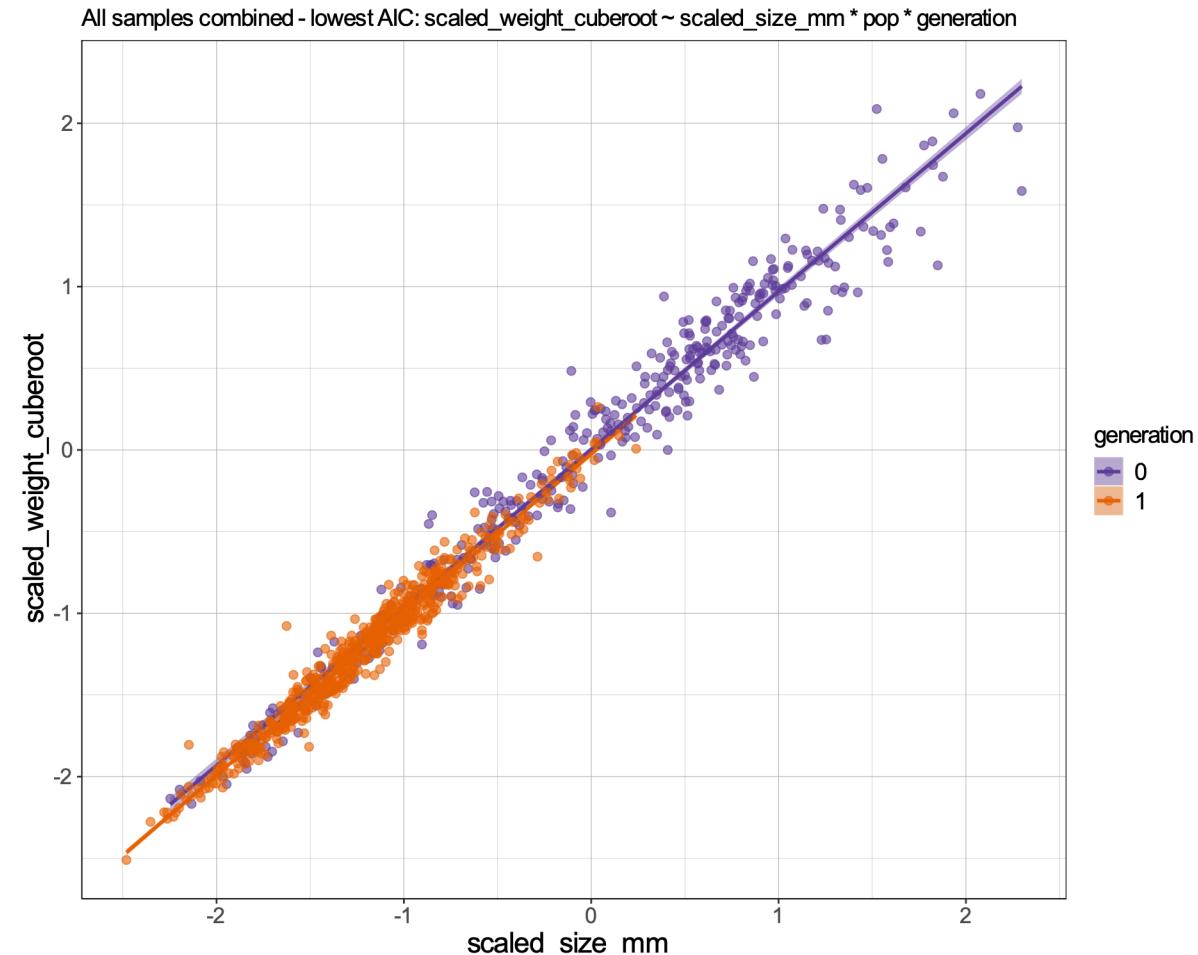


Figure 3c. Best model fit to the observed data with all samples combined after scaling. Showing only the generation effect.

WEIGHT

Predict what the mean ln weight for snails in each tank would be at the overall mean size of snails (after scaling = -0.74) for the experiment (with associated standard error).

generation 0 1

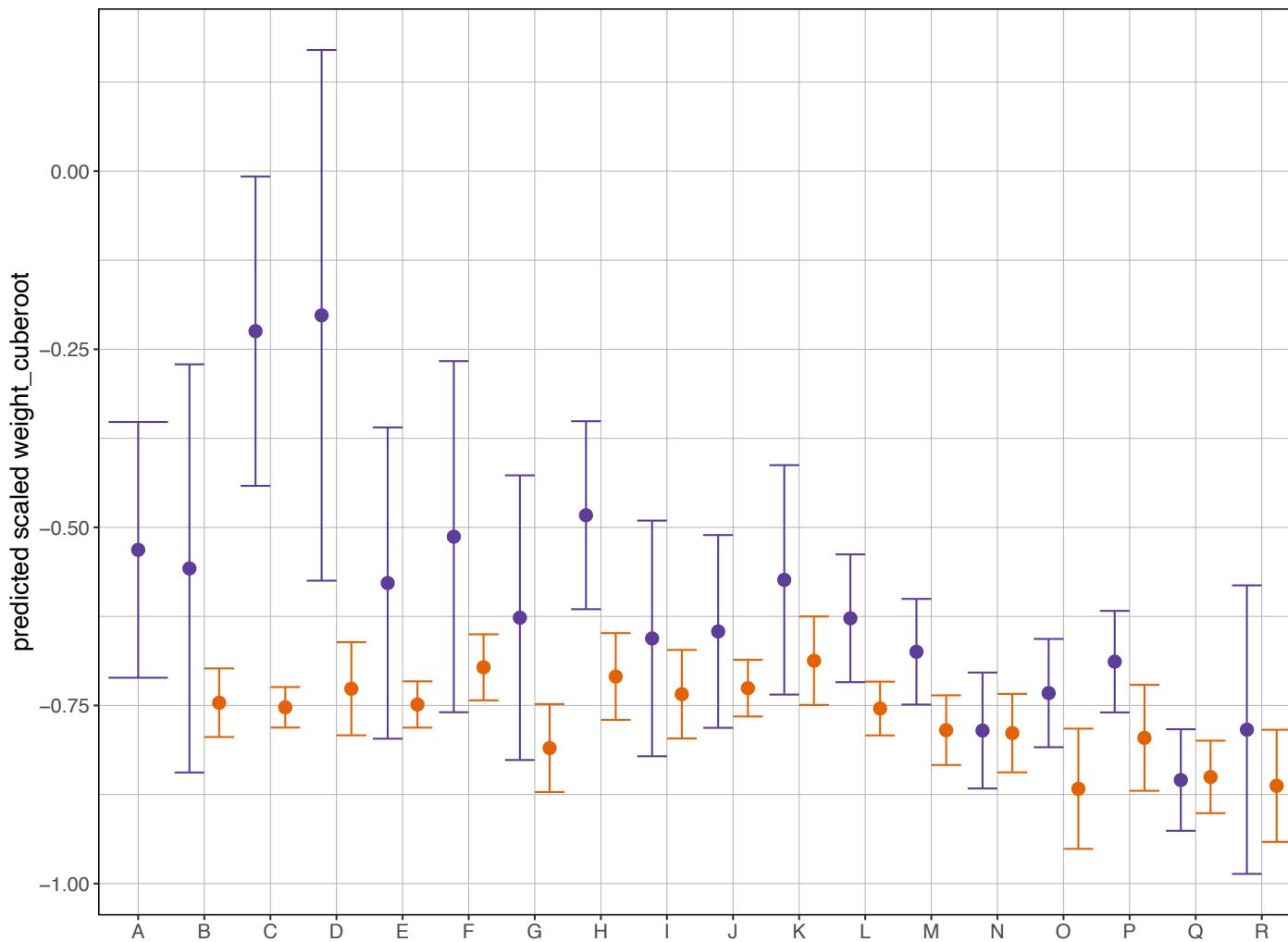


Figure 4. Predicted phenotype and 95% CIs for each population using the best model fit and the overall mean size of snails for the experiment.

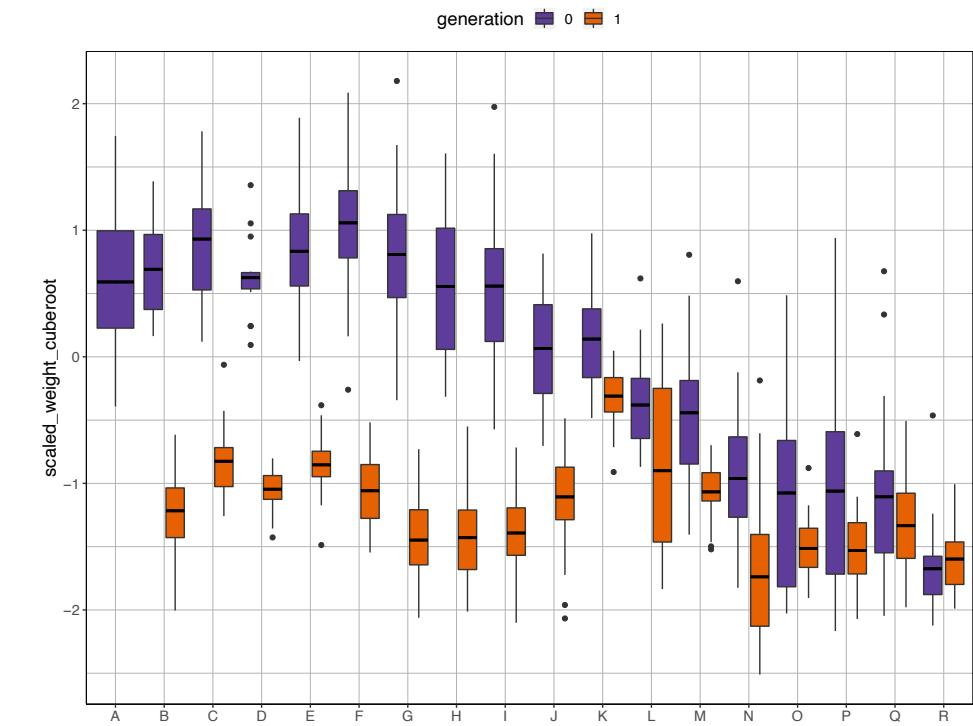
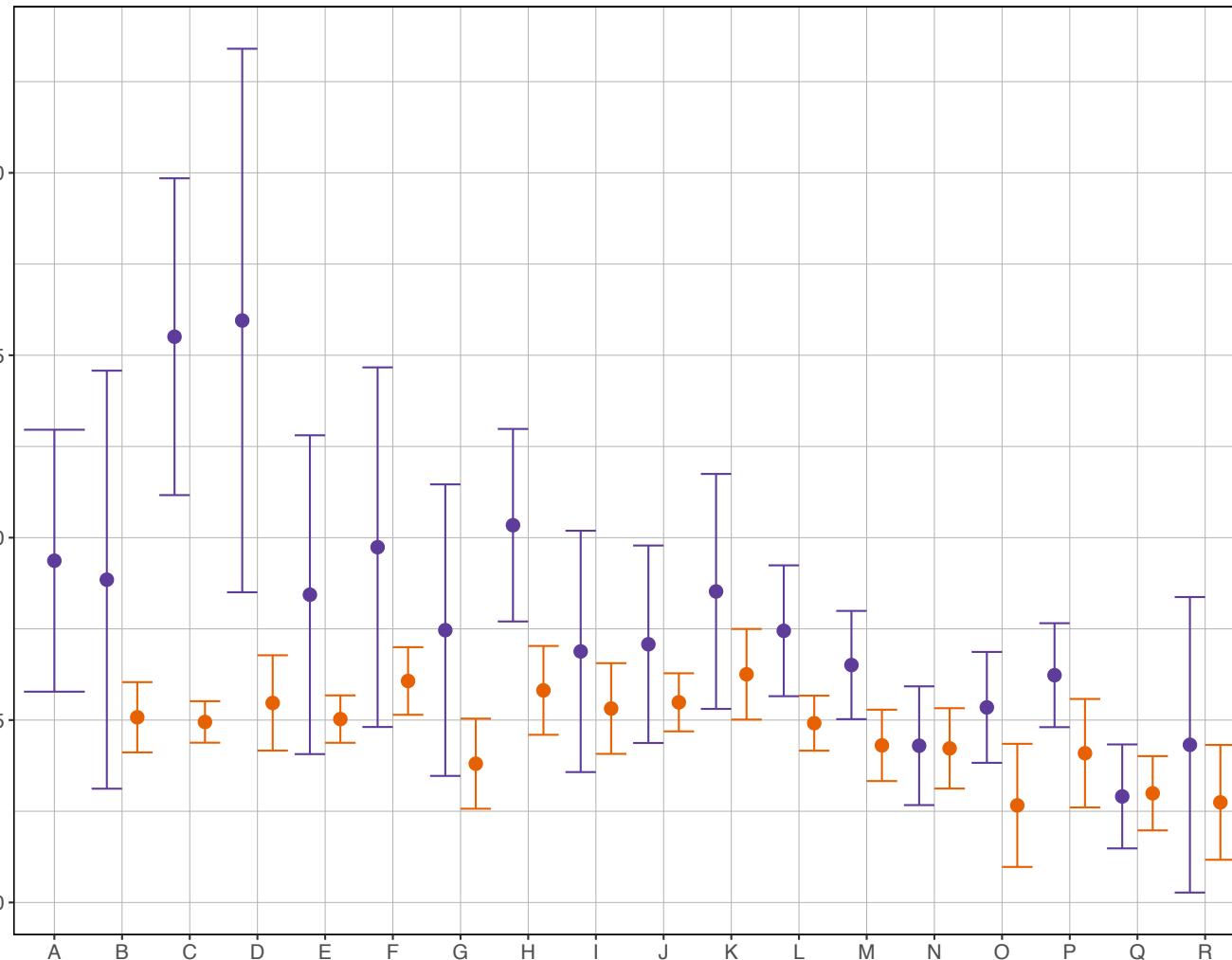


Figure 2. Boxplot of the scaled cube root of weight per generation and population. Phenotypic values have been scaled.

WEIGHT

Predict what the mean ln weight for snails in each tank would be at the overall mean size of snails (after scaling = -0.74) for the experiment (with associated standard error).

generation 0 1



gen0 does still show a weak weight cline (and maybe there is even a hint of a cline in gen1 - so incomplete plasticity). At first sight, this is inconsistent with the analyses in slides 6 and 7. However, 'population' in those analyses had a lot of degrees of freedom (16 or 17?). This makes it hard to get a significant effect (i.e. it lacks power). What we see here is a clinal effect on population means (4 degrees of freedom at most - centre, width and end means).

Despite the strong and apparently invariant relationship between size and weight, it really looks like there is weight variation across the cline. I wonder how much of that is explained by thickness.

Figure 4. Predicted phenotype and 95% CIs for each population using the best model fit and the overall mean size of snails for the experiment.

THICKNESS

generation 0 - lowest AIC: scaled_mean_thickness ~ scaled_size_mm + maturity + pop

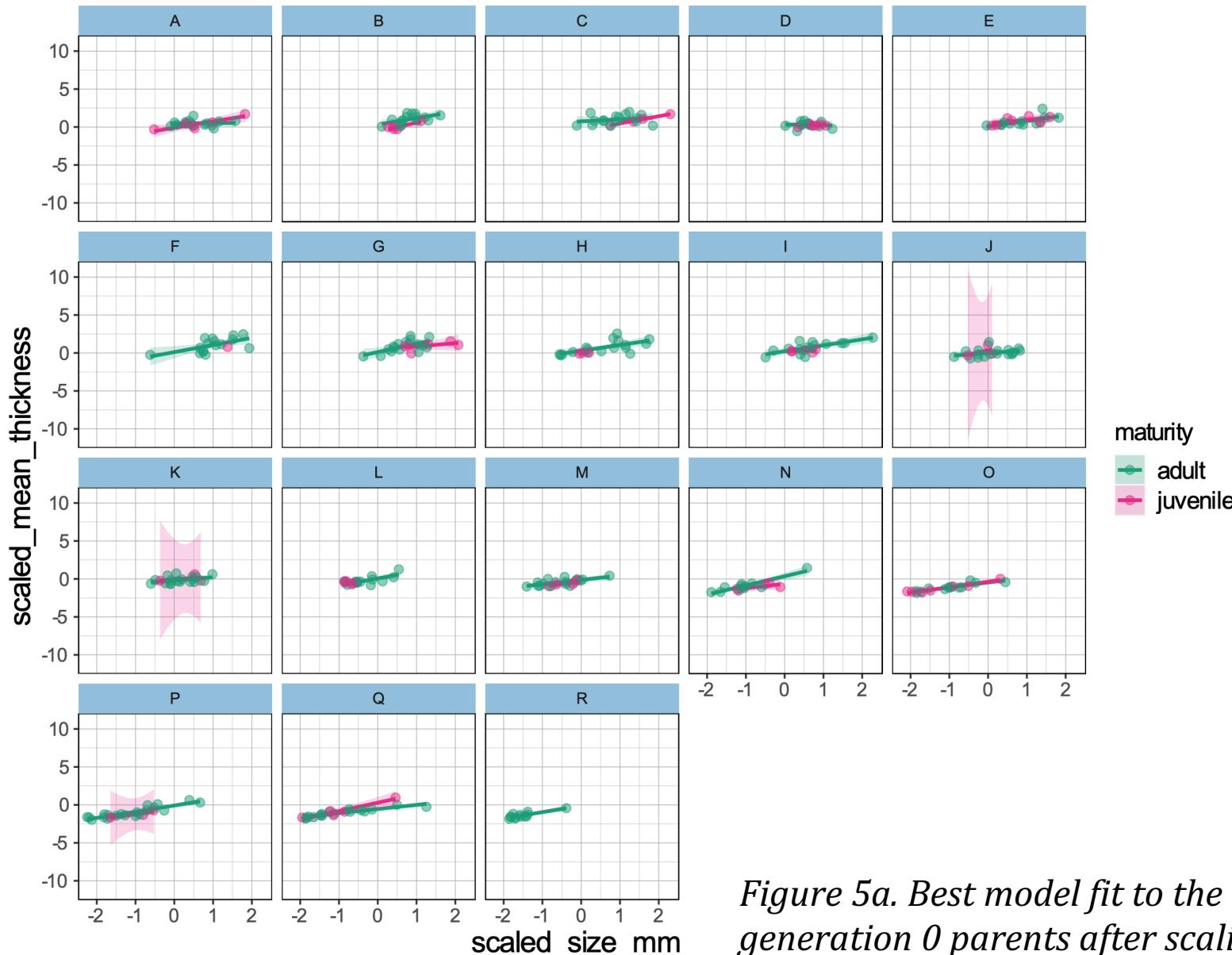


Table 2a. AIC of the models fitted to the observed data of generation 0 parents after scaling.

Formula	AIC
thick ~ size + maturity + pop	481.42
thick ~ size + pop	486.21
thick ~ size * pop	496.14
thick ~ size + maturity	499.30
thick ~ size * maturity	499.76
thick ~ size	504.13
thick ~ size * maturity * pop	509.96

Figure 5a. Best model fit to the observed data of generation 0 parents after scaling.

THICKNESS

generation 1 - lowest AIC: scaled_mean_thickness ~ scaled_size_mm + maturity + pop

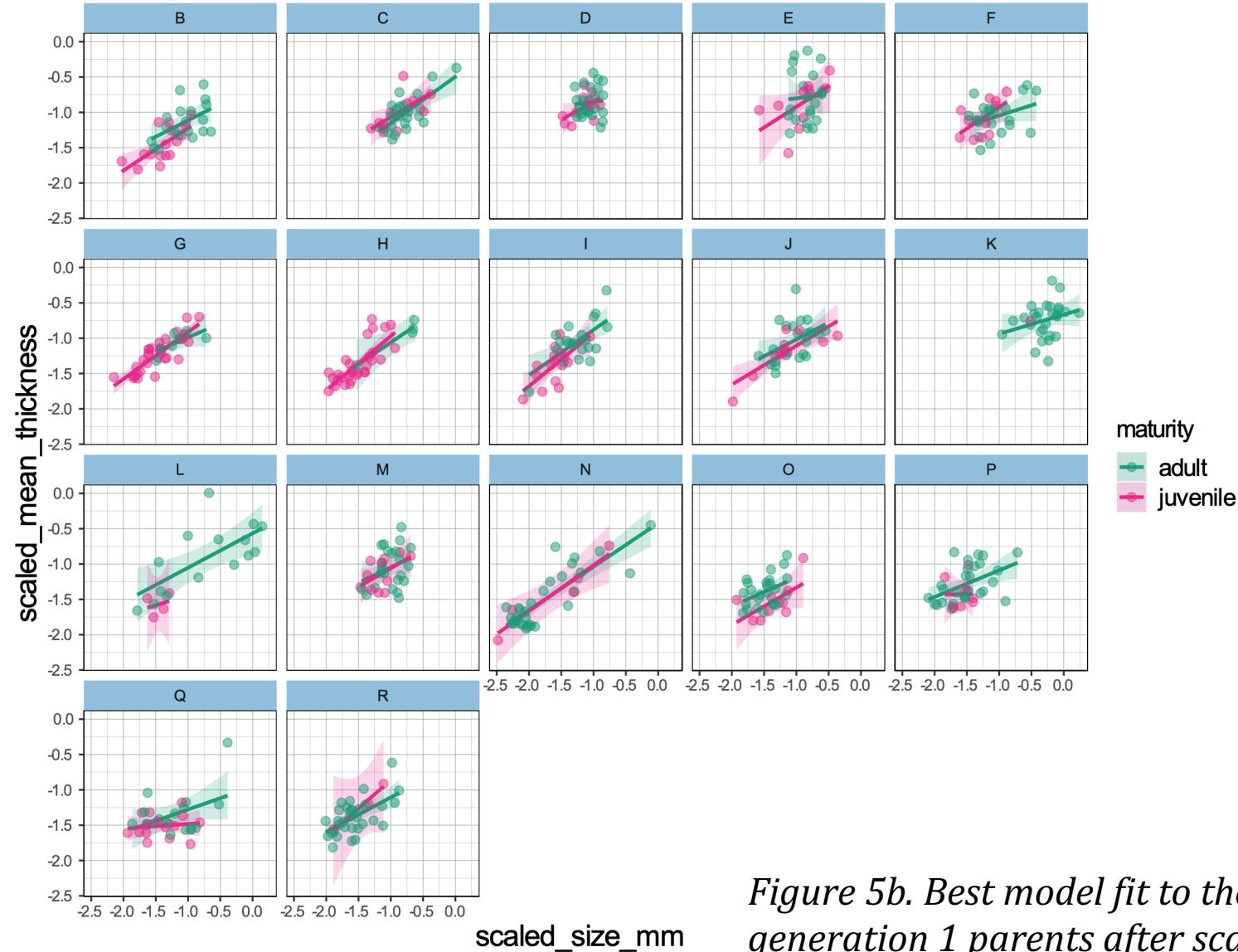


Table 2b. AIC of the models fitted to the observed data of generation 1 offspring after scaling.

Formula	AIC
thick ~ size + maturity + pop	-57.53
thick ~ size + pop	-52.83
thick ~ size * pop	-45.47
thick ~ size * maturity * pop	-9.91
thick ~ size * maturity	26.10
thick ~ size + maturity	27.40
thick ~ size	28.06

Figure 5b. Best model fit to the observed data of generation 1 parents after scaling.

THICKNESS

Comparison between slopes of generation 0 and generation 1.

Table 2c. AIC of the models fitted to the observed data with all samples combined after scaling.

Formula	AIC
thick ~ size x population x generation	669.1503
thick ~ size x generation	743.4834
thick ~ size x population	754.1876

All samples combined - lowest AIC: scaled_mean_thickness ~ scaled_size_mm * pop * generation

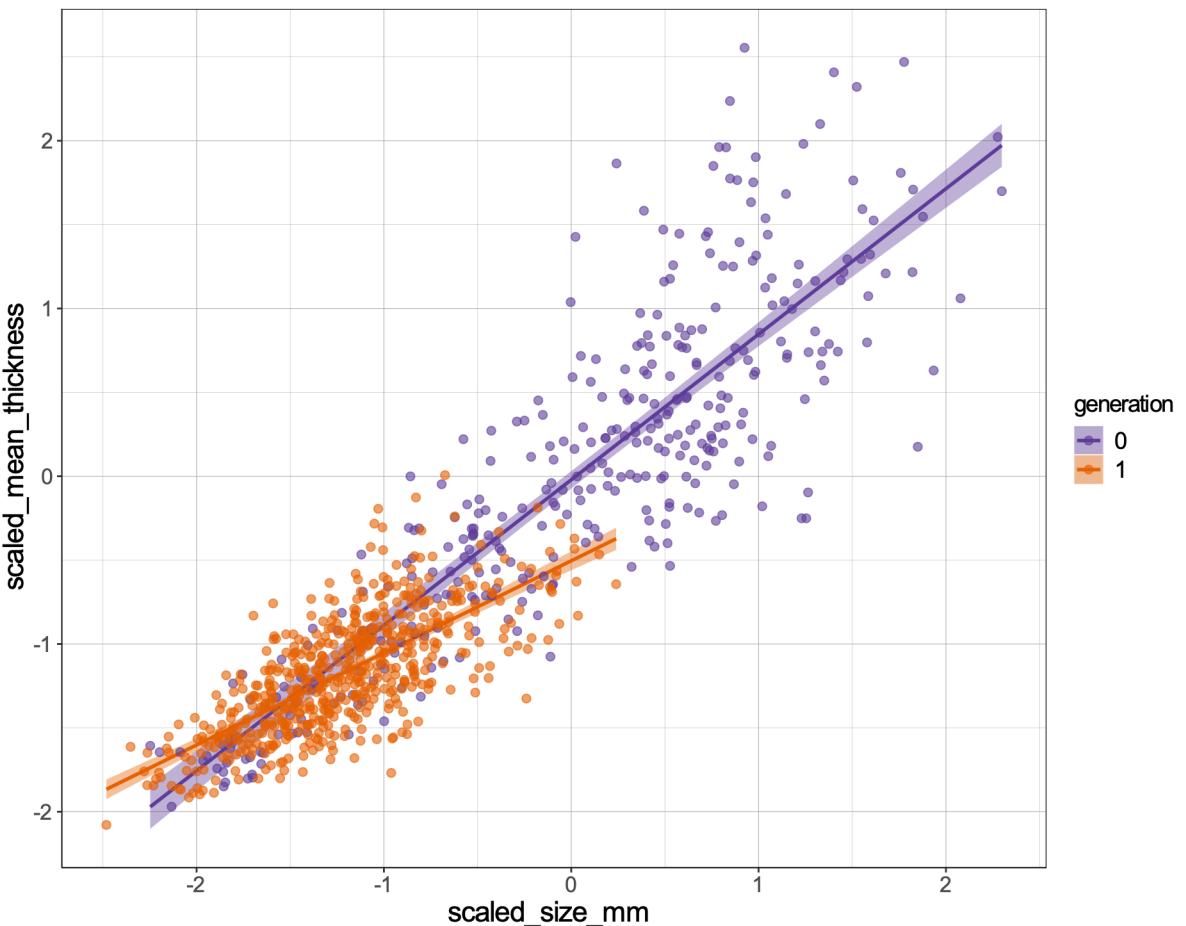


Figure 5c. Best model fit to the observed data with all samples combined after scaling. Showing only the generation effect.

If weight variation that is not accounted for by size is mainly caused by variation in thickness, then the results for weight and thickness should be very similar after size effects have been removed. You could look at this with simple correlations of the adjusted means (Fig. 6a-b) and regression coefficients (Table 3a-b).

WEIGHT and THICKNESS: Correlation of the adjusted means

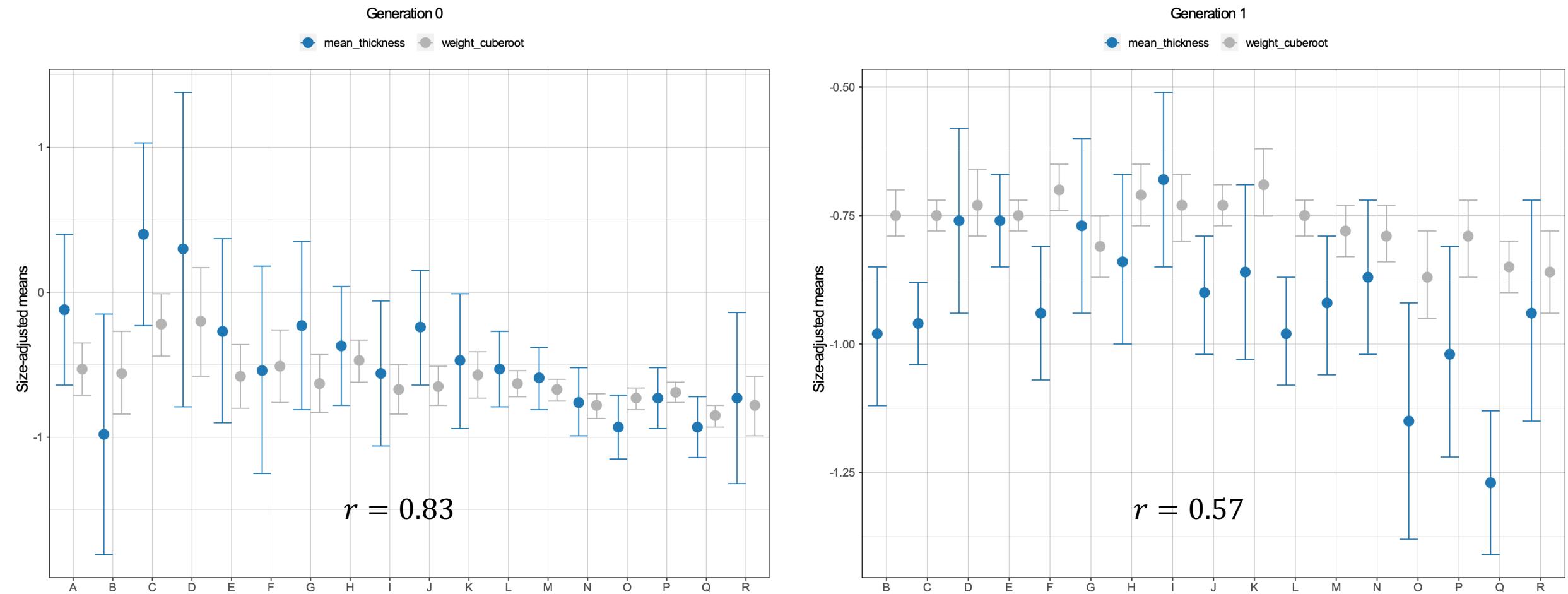


Figure 6a. Size-adjusted means of thickness and weight for each population of generation 0 parents. The model formula is $y \sim \text{size} \times \text{population}$ where y is either thickness or weight and size is the overall mean size. Both phenotypes as well as size were scaled before model predictions. Correlation of the adjusted means between thickness and weight is $r = 0.83$.

Figure 6b. Size-adjusted means of thickness and weight for each population of generation 1 offspring. The model formula is $y \sim \text{size} \times \text{population}$ where y is either thickness or weight and size is the overall mean size. Both phenotypes as well as size were scaled before model predictions. Correlation of the adjusted means between thickness and weight is $r = 0.57$.

WEIGHT and THICKNESS: Correlation of the regression coefficients

Table 3a. Estimates of the regression coefficients for thickness and weight. The model formula is $y \sim \text{size} \times \text{population}$ where y is either thickness or weight. Both phenotypes as well as size were scaled before model fitting. Only showing values of the regression coefficients with data from generation 0 parents. Correlation is 0.43 and if the intercept and offsets are also included, the correlation is 0.56.

Parameter	Thickness	Weight
size	0.44	0.87
size:B	0.74	-0.02
size:C	-0.12	-0.21
size:D	-0.46	-0.29
size:E	0.20	0.04
size:F	0.45	0.07
size:G	0.27	0.06
size:H	0.36	-0.07
size:I	0.39	0.05
size:J	-0.08	0.02
size:K	-0.03	-0.06
size:L	0.36	-0.08
size:M	0.13	0.15
size:N	0.54	0.05
size:O	0.23	0.16
size:P	0.36	0.17
size:Q	0.10	0.00
size:R	0.41	0.06

Table 3b. Estimates of the regression coefficients for thickness and weight. The model formula is $y \sim \text{size} \times \text{population}$ where y is either thickness or weight. Both phenotypes as well as size were scaled before model fitting. Only showing values of the regression coefficients with data from generation 1 offspring. Correlation is 0.85 and if the intercept and offsets are also included, the correlation is 0.62.

Parameter	Thickness	Weight
size	0.64	1.00
size:C	-0.04	-0.14
size:D	-0.30	-0.09
size:E	-0.22	-0.21
size:F	-0.30	-0.07
size:G	-0.01	-0.07
size:H	0.05	0.02
size:I	0.13	-0.02
size:J	-0.09	-0.01
size:K	-0.37	-0.14
size:L	-0.06	0.05
size:M	-0.15	-0.10
size:N	-0.01	-0.06
size:O	-0.25	-0.10
size:P	-0.26	-0.06
size:Q	-0.36	-0.14
size:R	-0.11	-0.11

One option would be to fit a model a bit like the one we discussed for size at maturity, in this case having a constant slope but allowing the intercept to vary clinally. However, it might be better to stick with a simpler option. Just use the size-adjusted weights you have plotted in slide 8 and take them through to the plasticity analysis.

The input data are the vectors x (size-adjusted means of generation 0 parents), sd_x (standard deviation calculated as $SD = SE \times \sqrt{n}$ where SE is the standard error for the predicted means and n is the number of parental individuals in each population) and y (size-adjusted means of generation 1 offspring). The length of the vectors is $N = 17$.

```
data {  
    int<lower=0> N;  
    vector[N] x;  
    vector[N] y;  
    vector<lower=0>[N] sd_x;  
}
```

The parameters accepted by the model are the vector x_lat of length N and the real numbers $alpha$, $beta$ and $sigma$. Finally, the parameter mu_yhat is a vector with a linear relationship with x_lat also of length N .

```
parameters {  
    vector[N] x_lat;  
    real alpha;  
    real beta;  
    real<lower=0> sigma;  
}  
  
transformed parameters {  
    vector[N] mu_yhat = alpha + beta * x_lat;  
}
```

Priors are defined for x_lat , $alpha$, $beta$ and $sigma$. We model x to be normally distributed with mean x_lat and standard deviation sd_x . We also model y to be normally distributed with mean mu_hat and standard deviation $sigma$.

```
model {  
    x_lat ~ normal(0, 10);  
    alpha ~ normal(0, 10);  
    beta ~ normal(1, 10);  
    sigma ~ cauchy(0, 2);  
  
    x ~ normal(x_lat, sd_x);  
    y ~ normal(mu_yhat, sigma);  
}
```

WEIGHT: Plasticity analysis

Input data

Table 4. Weight input data for the plasticity analysis. After scaling, cube root weights were size-adjusted for each population (Pop) in generation 0 parents (x) and in generation 1 offspring (y) separately. Standard deviations (sd_x and sd_y) were calculated as SD = SE × \sqrt{n} . Stan input data are highlighted in orange.

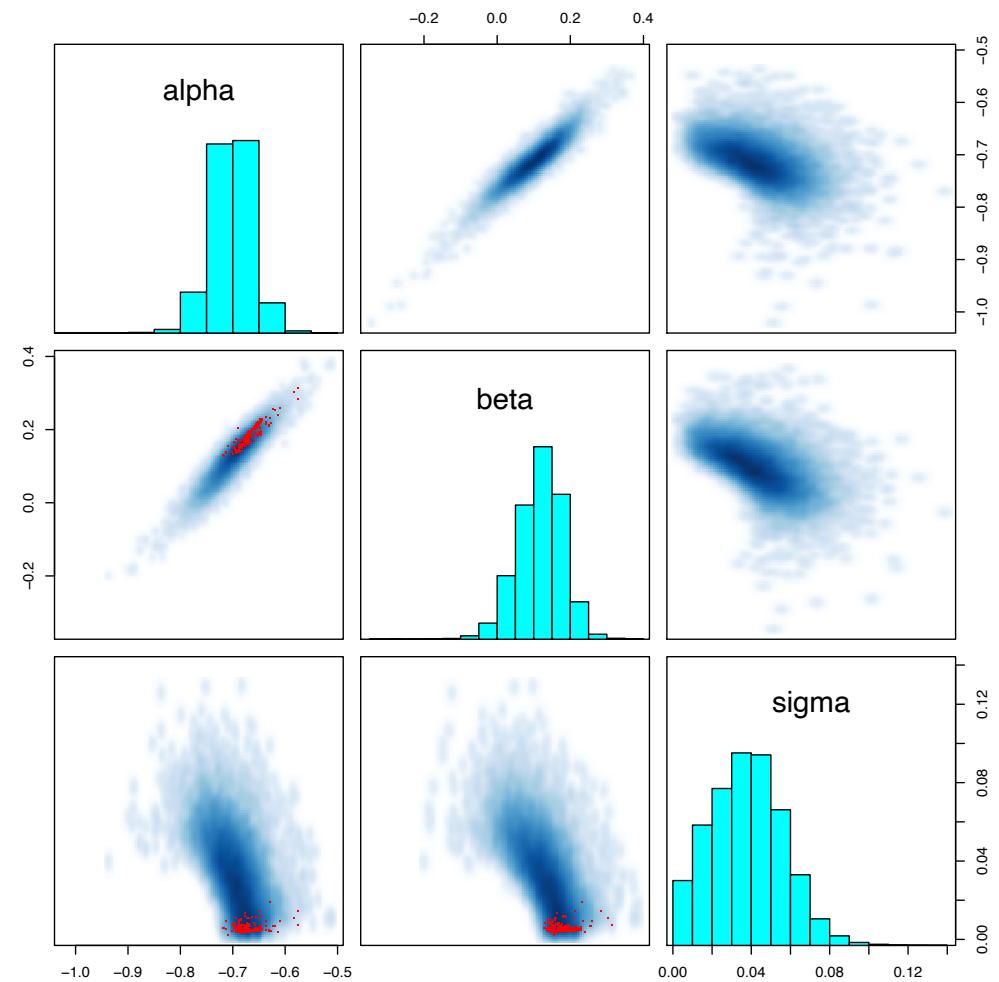
x	SE	n	Pop	Gen	sd_x
-0.56	0.15	20	B	0	0.65
-0.22	0.11	20	C	0	0.49
-0.20	0.19	17	D	0	0.78
-0.58	0.11	20	E	0	0.50
-0.51	0.13	17	F	0	0.52
-0.63	0.10	22	G	0	0.48
-0.48	0.07	21	H	0	0.31
-0.66	0.08	20	I	0	0.38
-0.65	0.07	21	J	0	0.32
-0.57	0.08	21	K	0	0.38
-0.63	0.05	19	L	0	0.20
-0.67	0.04	21	M	0	0.17
-0.79	0.04	16	N	0	0.17
-0.73	0.04	20	O	0	0.17
-0.69	0.04	23	P	0	0.17
-0.85	0.04	21	Q	0	0.17
-0.78	0.10	13	R	0	0.37

y	SE	n	Pop	Gen	sd_y
-0.75	0.02	35	B	1	0.14
-0.75	0.01	35	C	1	0.09
-0.73	0.03	36	D	1	0.20
-0.75	0.02	33	E	1	0.09
-0.70	0.02	33	F	1	0.14
-0.81	0.03	39	G	1	0.20
-0.71	0.03	34	H	1	0.18
-0.73	0.03	37	I	1	0.19
-0.73	0.02	35	J	1	0.12
-0.69	0.03	29	K	1	0.17
-0.75	0.02	19	L	1	0.08
-0.78	0.02	35	M	1	0.15
-0.79	0.03	36	N	1	0.17
-0.87	0.04	35	O	1	0.25
-0.80	0.04	37	P	1	0.23
-0.85	0.03	33	Q	1	0.15
-0.86	0.04	36	R	1	0.24

WEIGHT: Plasticity analysis

Parameters

Inference for Stan model. 4 chains, each with iteration = 12000; warmup = 4000; thin = 1; post-warmup draws per chain = 8000, total post-warmup draws = 32000.



Parameter	Mean	SD	2.5%	50%	97.5%	n_eff	R_hat
alpha	-0.702	0.038	-0.778	-0.701	-0.631	2002	1.002
beta	0.117	0.062	-0.010	0.122	0.226	1561	1.003
sigma	0.038	0.018	0.005	0.038	0.075	936	1.004

Figure 7. Posterior distributions of the parameters and diagnostics. Parameters x_{lat} and μ_{yhat} are not shown. Sampling problems are highlighted in red.

WEIGHT: Plasticity analysis

Warning messages

1: There were 501 divergent transitions after warmup.

Increasing adapt_delta above 0.99 may help. See

<http://mc-stan.org/misc/warnings.html#divergent-transitions-after-warmup>

2: There were 4 chains where the estimated Bayesian

Fraction of Missing Information was low. See

<http://mc-stan.org/misc/warnings.html#bfmi-low>

4: Tail Effective Samples Size (ESS) is too low, indicating posterior variances and tail quantiles may be unreliable.

Running the chains for more iterations may help. See

<http://mc-stan.org/misc/warnings.html#tail-ess>