Supplementary Material: Which line to follow? The utility of different line-fitting methods to capture the mechanism of morphological scaling

Demonstration that MA = $\boldsymbol{\mu_{k_2}/\mu_{k_1}}$ when $\boldsymbol{\sigma_{k_1}^2 = \sigma_{k_2}^2}$ and $\boldsymbol{\sigma_{i_1}^2 = \sigma_{i_2}^2}$

Recall that:

$$\alpha_{MA} = \left(\frac{\mu_{k_2}}{\mu_{k_1}}\right)\left(\frac{\sigma_{T_2}^2 - \sigma_{T_1}^2 + \sqrt{\left(\sigma_{T_2}^2 - \sigma_{T_1}^2\right)^2 + 4\sigma_{T_1,T_2}^2}}{2\sigma_S^2\mu_{k_2}^2}\right) \tag{1}$$

Note that:

$$\sigma_{T_2}^2 - \sigma_{T_1}^2 = \left(\sigma_S^2\sigma_{k_2}^2 + \sigma_S^2\mu_{k_2}^2 + \sigma_{k_2}^2\mu_S^2 + \sigma_{i_2}^2\right) - \left(\sigma_S^2\sigma_{k_1}^2 + \sigma_S^2\mu_{k_1}^2 + \sigma_{k_1}^2\mu_S^2 + \sigma_{i_1}^2\right) \tag{2}$$

When $\sigma_{k_1}^2 = \sigma_{k_2}^2 = \sigma_k^2$ and $\sigma_{i_1}^2 = \sigma_{i_2}^2 = \sigma_i^2$:

$$\begin{aligned}\sigma_{T_2}^2 - \sigma_{T_1}^2 &= \cancel{\sigma_S^2\sigma_k^2} + \sigma_S^2\mu_{k_2}^2 + \cancel{\sigma_k^2\mu_S^2} + \cancel{\sigma_i^2} - \cancel{\sigma_S^2\sigma_k^2} - \sigma_S^2\mu_{k_2}^2 - \cancel{\sigma_k^2\mu_S^2} - \cancel{\sigma_i^2} \\ &= \sigma_S^2\mu_{k_2}^2 - \sigma_S^2\mu_{k_1}^2\end{aligned} \tag{3}$$

And:

$$\left(\sigma_{T_2}^2 - \sigma_{T_1}^2\right)^2 = \sigma_S^4\mu_{k_1}^4 + \sigma_S^4\mu_{k_2}^4 - 2\sigma_S^4\mu_{k_1}^2\mu_{k_2}^2 \tag{4}$$

Recall that:

$$\sigma_{T_1,T_2} = \sigma_S^2\mu_{k_1}\mu_{k_2} \tag{5}$$

Thus:

$$4\sigma_{T_1,T_2}^2 = 4\sigma_S^4\mu_{k_1}^2\mu_{k_2}^2 \tag{6}$$

Thus:

$$\left(\sigma_{T_2}^2 - \sigma_{T_1}^2\right)^2 + 4\sigma_{T_1,T_2}^2 = \sigma_S^4\mu_{k_1}^4 + \sigma_S^4\mu_{k_2}^4 + 2\sigma_S^4\mu_{k_1}^2\mu_{k_2}^2 = \left(\sigma_S^2\mu_{k_2}^2 + \sigma_S^2\mu_{k_1}^2\right)^2 \tag{7}$$

Inserting into Eqn 9:

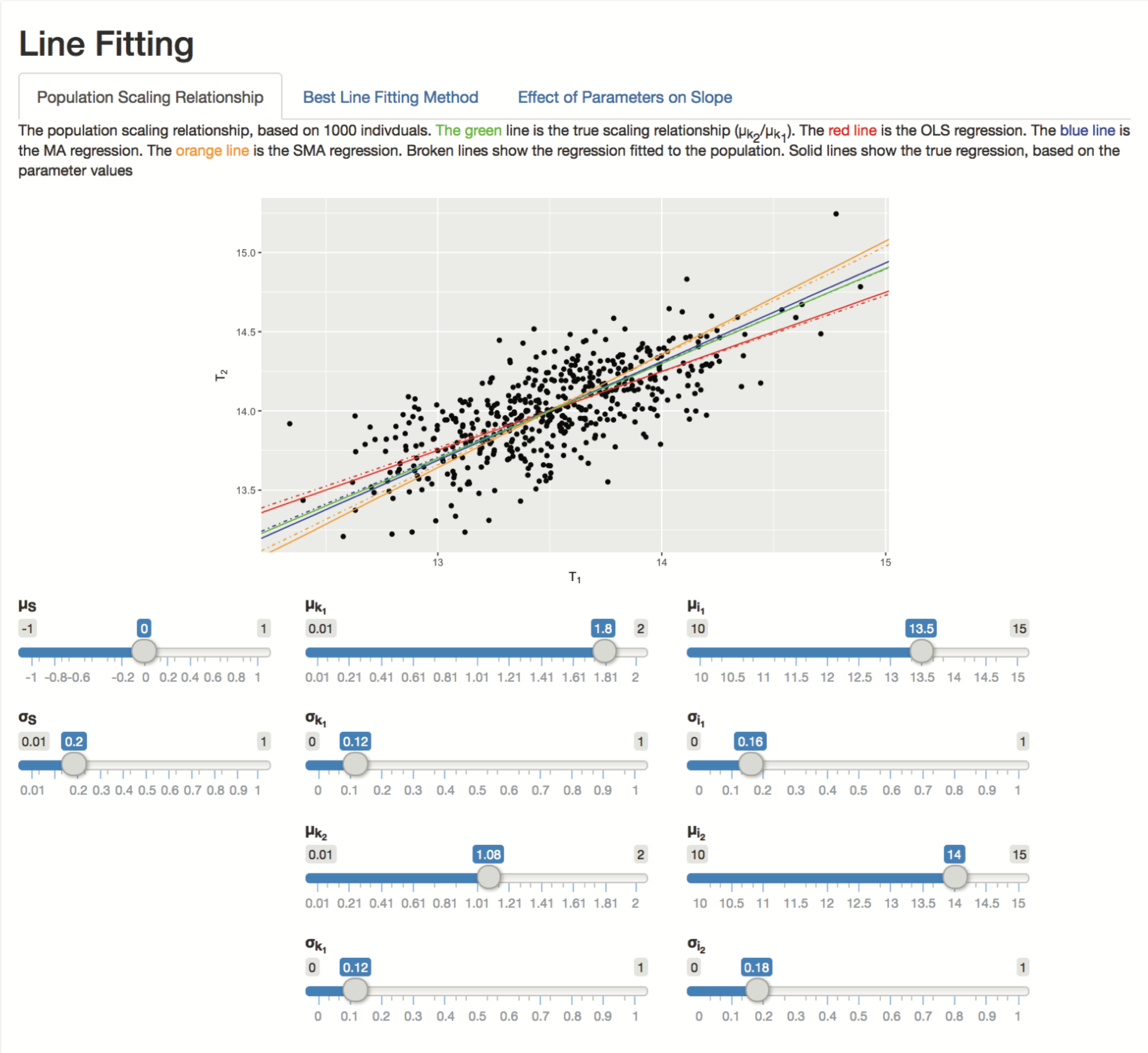
$$\begin{aligned}\alpha_{MA} &= \left(\frac{\mu_{k_2}}{\mu_{k_1}}\right)\left(\frac{\sigma_S^2\mu_{k_2}^2 - \sigma_S^2\mu_{k_1}^2 + \sqrt{\left(\sigma_S^2\mu_{k_2}^2 + \sigma_S^2\mu_{k_1}^2\right)^2}}{2\sigma_S^2\mu_{k_2}^2}\right) \\ &= \left(\frac{\mu_{k_2}}{\mu_{k_1}}\right)\left(\frac{\sigma_S^2\mu_{k_2}^2 \cancel{- \sigma_S^2\mu_{k_1}^2} + \sigma_S^2\mu_{k_2}^2 \cancel{+ \sigma_S^2\mu_{k_1}^2}}{2\sigma_S^2\mu_{k_2}^2}\right)\end{aligned} \tag{8}$$

$$= \left(\frac{\mu_{k_2}}{\mu_{k_1}}\right)\left(\frac{2\sigma_S^2\mu_{k_2}^2}{2\sigma_S^2\mu_{k_2}^2}\right)$$

$$= \left(\frac{\mu_{k_2}}{\mu_{k_1}}\right)1$$

The Line Fitting *ShinyApp* interface

The line fitting *shinyapp* has three tabs that allows the user to explore the influence of model parameters on how well each line fitting method captures μ_{k_2}/μ_{k_1} (Supplementary Figure 1). The user can adjust all the model parameter using sliders at the bottom of the display. The first tab displays the relationship between T_1 and T_2 among individuals in a population of 500 individuals, along with the average individual scaling relationship (μ_{k_2}/μ_{k_1}), and the population scaling relationship as estimated by OLS, MA and SMA regression. For each of the regression analyses, the plot displays both the regression line estimated from the sampled data, and the regression line calculated from the parameters used to generate the sample data. The second tab displays which line fitting method best captures μ_{k_2}/μ_{k_1} across combinations of parameter values, either different levels of σ_{i_1} and σ_{i_2} or σ_{k_1} and σ_{k_2}. The criterion used for 'best' is the line-fitting method that produces a slope closest to μ_{k_2}/μ_{k_1}. The third tab displays the relationship between different parameter values ($\mu_S, \sigma_S, \mu_{k_1}, \sigma_{k_1}, \mu_{k_2}, \sigma_{k_2}, \sigma_{i_1}, \sigma_{i_2}$) versus the average individual scaling relationship (μ_{k_2}/μ_{k_1}), or the population scaling relationship as estimated by OLS, MA or SMA regression. These charts allow the user to determine how sensitive different line-fitting methods are to changes in specific parameter values. The range of possible parameter values was chosen so that the population scaling relationship resembled the relationship between log(wing size) and log(pupal size) in *Drosophila*. Users can adjust the initial parameter values and their ranges by editing the original *R*-scripts and running the application locally.



Line Fitting

Population Scaling Relationship | Best Line Fitting Method | Effect of Parameters on Slope

The population scaling relationship, based on 1000 indivduals. The green line is the true scaling relationship (μ_{k_2}/μ_{k_1}). The red line is the OLS regression. The blue line is the MA regression. The orange line is the SMA regression. Broken lines show the regression fitted to the population. Solid lines show the true regression, based on the parameter values

μ_s: 0 (-1 to 1)

σ_s: 0.2 (0.01 to 1)

μ_{k_1}: 1.8 (0.01 to 2)

σ_{k_1}: 0.12 (0 to 1)

μ_{k_2}: 1.08 (0.01 to 2)

σ_{k_1}: 0.12 (0 to 1)

μ_{i_1}: 13.5 (10 to 15)

σ_{i_1}: 0.16 (0 to 1)

μ_{i_2}: 14 (10 to 15)

σ_{i_2}: 0.18 (0 to 1)

Supplementary Figure 1: Screen shot of the application that allows users to explore the effect model parameters on the ability of different line-fitting methods to capture the slope of morphological scaling relationships. The first tab shows the population scaling relationship for two traits in a population of 500 individuals. The red, blue, and orange line shows the theoretical (sold line) and sampled (broken line) OLS, MA and SMA regression. The green line shows the average individual scaling relationship μ_{k_2}/μ_{k_1} for the population. The application is available at:https://shingletonlab.shinyapps.io/linefitting/

Incorporating developmental time into the model

We will assume that traits grow exponentially throughout development, and that both growth rate and developmental time are positively and negatively regulated by a systemic growth factor, respectively. Individual trait size can be modelled as:

$$\log t = T = (Sk + i)(1 - Sd) \tag{9}$$

where *d* described the effect of the systemic growth factor on developmental time. Here, the mean developmental time is set as 1, and changes in *S* either increase of decrease development time. Note that *d* must be small, such that *Sd*<1.

Rearranging Eqn 9:

$$T = -dkS^2 + (k - id)S + i$$

$$0 = -dkS^2 + (k - id)S - (T - i)$$

$$0 = kS^2 + \frac{(id - k)}{d}S + \frac{(T - i)}{d} \tag{10}$$

Using the quadratic formula.

$$S = \frac{-\left(\frac{(id-k)}{d}\right) + \sqrt{\left(\frac{(id-k)}{d}\right)^2 + 4k\left(\frac{i-T}{d}\right)}}{2k}$$

$$= \frac{-\left(\frac{(id-k)}{d}\right) + \sqrt{\left(\frac{(id-k)}{d}\right)^2 + 4k\left(\frac{i-T}{d}\right)}}{2k}$$

$$= \frac{-\left(\frac{(id-k)}{d}\right) + \sqrt{\left(\frac{(id-k)}{d}\right)^2 . \left(1 + 4k\left(\frac{i-T}{d}\right) . \left(\frac{d}{(id-k)}\right)^2\right)}}{2k}$$

$$= \frac{-\left(\frac{(id-k)}{d}\right) + \left(\frac{(id-k)}{d}\right)\sqrt{1 + 4k\left(\frac{i-T}{d}\right) . \frac{d^2}{(id-k)^2}}}{2k}$$

$$= \frac{-\left(\frac{(id-k)}{d}\right) + \left(\frac{(id-k)}{d}\right)\sqrt{1 + \frac{4k(i-T)d}{(id-k)^2}}}{2k} \tag{11}$$

Using Taylor series, consider the function $f(x) = \sqrt{(1+x)}$, then:

$$f(x) = 1 + \frac{x}{2} + Err(x) \tag{12}$$

Where:

$$Err(x) = -\frac{x^2}{8(1+c)^{3/2}} \tag{13}$$

Which has absolute value bounded by $x^2/8$.

If $\frac{4k(i-T)d}{(id-k)^2}$ is small, then we get a small error for *f*(*x*). Note that with $-1 \le S \le 1$, $i\approx 13$, $k\approx 1$and $d\approx .0.01$, then $\frac{4k(i-T)d}{(id-k)^2} \approx 0.25$. So the error for *f*(*x*) is bound by 1/128.

Therefore, suppose e $\sqrt{(1+x)} = 1 + \frac{x}{2}$, then Eqn 3 simplifies to:

$$\begin{aligned} S &\approx \frac{-\left(\frac{(id-k)}{d}\right) + \left(\frac{(id-k)}{d}\right)\left(1 + \frac{2k(i-T)d}{(id-k)^2}\right)}{2k} \\ &\approx \frac{-\left(\frac{(id-k)}{d}\right) + \left(\frac{(id-k)}{d}\right)\left(1 + \frac{2k(i-T)d}{(id-k)^2}\right)}{2k} \\ &\approx \frac{-\left(\frac{(id-k)}{d}\right) + \left(\frac{(id-k)}{d}\right) + \left(\frac{2k(i-T)d}{(id-k)^2}\right)\left(\frac{(id-k)}{d}\right)}{2k} \\ &\approx \frac{i-T}{id-k} \end{aligned} \tag{14}$$

For two traits:

$$\begin{aligned} T_1 &= (Sk_1 + i_1)(1 - Sd_1) \\ T_2 &= (Sk_2 + i_2)(1 - Sd_2) \end{aligned} \tag{15}$$

Using Eqn 14:

$$T_2 \approx -d_2 k_2 \left(\frac{i_1 - T_1}{i_1 d_1 - k_1}\right)^2 + (k_2 - i_2 d_2)\left(\frac{i_1 - T_1}{i_1 d_1 - k_1}\right) + i_2 \tag{16}$$

Since many morphological scaling relationships can be modelled using a linear equations, we may assume that the quadratic term $d_2 k_2 \left(\frac{i_1 - T_1}{i_1 d_1 - k_1}\right)^2$ is a small. Eqn 8 therefore simplifies to:

$$
\begin{aligned}
T_2 &\approx k_2\left(\frac{i_1 - T_1}{i_1 d_1 - k_1}\right) - i_2 d_2\left(\frac{i_1 - T_1}{(i_1 d_1 - k_1)}\right) + i_2 \\
&\approx T_1\left(\frac{i_2 d_2 - k_2}{i_1 d_1 - k_1}\right) - i_1\left(\frac{i_2 d_2 - k_2}{i_1 d_1 - k_1}\right) + i_2
\end{aligned} \qquad (17)
$$

The slope of the individual scaling relationship is therefore controlled by the relative sensitivity of the trait's growth rate and growth duration to changes in the systemic growth factor, that is *k* and *d* respectively. However, these parameters have opposing effects on the slope of the individual scaling relationship. As *k* increases, trait size will become more sensitive to changes in *S*, because that trait will grow faster at a higher *S*. In contrast, as *d* becomes larger, trait size will become less sensitive to changes in *S*, because, while it will be growing at a faster rate, it will grow for an increasingly short period of time.

The model requires that *d*, the sensitivity of growth duration to changes in the systemic growth regulator, be small relative to *S*. There are two reasons for this. First, $Sd < 1$, or the duration of growth will be negative. Second, as *d* increases, the quadratic term of the individual scaling relationship (Eqn 8), become larger and the scaling relationship becomes markedly non-linear.

The requirement that *d* be small appears to be inconsistent with data that show that growth duration is very sensitive to systemic growth regulators. For example, suppression of insulin/insulin-like growth factor signaling (IIS) can more than double egg-to-adult developmental time in *Drosophila* (Shingleton et al. 2005). However, in *Drosophila* adult body size is only affected by changes IIS during a very short period of larval development, called the terminal growth period (TGP) (Shingleton et al. 2005). In wild-type flies reared at 25˚C the TGP is ~30h, and is only moderately affected by nutrition (Stieper et al. 2008). The same appears to be true in *Manduca sexta* (Davidowitz et al. 2004). Thus, while a systemic growth regulator may ostensibly have a substantial effect on the duration of growth, the effect of the growth regulator on the period of growth that influences final body size may be very different.

References

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