Supplementary Information

Data Analysis

We have written an *R* script that extracts a number of summary statistics for the data generated by our image, available on Dryad. These summary statistics capture the maximum and minimum level of pigmentation across the cuticle, the width of all pigmented cuticle (a2-a5), which is a proxy for tergite size, and the width of the pigment band (a4-a5), for the third and fourth abdominal tergite. Briefly, the script first calculates and plots a cubic spline $S(x)$ that describes the change in average pigment value (y-axis) from the anterior (x=0) to the posterior edge of the ROI defined in Step 4.9 (Figure 2D). The script next calculates the first derivative $S'(x)$ (Figure 2E) and second derivative $S''(x)$ (Figure 2F) of this spline, and uses them to define three points along the spline, moving from posterior to anterior: the posterior edge of a5 cuticle ($x = T_3$), the anterior edge of the a4 cuticle ($x = T_2$) and the point of minimum pigmentation ($x = T_1$). The user has already defined the anterior edge of the a2 cuticle ($x = 0$)

The posterior edge of the pigment band, T_3, is where the level of pigmentation peaks, which the script finds as the first point at which the $S'(x)$ transitions from <0 to >0, moving anteriorly from the posterior of the spline (Figure 2E). This only works, however, if the posterior edge of the ROI, defined in Step 4.9, contains a small unpigmented portion either of a6-p1 cuticle, or even a1-a2 cuticle of the next posterior tergite. If it does not, there will be no position along the spline where $S'(x)$ transitions from <0 to >0. In this case, the script assumes the posterior edge of the spline is the posterior edge of the pigment band.

The second point is the anterior edge of the pigment band, T_2. This is where the the rate of decline in pigmentation is maximum, when moving from posterior to anterior. This can be calculated as the point where $S'(x)$ is maxmimum moving anteriorly from the posterior edge of the spline (Figure 2E). If the script can not find this point, it then uses the first point where $S''(x)$ transitions from <0 to >0 when moving anteriorly from the posterior edge of the spline (Figure 2F′).

The third point is the the posterior edge of the a2 cuticle, which we use as the point of minimum pigmentation T_1. The a2 cuticle has the same level of light pigmentation as the a3 cuticle, but the bristles on the a3 cuticle make it difficult to measure the latter's pigmentation. The posterior edge of the a2 cuticle is the first point where $S'(x)$ transitions from >0 to <0 when moving moving anteriorly from the anterior edge of the pigment band, T_2. If the script can not find this point (Figure 2E′) it then uses the first point where $S''(x)$ transitions from >0 to <0 when moving anteriorly from the anterior edge of the pigment band, T_2(Figure 2F′).

Note that the difference in pigmentation between cuticle a1 and a2 is typically small, which means that the pigmentation profile can not be reliably used to define the anterior edge of the a2 cuticle. For this reason, this position is define by the user.

The script then uses these points to determine maximum (P_{max}) and minimum (P_{min}) pigmentation at points T_2 and T_4 respectivly, width of pigment band ($W_{band} = T_3 - T_2$), and width of the pigmented cuticle ($W_{tergite} = T_3$).

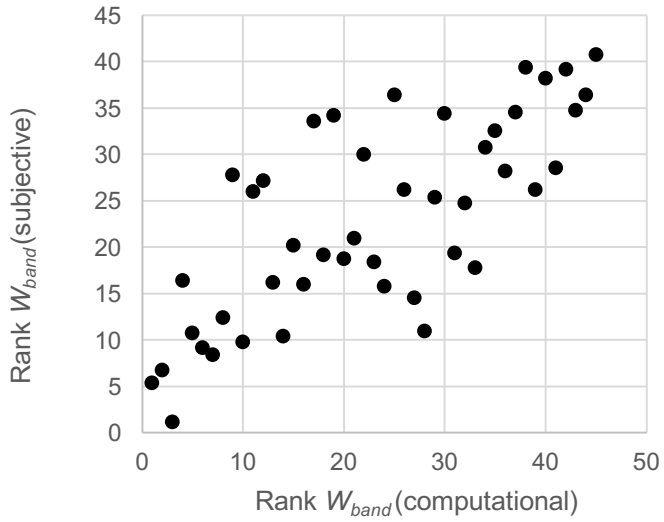
Session Correction

For each pigmentation measure (P_{max} and P_{min}) for each tergite, we then correct for any nuisance factors arising due to session effects, using the images of the same 15 tergites taken across temporally adjacent sessions. First, the data from the third and fourth tergites are seperated and corrected independently. Pigmentation measurements from each session are corrected by adding a correction factor (C) such that:

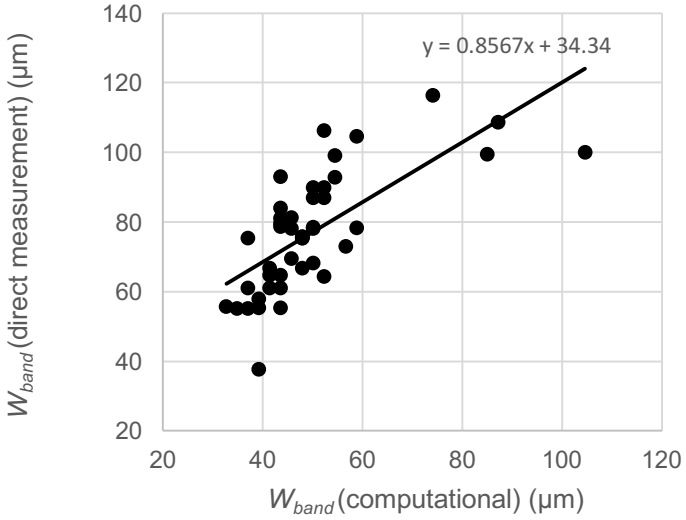
$$C_s = \bar{M}_{s-1} - \bar{M}_s$$

where C_s is the correction value for session s, $\bar{M}_s$ and $\bar{M}_{s-1}$ are the mean pigmentation measurements across repeatedly measured tergites in session s and $s-1$, respectivly. Thus C_s is the average change in pixel value between subsequent sessions due to nuisance factors. This correction is then added to all the pigmentation measures made in session s. The correction is applied in a 'daisy chain': the second session is corrected relative to the first session, the third session is corrected relative to the (corrected) second session, the fourth session is corrected relative to the (corrected) third session, and so on**.** After all the data have been corrected, the script then removes all duplicated data, such that the only data that are retained are from the first time a specimen is imaged.

Supplementary Figures



Supplementary Figure 1. The relationship between the W_{band} width ranked computationally and by eye. The relationship is significant (Spearman's Rank, $\rho = 0.7342$, $P<0.0001$).



Supplementary Figure 2. The relationship between the W_{band} width measured computationally and by eye. The relationship is significant (OLS, $r^2 = 0.49$, $P<0.0001$).