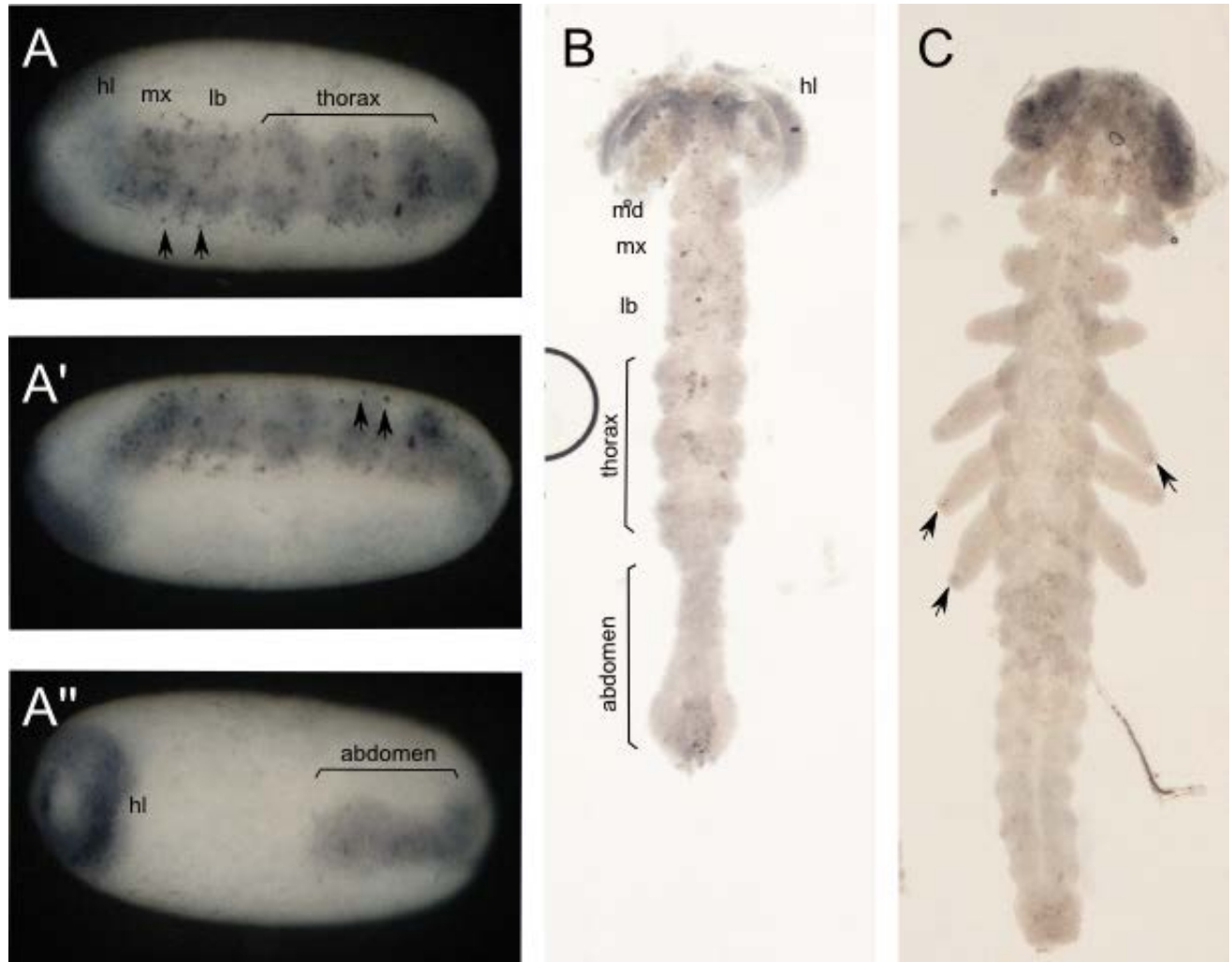
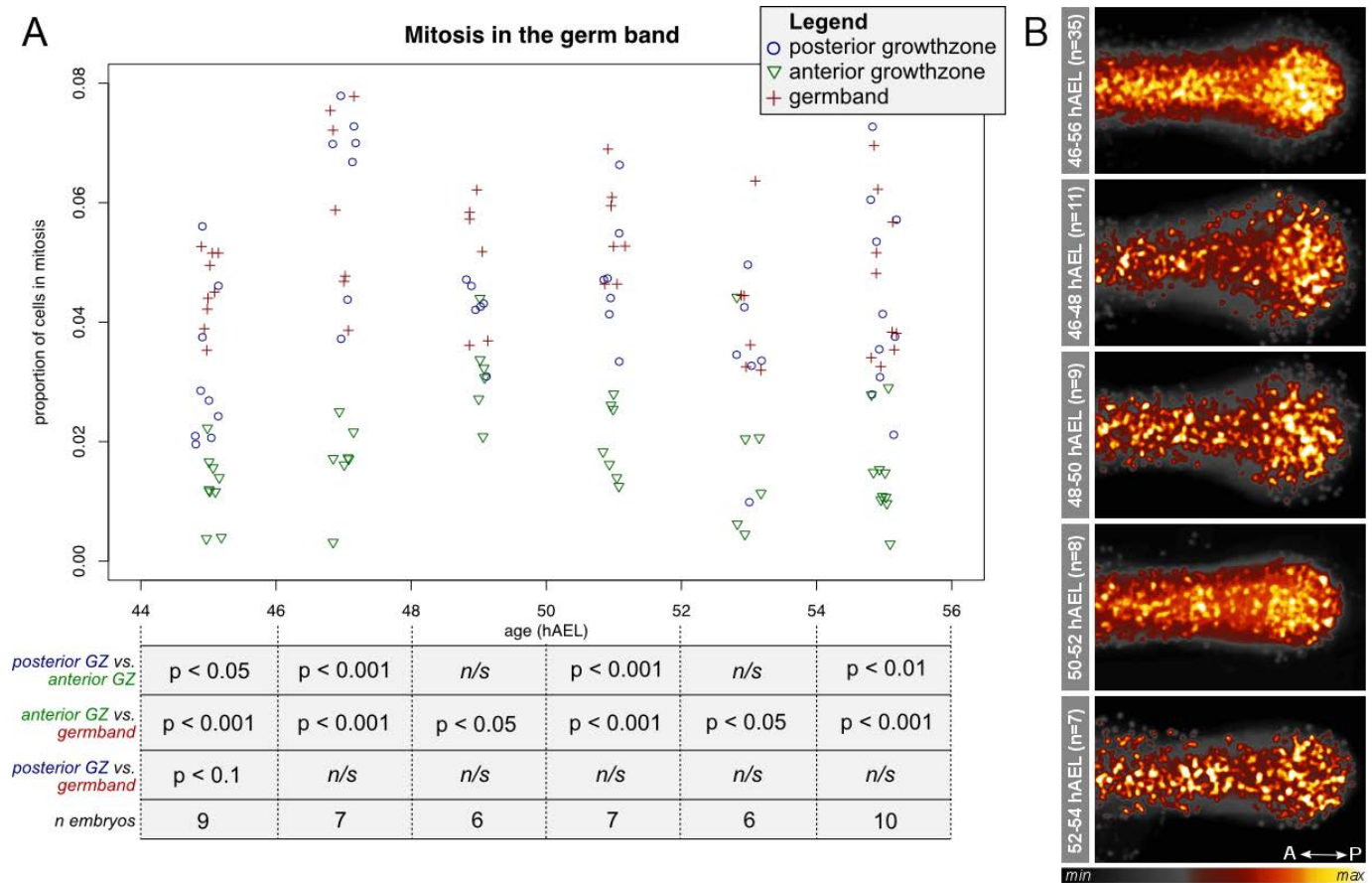


**Fig. S1. Growth zone measurements in *Oncopeltus fasciatus* germband embryos.** (A) Map of all measurements performed on *O. fasciatus* embryos, and their corresponding names in Tables S1 and S2. (B) Correlations between these measurements are shown as a heatmap of pairwise  $R^2$  scores.



**Fig. S2. Apoptosis in the growth zone of *O. fasciatus*.** In order to confirm whether apoptosis has a role in the changing size of the growth zone, we stained for the apoptotic marker caspase. We use the same protocol as for anti-PH3 staining (see Materials and Methods) but with anti-caspase 3 (Abcam 13847; 1:2000) primary antibody and anti-rabbit HRP (DAB substrate) secondary antibody. Examining the embryo while still in the yolk reveals an abundance of apoptotic cells surrounding the embryo, presumably in extra-embryonic tissues. This can be seen in the undissected embryo from different angles (A-A'') (examples indicated by arrows). However, when embryos are dissected out of the yolk, the embryonic tissue is seen to be almost completely free of apoptotic cells: (B) early germband, approximately 48 hAEL; (C) late germband, approximately 64 hAEL. Very few apoptotic cells are observed in the gnathal, thoracic or abdominal segments. A somewhat greater amount of apoptosis is detected in the head lobe, and may be associated with the formation of the nervous system. In later embryos, we note apoptosis concentrated in the tip of the developing limbs (C, arrows). Very few apoptotic cells are scattered in the mesoderm cells of the growth zone. The number of apoptotic cells in the growth zone is more than an order of magnitude lower than the number of dividing cells (i.e.  $43.03 \pm 16.50$ ,  $n=68$ ). We thus consider apoptosis to be a negligible factor in growth zone dynamics. hl, head lobes; md, mandibular segment; mx, maxillary segment; lb, labial segment.



**Fig. S3. Mitosis in the growth zone of *O. fasciatus*.** (A) Ratio of dividing cells in distinct areas of the growth zone, as shown in Fig. 6C, separated in different time compartments. This image shows that no considerable change occurs in the relative ratios of dividing cells over the 44-56 hAEL time period studied in this work. Results of paired one-way ANOVAs are reported below the plot (see Materials and Methods; the linear model controlled for individual embryos, and *P*-values were corrected for multiple testing using the Holm procedure). Note that staging was not possible because this requires *engrailed* staining, which would have conflicted with *eve* necessary for the division into relevant sections. (B) Heatmap of dividing cells in the growth zone, as also shown in Fig. 6A, separated into different time compartments.

# Appendix S1

## Variability in the data

In the dataset, we see a large amount of variation in the sizes of embryos at each stage. This is an intriguing observation, as the process of development needs to be robust to these size variations, if they are real. To determine whether this is the case, we need to assess the amount of experimental noise, and its influence on the variance in our measurements. This means we need to assess (1) the amount of variability between embryos, and (2) to what extent this can be attributed to experimental parameters, such as (a) measurement error or (b) mounting error.

Measurement errors (a) were accounted for by performing three separate measurements on each image. Each datapoint in the dataset used is the average of these measurements.

To account for mounting error (b), we need to assess what part of the variance between measurements can be attributed to different slides that may have mounting differences between them. To start, we need to find a parameter that is not dynamic over the development of the embryo. A good candidate is segment width, which does not change significantly in the transition from the second to third stripe in any stage (Fig 1F), making this measurement our best proxy for variability.

## Assessing segment width as a proxy for embryonic size

Without live imaging we cannot fully exclude a cyclical dynamic, whereby the width of the segment decreases and increases again (or increases and decreases again) within a single stage. However, if this were the case, we would expect a strong correlation between segment length (which increases within the stage) and segment width, in a second order polynomial regression.

*#between stripe2 and seg2*

```
M1 <- lm(stripe.2.width~X2nd.segment.length+(X2nd.segment.length)^2+
        X2nd.segment.length:segments+segments,data=sup1b)
summary(M1)
```

```
##
## Call:
## lm(formula = stripe.2.width ~ X2nd.segment.length + (X2nd.segment.length)^2 +
##     X2nd.segment.length:segments + segments, data = sup1b)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -34.849 -10.119   0.878   8.605  37.045
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      87.723     25.031   3.505 0.000579 ***
## X2nd.segment.length
##      5.070         2.912   1.741 0.083405 .
## segments4
##      40.501        47.976   0.844 0.399705
## segments5
##      115.175        36.309   3.172 0.001783 **
## segments6
##      60.936        34.061   1.789 0.075313 .
## segments7
##      34.585        36.927   0.937 0.350242
## segments8
##      73.524        42.761   1.719 0.087276 .
## segments9
##      66.597        38.969   1.709 0.089198 .
## X2nd.segment.length:segments4
##      -5.847         5.714  -1.023 0.307545
## X2nd.segment.length:segments5
##     -12.968         4.134  -3.137 0.001997 **
```

```

## X2nd.segment.length:segments6  -7.451      3.761  -1.981  0.049119 *
## X2nd.segment.length:segments7  -5.580      4.173  -1.337  0.182842
## X2nd.segment.length:segments8  -8.279      4.941  -1.676  0.095546 .
## X2nd.segment.length:segments9  -8.674      4.629  -1.874  0.062613 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 13.87 on 178 degrees of freedom
## (43 observations deleted due to missingness)
## Multiple R-squared:  0.1851, Adjusted R-squared:  0.1256
## F-statistic:  3.11 on 13 and 178 DF,  p-value: 0.000342
#between stripe3 and seg2
M2 <- lm(stripe.3.width~X2nd.segment.length+(X2nd.segment.length)^2+
          X2nd.segment.length:segments+segments,data=sup1b)
summary(M2)

##
## Call:
## lm(formula = stripe.3.width ~ X2nd.segment.length + (X2nd.segment.length)^2 +
##     X2nd.segment.length:segments + segments, data = sup1b)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -3.5254 -0.8557  0.0152  0.7966  3.2543
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    10.94128    2.27240   4.815 3.13e-06 ***
## X2nd.segment.length
## segments4      -2.30041    4.35544  -0.528  0.5980
## segments5       8.09270    3.29621   2.455  0.0150 *
## segments6       2.33037    3.09216   0.754  0.4521
## segments7       0.05067    3.35236   0.015  0.9880
## segments8       0.98640    3.88195   0.254  0.7997
## segments9       5.11996    3.53773   1.447  0.1496
## X2nd.segment.length:segments4  0.18858    0.51875   0.364  0.7167
## X2nd.segment.length:segments5 -0.94053    0.37526  -2.506  0.0131 *
## X2nd.segment.length:segments6 -0.31768    0.34144  -0.930  0.3534
## X2nd.segment.length:segments7 -0.11463    0.37880  -0.303  0.7625
## X2nd.segment.length:segments8 -0.20925    0.44854  -0.467  0.6414
## X2nd.segment.length:segments9 -0.67431    0.42026  -1.605  0.1104
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.259 on 178 degrees of freedom
## (43 observations deleted due to missingness)
## Multiple R-squared:  0.1059, Adjusted R-squared:  0.04061
## F-statistic:  1.622 on 13 and 178 DF,  p-value: 0.08271

```

In fact, the  $R^2$  of these regressions is 0.04 between the length of the penultimate segment and the stripe preceding it ( $p < 0.1$ ), and 0.13 ( $p < 0.001$ ) between the length of the penultimate segment and the stripe that follows. Thus, it is unlikely that a cyclical pattern exists and is responsible for intra-stage variability.

## Quantifying variation

Using variation in the penultimate stripe as a proxy for overall variability, we see that, indeed, this variation is extensive. The total mean and variance of this parameter is:

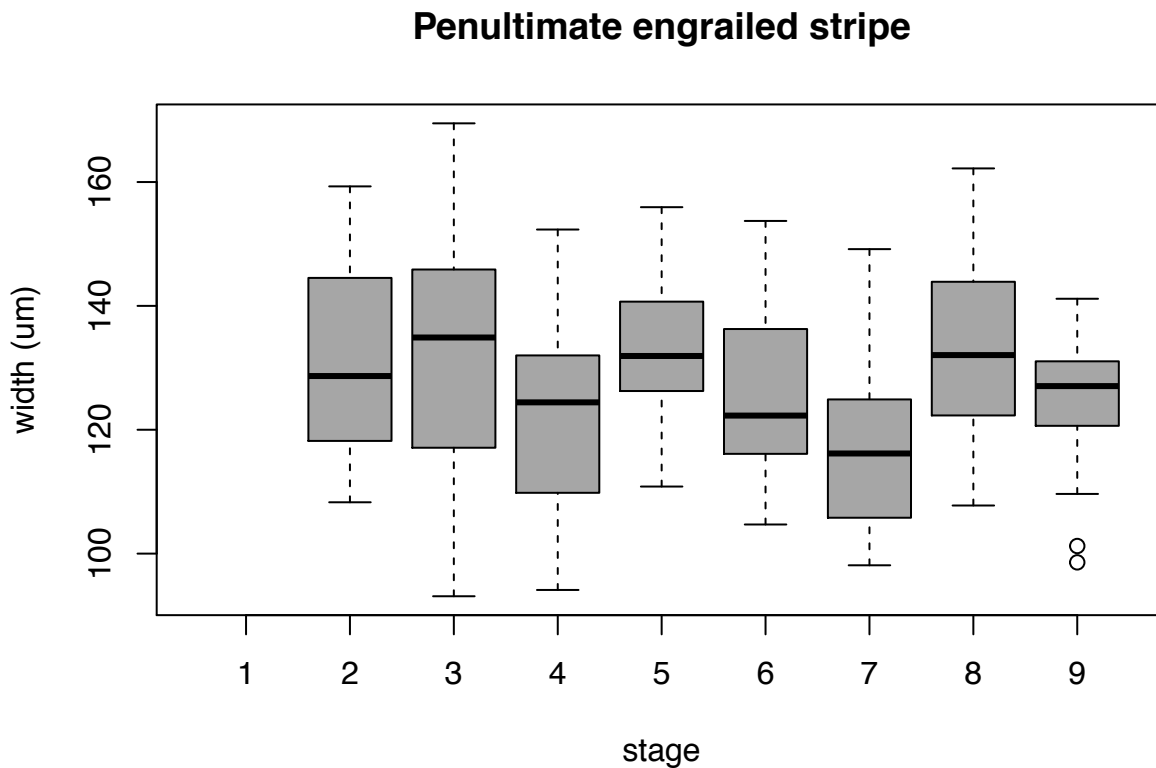
```
# mean stripe 2 width:
mean(sup1b$stripe.2.width,na.rm=TRUE)
```

```
## [1] 127.2376
```

```
# total variance in stripe 2 width
var(sup1b$stripe.2.width,na.rm=TRUE)
```

```
## [1] 225.2739
```

Separated by stage, the metric looks like this:



## The effect of mounting on variance in segment width

Fitting the parameter in a linear mixed model, with segments and slide as random effects, can tell us what part of the variance can be attributed to differential mounting (i.e. slides).

```
library(lme4)
```

```
## Loading required package: Matrix
```

```
M <- lmer(stripe.2.width~1+(1|segments)+(1|slide), data=sup1b)
summary(M)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: stripe.2.width ~ 1 + (1 | segments) + (1 | slide)
## Data: sup1b
```

```

##
## REML criterion at convergence: 1776.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.56231 -0.65085  0.02652  0.59947  2.64669
##
## Random effects:
##  Groups   Name            Variance Std.Dev.
##  slide    (Intercept)    48.54    6.967
##  segments (Intercept)    20.97    4.579
##  Residual                    173.32   13.165
## Number of obs: 219, groups:  slide, 17; segments, 8
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)  125.082      2.608   47.96

```

The model indicates that the variance attributable to slide ID is 21.55% (48.54/225.27; 225.27 is the total variance for stripe width, see ‘Quantifying variation’). Segments account for a further 9.31% (20.97/225.27) of variance.

## Conclusion

Using the residual standard deviation of this model, the distribution of stripe width is  $127.24 \pm 13.17$   $\mu\text{m}$  (127.24 is the mean stripe width, see ‘Quantifying variation’); this means that 95% of the measurements are between 100.9  $\mu\text{m}$  and 153.58  $\mu\text{m}$  (2 standard deviations from the mean), giving a 53% increase in size between the smallest and largest measurement of 95% of embryos, when accounting for measurement error and mounting errors.

**Table S1.** Raw data after measurements on imaged *O. fasciatus* embryos. Each image was measured three times, consecutively. The location of the measurements (a1-a3; w1-w4; l5-l8) are indicated in Fig. S1A. All units are in  $\mu\text{m}$ .

[Click here to Download Table S1](#)

**Table S2.** Processed data from measurements on imaged *O. fasciatus* embryos: entries are averaged from three measurements done in the raw data. The measurements are indicated in Fig S1A. All units are in  $\mu\text{m}$ .

[Click here to Download Table S2](#)

**Table S3.** Additional data on segment number and age of *O. fasciatus* embryos, used for the calculation of segmentation rate.

[Click here to Download Table S3](#)



**Table S4.** The data of all measurements on mitosis in the *O. fasciatus* growth zone and germband, collected as described in the methods section of the main text. Regions are visualized in Fig. 6B. DAPI indicates the number of nuclei detected in the relevant area; PH3 indicates the number of dividing cells detected.

[Click here to Download Table S4](#)

**Table S5.** Primers used to design probes for *in situ* hybridization.

**eve:**

**F:** AGGGTGGTGGAGCGGAGGGG

**R:** GGCGCAGGACAACCTTGATT

**Delta:**

**F:** AGTGCCCTTCCATCCGCTGT

**R:** CGTGTTGACGCTCTCCTTGG

**cad:**

**F:** TCACACCCGACTCCAGGAAA

**R:** AAACAGTGCTGAAAAGATAC

**inv:**

**F:** TCAATCGGATGTAGTGAGGA

**R:** TCGGCAACGGTTCTTGCCAT