bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

1 **Long title:** A revised understanding of *Tribolium* morphogenesis further
2 reconciles short and long germ development
3
4 **Short title:** A significant revision to our understanding of short germ
5 embryogenesis
6
7
8 **Author:** Matthew A. Benton
9
10 **Author affiliation:** Department of Zoology, University of Cologne, Cologne, NRW, Germany
11
12 **Corresponding author:** Matthew A. Benton, matthewabenton@gmail.com
13

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

14 **Author summary**

15 In many animals, certain groups of cells in the embryo do not directly contribute to adult
16 structures. Instead, these cells generate so-called 'extra-embryonic tissues' that support and
17 facilitate development, but degenerate prior to birth/hatching. In most insect species,
18 embryos are described as having two major extra-embryonic tissues; the serosa, which
19 encapsulates the entire embryo and yolk, and the amnion, which covers one side of the
20 embryo. This tissue structure has been widely reported for over a century, but detailed
21 studies on the amnion are lacking. Working in the beetle Tribolium castaneum, I used long-
22 term fluorescent live imaging, cell tracking and differential cell labelling to investigate
23 amnion development. In contrast to our current understanding, I show that most cells
24 previously thought to be amnion actually form large parts of the embryo. In addition, I show
25 how these cells 'flow' as a whole tissue and contribute to elongation of the embryo, and how
26 only a relatively small number of cells form the actual amnion. Lastly, I describe how my
27 findings show that despite exhibiting substantial differences in overall structure, embryos of
28 Tribolium and the fruit fly, Drosophila melanogaster, utilise a conserved set of
29 morphogenetic processes.

30

31 **Abstract**

32 In *Drosophila melanogaster*, the germband forms directly on the egg surface and solely
33 consists of embryonic tissue. In contrast, most insect embryos undergo a complicated set of
34 tissue rearrangements to generate a condensed, multi-layered germband. The ventral side
35 of the germband is embryonic, while the dorsal side is thought to be an extraembryonic
36 tissue called the amnion. While this tissue organisation has been accepted for decades, and
37 has been widely reported in insects, its accuracy has not been directly tested in any species.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

38 Using live cell tracking and differential cell labelling in the short germ beetle *Tribolium*
39 *castaneum*, I show that most of the cells previously thought to be amnion actually give rise
40 to large parts of the embryo. This process occurs via the dorsal-to-ventral flow of cells and
41 contributes to germband extension. In addition, I show that true 'amnion' cells in *Tribolium*
42 originate from a small region of the blastoderm. Together, my findings show that
43 development in the short germ embryos of *Tribolium* and the long germ embryos of
44 *Drosophila* is more similar than previously proposed. Dorsal-to-ventral cell flow also occurs
45 in *Drosophila* during germband extension, and I argue that the flow is driven by a conserved
46 set of underlying morphogenetic events in both species. Furthermore, the revised *Tribolium*
47 fatemap that I present is far more similar to that of *Drosophila* than the classic *Tribolium*
48 fatemap. Lastly, my findings show that there is no qualitative difference between the tissue
49 structure of the cellularised blastoderm and the short/intermediate germ germband. As
50 such, the same tissue patterning mechanisms could function continuously throughout the
51 cellularised blastoderm and germband stages, and easily shift between them over
52 evolutionary time.

53

54 Introduction

55 Insects are the most speciose phylum of animals and display remarkable diversity in adult
56 morphology [1]. Insect embryo development is also very diverse, particularly in the stages
57 leading to the formation of the elongated, segmented embryo (called the germband) [2].
58 The molecular and morphogenetic basis of this process is best understood in the fly
59 *Drosophila melanogaster*. In this species, a predominantly hierarchical chain of patterning
60 events specifies nearly all segments more-or-less simultaneously at the syncytial blastoderm
61 stage [3]. Cellularisation takes place near the end of this process, after which point

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

62 morphogenetic events such as germband extension (GBE) occur (see Fig 1 for schematic
63 summary). The *Drosophila* mode of development is termed long germ development and is
64 fairly representative of most true flies [4]. In contrast, the vast majority of insects undergo
65 short or intermediate germ development, meaning that only a handful of segments are
66 specified at the blastoderm stage and the remaining segments are specified sequentially as
67 the germband elongates [5].

68 Short germ development has been best studied in the beetle *Tribolium castaneum,*
69 and recent research has shown that development in this species is more similar to
70 *Drosophila* than previously thought. In *Drosophila*, GBE is predominantly driven by the
71 mediolateral intercalation of ectodermal cells (i.e. convergent extension), although cell
72 deformation along the anterior-posterior (AP) axis and cell divisions are also involved [6–11].
73 In contrast to this, *Tribolium* germband elongation was previously thought to be driven by
74 the so-called ‘growth zone’ at the posterior of the germband [12]. Now, however, it is clear
75 that *Tribolium* germband elongation is also predominantly driven by mediolateral cell
76 intercalation (see Fig 1 for schematic summary of *Tribolium* development) [13–15].
77 Furthermore, in both *Tribolium* and *Drosophila*, this intercalation requires the striped
78 expression of a specific group of Toll genes (so-called Long Toll/Loto class genes) [16,17].
79

80 **Fig 1. Schematics of development in *Drosophila* and *Tribolium*.** The two left columns show
81 schematics of *Drosophila* embryos from the uniform blastoderm stage to the extended
82 germband stage. The right three columns show schematics of *Tribolium* embryos at
83 comparable developmental stages. The schematics in the right-most column depict
84 dissected, flatmounted embryos. Red arrows display cell/tissue movement. The question
85 marks highlight two regions (the *Drosophila* embryo/amnioserosa border in the cephalic

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

86 furrow region, and the dorsoventral position of the *Tribolium* embryo/amnion border) where
87 the tissue boundaries are unknown/undescribed. Several features have been omitted,
88 including the yolk, mesoderm gastrulation, anterior gut formation and appendage
89 formation. The *Drosophila* fatemap is based on data from [18] and the references therein.
90 Refer to text for additional details.
91

92 It is highly likely that germband elongation mediated by cell intercalation is
93 homologous in these two species, and probably in other arthropods, as well [17]. As such, I
94 will hereafter refer to *Tribolium* 'germband elongation' as 'germband extension'/GBE,
95 unifying the *Drosophila*/*Tribolium* terminology. In addition, as there is no evidence for a
96 qualitatively different 'growth zone' in *Tribolium* (i.e. a specialised zone of volumetric
97 growth), I will refer to the posterior unsegmented region as the 'segment addition zone'
98 (SAZ) [19–21].

99 Despite the similarities described above, there are substantial differences in the
100 embryonic fatemaps of these two species (Fig 1). In *Drosophila*, almost the entire
101 blastoderm is fated as embryonic tissue, and only a small dorsal region is fated as
102 extraembryonic tissue (termed the amnioserosa) [18]. In contrast, in *Tribolium*, roughly the
103 anterior third of the blastoderm gives rise to an extraembryonic tissue called the serosa [22].
104 Of the remaining blastoderm, a large dorsal region is thought to give rise to a second
105 extraembryonic tissue called the amnion, with only the remaining ventral tissue giving rise to
106 the embryo itself [23–25]. Like the amnioserosa, the serosa and the amnion are proposed to
107 support the embryo during development, but are thought to degenerate prior to hatching
108 and not contribute to any larval or adult structures [19,26,27].

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

109 *Drosophila* and *Tribolium* also exhibit dramatic differences in the morphogenetic
110 events occurring during early development (Fig 1). When GBE occurs in *Drosophila*, the
111 germband stays at the surface of the egg and the amnioserosa largely remains in place. In
112 *Tribolium*, on the other hand, germband extension begins with a process called embryo
113 condensation, during which the embryonic ectoderm and presumptive amnion (together
114 termed the 'germ rudiment') form the germband (see Fig 1; for a detailed description see
115 [14,28]). Several concurrent morphogenetic events underlie embryo condensation. The
116 embryonic ectoderm condenses towards the ventral side of the egg via both mediolateral
117 cell intercalation and a cuboidal-to-columnar cell shape transition. Simultaneously, epithelial
118 folding and tissue involution occurs, causing the presumptive amnion to fold over the
119 embryonic ectoderm. During these movements, the serosa cells undergo a cuboidal-to-
120 squamous transition to spread over the entire egg surface. The final stage of embryo
121 condensation coincides with closure of the serosa (serosa window stage), which appears to
122 involve a supracellular actomyosin cable [14].
123 The differences in fatemap and tissue folding described above show that both
124 fatemap shifts and reductions in early morphogenetic events have contributed to the
125 evolution of the long germ mode of development found in *Drosophila*. However, it is
126 important to note that *Drosophila*, regarding the extraembryonic tissues, represents an
127 extreme case of reductive evolution, which is characteristic only for higher cylorrhaphan flies
128 [29]. More basally branching flies form both an amnion and a serosa, while still exhibiting
129 the long germ mode of development (for a review see [26]). For example, in the scuttle fly
130 *Megaselia abdita*, both an amnion and serosa form, but while the serosa spreads over the
131 egg surface as in *Tribolium*, the amnion remains at the dorsal side of the embryo, similar to
132 the *Drosophila* amnioserosa [30–33]. Such intermediate topologies help to explain the

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

133 evolution of the situation in *Drosophila,* where all extraembryonic cells remain at the dorsal
134 side.

135 Understanding how these differences evolved is integral to understanding the short-
136 to-long germ transition, but in order to study how this occurred, we first need to understand
137 how these tissues develop in each species. The form and function of the *Tribolium* serosa has
138 been analysed in several studies [22,34,35]. The amnion, on the other hand, has proven
139 harder to analyse, and the precise embryo/amnion boundary at the blastoderm stage is
140 unknown. However, a defined boundary between embryo and amnion has been proposed to
141 exist from when the germband forms (Fig 1) [23]. Cells in the ventral half of the germband
142 (ventral with respect to the germband dorsoventral [DV] polarity, but dorsal with respect to
143 the egg) are thought to give rise to all embryonic structures, while cells in the dorsal half of
144 the germband (dorsal with respect to the germband DV polarity, but ventral with respect to
145 the egg) are thought to form the amnion [25,36,37]. This germband structure has been
146 described in many insects over the past century and is proposed to represent the core
147 conserved structure of short/intermediate germ embryos (reviewed in [2,38,39]). However,
148 the proposed boundary between cells fated to become embryo and those fated to become
149 amnion has not been directly tested.

150 Here, I investigate the development of the presumptive amnion in *Tribolium* using a
151 combination of fluorescent live imaging and fate mapping techniques. To my great surprise, I
152 find that the majority of the cells previously described as 'amnion' actually form large parts
153 of the embryo proper. Using fate-mapping experiments, I show that true 'amnion' cells
154 originate from a very small domain of the blastoderm, just as the *Drosophila* amnioserosa
155 cells do. I also show that the movement of cells from the 'amnion' side of the germband to
156 the 'embryo' side of the germband occurs via the large scale flow of the ectodermal

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

157 epithelium. Lastly, I describe the underlying causes of this flow, and show how this tissue
158 movement is likely homologous to the dorsal-to-ventral tissue flow that occurs during
159 *Drosophila* GBE.
160

161 ## Results

162 **Live cell tracking reveals movement of 'amnion' cells into the embryo**

163 To examine the development of the *Tribolium* presumptive amnion in detail, I carried out
164 high resolution live imaging of embryos transiently labelled [14] with a fluorescent histone
165 marker (H2B-venus) to label nuclei. My goal was to track presumptive amnion cells from the
166 blastoderm stage onwards. However, it was not possible to accurately track the majority of
167 cells throughout embryo condensation and GBE, due to the extensive morphogenetic
168 rearrangements that take place during this process. Instead, I focused on the stage
169 immediately following condensation when the germband has formed, and analysed the
170 embryonic region where the presumptive amnion is closest to the surface of the egg.
171 Specifically, I tracked over 200 presumptive amnion cells from the central region of the
172 germband from the closure of the serosa window until after the formation of the thoracic
173 segments (over 11 hours of development; Fig 2 and S2 Movie). As previously described [14],
174 the germband and yolk exhibit pulsatile movements during this period, as well as rotating
175 within the serosa (S1 Movie).

176 The presumptive amnion initially consists of many tightly packed cells, which become
177 increasingly spread out during GBE (S2 Movie, Fig 2(A-C)). However, rather than remaining
178 restricted to the 'amnion territory', many of the tracked cells moved around the edge of the
179 germband into the 'embryo territory'. Differential labelling of tracked cells clearly showed
180 that these cells that moved around the germband edge became part of the embryo proper

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

181 (S2 Movie and Fig 2(A-C)). The cells that joined the 'embryo territory' became tightly packed,
182 continued to divide, and formed embryonic structures (S3 Movie and Fig 2(D-F)). In contrast,
183 cells that remained in the 'amnion territory' became squamous and stopped dividing. The
184 nuclei of these latter cells became enlarged (S3 Movie and Fig 2(D-F)), suggesting that they
185 underwent endoreplication to become polyploid, as seen in the *Tribolium* serosa and in the
186 *Drosophila* amnioserosa [18,24]. In addition, several germband nuclei underwent apoptosis
187 (S3 Movie) as has been described in fixed embryos [40]. These results show that many of the
188 cells previously thought to constitute extraembryonic amnion give rise to embryonic
189 structures.
190 Since the epithelium formerly termed 'amnion' is made up cells that will variously
191 form amnion, dorsal ectoderm and dorsolateral ectoderm, it is not accurate for the entire
192 tissue to be called 'amnion'. Therefore, I will refer to this part of the germband as the 'dorsal
193 epithelium', based on the tissue's location at the dorsal side of the germband (with respect
194 to the DV polarity of the germband rather than the egg). This term 'dorsal epithelium' is
195 simply a spatial designation, and comes with no implicit assumptions about the identity of
196 the tissue nor the final fate of the tissue. It is also important to keep in mind that the dorsal
197 epithelium is continuous with the ventral epithelium.
198
199 **Fig 2. Live cell tracking reveals contribution of 'amnion' cells to embryonic tissue.** (A-F)
200 Time series from fluorescent live imaging of a *Tribolium* embryo expressing H2B-venus. The
201 serosa nuclei located above the germband have been manually removed from these frames
202 (by deleting them from individual z-stack slices), but left in the surrounding territory
203 (arrowhead in (A+A')). (A'-C') show optical transverse sections of the respective frame at the
204 position shown by the dashed line (the surface of the egg is to the left). In (A-C), all nuclei

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

205 that lie in a region of the 'amnion territory' in (A) have been tracked and differentially
206 labelled depending on whether they become part of the embryo (magenta; labels disappear
207 when nuclei join the germband), become located at the edge of the germband (yellow) or
208 remain in the 'amnion territory' (cyan). In (D-F), a line of nuclei that lie in the 'amnion
209 territory' in (D) have been tracked and differentially labelled depending on whether they
210 become part of the embryo (coloured points; daughter cells are labelled in same colour as
211 parent) or remain in the 'amnion territory' (cyan; no division takes place). Note that in panel
212 (D), the orange spot is mostly hidden below the yellow spot because the nuclei in that region
213 are partially overlapping when viewed as projections. The first frame of the timelapse was
214 defined as timepoint 0. In (A-F), embryos are oriented based on the AP/DV polarity of the
215 egg with anterior to the left and dorsal to the top. (A-C) are maximum intensity projections
216 of one egg hemisphere. (D-F) are average intensity projections of 46 microns to specifically
217 show the germband. Scale bars are 50 μm.
218
219 **Differential cell labelling confirms widespread dorsal-to-ventral cell movement**
220 My next question was whether the movement of cells from the dorsal epithelium to the
221 ventral epithelium occurs throughout the AP axis or is just limited to the thoracic region. The
222 extensive movements of the germband made it difficult to track individual cells accurately at
223 the anterior and posterior poles. To overcome this problem, I combined differential cell
224 labelling with long term fluorescent live imaging to follow small groups of nuclei throughout
225 development. Specifically, I microinjected mRNA encoding a nuclear-localised
226 photoconvertable fluorescent protein (NLS-tdEos) into pre-blastoderm embryos to uniformly
227 label all nuclei, then photoconverted a small patch of nuclei at different positions along the
228 AP axis at the final uniform blastoderm stage. I then performed long term confocal live

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

229 imaging of both the unconverted and photoconverted forms of the fluorescent protein
230 throughout the period of GBE (or longer). Unlike that of *Drosophila*, the *Tribolium* egg shell
231 does not show any dorsoventral (DV) polarity, and I was therefore unable to specifically
232 target particular locations along the DV axis. Instead, I opted for a brute-force approach and
233 performed the photoconversion experiment at unknown DV positions for 50-150 embryos at
234 each of the following AP positions: 75% egg length (EL) from the posterior pole, 50% EL, 25%
235 EL, and close to the posterior pole. I then used the resulting live imaging data to determine
236 the approximate DV position of the photoconverted cells. Using a new live imaging set up
237 (see Materials and Methods), I obtained the same range of hatching rates as I typically
238 obtain for other microinjection experiments (approximately 80%, [14]), even after
239 continuous confocal live imaging for almost the entirety of *Tribolium* embryonic
240 development (3.5 days; S4 Movie). Both unconverted and photoconverted protein persisted
241 throughout germband extension and retraction, although fluorescent signal faded over time.
242 I have included various examples from this data set in S1-S3 Figures. In addition, I have made
243 the raw confocal data for a large number of timelapses available online (>300 embryos, >700
244 GB of data [41]) for the benefit of the community. This data will likely prove valuable for a
245 wide range of research projects.

246 When I examined clones initially located in the dorsal epithelium, I found that
247 movement of cells from the dorsal epithelium to the ventral epithelium occurred throughout
248 the posterior of the embryo during GBE (Fig 3(A-F), S5 Movie). I also observed the same
249 movements at the anterior of the germband (Fig 3(G-J)), although I have focused my analysis
250 on the middle and posterior parts of the embryo. Together with the cell tracking data, these
251 results show that most of what was previously thought to be ‘amnion’ is in fact embryonic

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

252 tissue, and that cells move from the dorsal epithelium to the ventral epithelium throughout
253 the germband (summarised in Fig 3(K,L)).
254
255 **Fig 3. Differential cell labelling reveals widespread movement of cells from the dorsal**
256 **epithelium to the ventral epithelium.** (A-J) Time series from fluorescent live imaging of two
257 *Tribolium* embryos expressing NLS-tdEos showing unconverted protein (magenta) and
258 photoconverted protein (cyan). In (A-F') a patch of nuclei at the posterior-dorsal region of
259 the blastoderm were photoconverted. Panels (A-F) show the posterior region of the
260 germband during late GBE and panels (A'-F') show optical transverse sections made at the
261 position of the dashed line at each timepoint (roughly following the same nuclei). Serosa
262 nuclei are marked by white crosses in the transverse sections. In (G-J), a patch of nuclei at
263 the anterior-lateral region of the blastoderm were photoconverted. Panels (G-J) show the
264 anterior of the germband during condensation and GBE. In both embryos, all converted
265 nuclei are initially located in the dorsal epithelium, but most move into the ventral
266 epithelium during GBE. (K-L) Schematics showing the classic and revised models of the
267 *Tribolium* germband (presumptive amnion is shown in purple, presumptive embryo is shown
268 in grey, red arrows show the newly discovered tissue flow). The first frame of the timelapses
269 was defined as timepoint 0. In (A-J), embryos are oriented based on the AP/DV polarity of
270 the egg with anterior to the left and dorsal to the top. In (A'-F'), the surface of the egg is
271 oriented to the left. In (K-L), schematics show flatmounted germbands with the focus on the
272 dorsal epithelium, the anterior to the left and the orthogonal sections are oriented with the
273 dorsal half of the germband to the left. (A-J) are maximum intensity projections of one egg
274 hemisphere. Scale bars are 50 µm.
275

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

276 **Mediolateral cell intercalation occurs throughout GBE**

277 During my live imaging, ectodermal cell clones became elongated along the AP axis over
278 time, as previously reported in a *Tribolium* study that used a non-live imaging cell clone
279 method [15]. However, this study found that "labelled ectodermal cells … rarely mix with
280 unlabelled cells" even as clones became greatly elongated [15]. In contrast, I frequently
281 observed non-converted nuclei in the midst of labelled nuclei (Fig 3, S1-S3 Figs).

282 To test whether the pattern I observed was caused by mediolateral cell intercalation,
283 I tracked the nuclei of abutting rows of ectodermal cells in the SAZ during formation of the
284 abdominal segments (50 cells in total, tracked for 3.5 hours; Fig 4 and S6 Movie). This
285 analysis clearly showed that, as during embryo condensation [14], cells intercalated between
286 their dorsal and ventral neighbours. Together with the photoconversion dataset, these
287 results show that extensive mediolateral cell intercalation takes place throughout GBE to
288 drive the convergent extension of the ectoderm.

289

290 **Fig 4. Mediolateral cell intercalation occurs in the SAZ during GBE.** Time series from
291 fluorescent live imaging of a *Tribolium* embryo expressing NLS-tdEos showing the SAZ during
292 abdominal segment formation. Coloured points mark tracked nuclei. During the timelapse,
293 nuclei underwent apicobasal movement but I observed no cell delamination. Note that the
294 same embryo is shown in Fig 3(A-F). The first frame of the timelapse was defined as
295 timepoint 0. The embryo is oriented based on polarity of the visible region of the germband.
296 Panels show maximum intensity projections of 15 µm to specifically show the germband.
297 Abbreviations as follows: germband (GB); anterior (ant); posterior (post); medial (med);
298 lateral (lat). The scale bar is 50 µm.

299

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

300 ***Tribolium serpent* may mark true 'amnion'**

301 As described earlier, cells that remained in the dorsal epithelium became squamous, and this
302 cell shape change occurred progressively along the AP axis (Fig 5(A)). This change in cell
303 shape may be a sign of maturation of true 'amnion'. While characterising cell fate markers, I
304 found that the *Tribolium* ortholog of the GATA factor *serpent* (*Tc-srp*) exhibited spatial and
305 temporal expression dynamics that were very similar to those of the potential 'amnion' (i.e.
306 progressive flattening of cells, Fig 5(B), S4 Fig).

307 At the end of GBE, all but the most posterior cells of the dorsal epithelium were
308 squamous and *Tc-srp* seemed to be expressed in dorsal epithelium cells along nearly the full
309 length of the germband (S4 Fig(I_2)). However, this latter finding was difficult to confirm as
310 most of the dorsal epithelium is lost during embryo fixation at this embryonic stage
311 (presumably due to the fragility of the tissue). I also found *Tc-srp* to be expressed in several
312 other domains, including in the presumptive endoderm (S4 Fig).

313 In *Drosophila*, *serpent* is also expressed in extraembryonic tissue (the amnioserosa)
314 [42–45], and, therefore, *Tc-srp* may mark 'true' extraembryonic amnion. However, future
315 work is required to confirm whether this putative amnion degenerates prior to hatching (as
316 is required to be defined as extraembryonic). For simplicity, I will refer to this tissue as
317 'amnion' for the remainder of this text.

318

319 **Fig 5. Development of the putative amnion.** (A-A'') *Tribolium* embryo transiently expressing
320 the membrane marker GAP43-YFP. (A) shows an overview of the whole egg, (A') shows the
321 dorsal epithelium of the same embryo at the position of the white box, (A'') is an optical
322 sagittal section at the position of the dashed line in (A') showing the apical-basal height of
323 cells of the dorsal epithelium. (B) *Tc-srp* (red) expression in a flatmounted *Tribolium*

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

324 germband also showing nuclei (DAPI, blue). The strong *Tc-srp* signal in nuclei may suggest
325 nuclear or peri-nuclear localisation of the transcript, or it may be due to the cell body being
326 flattened. Aside from the strong patch of anterior medial expression (which is from cells
327 beneath the embryonic ectoderm), all visible expression is in the putative amnion
328 epithelium. (C-E) Extended germband stage *Tribolium* embryos transiently expressing NLS-
329 tdEos showing unconverted protein (magenta) and photoconverted protein (cyan). In each
330 embryo, the clone of converted cells spans the entire amnion. (C_{1-2}) show both sides of the
331 same embryo in which a 6 nuclei wide patch of dorsal-most cells located at 50% EL were
332 photoconverted at the blastoderm stage. (D) shows an embryo in which a 3 nuclei wide
333 patch of dorsal-most cells located at 25% EL were photoconverted at the blastoderm stage.
334 (E) shows an embryo in which a 3 nuclei wide by 6 nuclei long patch of dorsal-most cells
335 located at roughly 2-10% EL were photoconverted at the blastoderm stage. In (A) and (C-E),
336 embryos are oriented based on the AP/DV polarity of the egg with anterior to the left and
337 dorsal to the top. In (A''), the surface of the egg is oriented to the bottom. In (B), the anterior
338 of the germband is to the left. (A) is an average intensity projection of one egg hemisphere.
339 (A') is an average intensity projection of 6 µm to specifically show the dorsal epithelium. (B)
340 is a maximum intensity projection of the whole germband. (C-E) are maximum intensity
341 projections of one egg hemisphere. Abbreviations are: ventral ectoderm (VE) and dorsal
342 ectoderm (DE). Scale bars are 50 µm.
343

344 **A revised *Tribolium* amnion fatemap**

345 To determine which blastoderm cells give rise to the amnion, I analysed 85 embryos in which
346 the dorsal and dorsolateral blastoderm cells were labelled by NLStdEos photoconversion as
347 described above. As I was unable to determine the precise DV position of the

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

348 photoconverted cells at the blastoderm stage, I (1) examined embryos at the extended
349 germband stage (when the mature amnion had formed), (2) determined the embryos in
350 which photoconverted nuclei spanned the full DV width of the amnion (but were not
351 observed in the embryonic tissue), and (3) checked the initial size of the photoconverted
352 patch of nuclei (for a schematic of this approach, see S5 Fig).

353 I found that amnion cells arose from a very small domain of dorsal-most cells (that
354 tapers from its anterior to posterior extent) and from a narrow strip of cells between the
355 presumptive embryo and presumptive serosa (summarised in Fig 6 and S6 Fig). At 50% EL,
356 only approximately the 6 most dorsal cells (approximately 8% of the circumference of the
357 blastoderm) gave rise to all amnion cells stretching from one side of the thorax to the other
358 (Fig 5(C), S1 Fig). Nearer to the posterior of the blastoderm (25% EL), even fewer cells gave
359 rise to amnion (approximately 3 of the most dorsal cells; approximately 6% of the
360 circumference; Fig 5(D), S2 Fig). The posterior limit of the amnion was difficult to define, as
361 although some cells from approximately 5-10% EL appeared to become amnion (Fig5(E)),
362 these cells condensed posteriorly towards the hindgut during germband retraction, and
363 might have contributed to the hindgut tissue (S3 Fig, S7 Movie). I was unable to
364 unambiguously determine the fate of these cells. At the anterior of the embryo, I found that
365 a narrow strip of 1-2 cells between the presumptive embryo and presumptive serosa also
366 gave rise to amnion (Fig 3(G-J)). While substantial additional work will be required to define
367 a complete blastoderm fatemap for *Tribolium*, my findings clearly demonstrate that the
368 'amnion' domain is drastically smaller than previously proposed.

369

370 **Fig 6. Schematics showing the revised *Tribolium* amnion fatemap and germband model.**
371 Schematics drawn as in Fig 1 to show the revised fatemap (top row; drawn directly from the

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

372 numbers described in the text) and germband model based on the results of this manuscript.
373 Note that the posterior amnion/embryo boundary is unclear. The schematics of the
374 flatmounted germbands are drawn with the focus on the dorsal epithelium. See text for
375 additional details, and S6 Fig for an extended figure with the classic and revised models side-
376 by-side.
377

378 Discussion

379 In this article, I have shown that a majority of the cells currently thought to be
380 extraembryonic amnion actually give rise to embryonic tissue. Movement of these cells from
381 the dorsal side of the germband to the ventral side was visible in live cell tracking and
382 differential cell labelling experiments. My results also indicate that the true amnion region
383 differentiates progressively along the AP axis during GBE, as evidenced by differences in cell
384 behaviour and the expression of the gene *Tc-srp*. Lastly, presumptive amnion cells
385 predominantly originate from a small domain on the dorsal side of the blastoderm.
386

387 A revised understanding of the short germ embryo

388 The revision to the *Tribolium* blastoderm fatemap that I describe is essentially a quantitative
389 shift in our understanding of where cell fate boundaries lie along the DV axis. In the revised
390 fatemap (Fig 6, S6 Fig), the proportion of the blastoderm that gives rise to the presumptive
391 amnion is much smaller than previously thought. The presumptive amnion domain is,
392 therefore, remarkably similar in size to the amnioserosa domain of the *Drosophila*
393 blastoderm fatemap [18]. However, it is important to recognize that fatemaps such as those
394 presented here show a static picture of a dynamic process. There is no evidence that the
395 presumptive amnion is specified at the blastoderm stage in *Tribolium*. Instead, the

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

396 progressive changes in cell shape and activation of *Tc-srp* expression in the dorsal epithelium
397 of the germband suggest that the amnion is specified progressively along the AP axis during
398 GBE. Progressive specification of DV cell fates during GBE fits with previous hypotheses
399 [36,46], and analysis of how this process occurs represents an exciting avenue of future
400 research (a possible mechanism for DV patterning during GBE is discussed in S1 Text).

401 In contrast to the fatemap revision, the observation that cells move from the dorsal
402 half of the germband to the ventral half of the germband represents a qualitative shift in our
403 understanding of development in short/intermediate germ insects. In the classic model of
404 short/intermediate germ development, the germband was thought of as a more-or-less flat
405 sheet of ectodermal cells (with mesoderm underneath) covered by the extraembryonic
406 amnion. Because of this, the entire dorsal epithelium is routinely removed during embryo
407 preparation, or not included in descriptions of gene expression patterns and embryonic
408 phenotypes. Based on the new data presented here, it is obvious that we have been
409 discarding or ignoring large parts of the embryo. Furthermore, the movement of cells from
410 the dorsal epithelium into the ventral epithelium must be contributing to GBE, and is,
411 therefore, a key aspect of the extension and overall development of the germband that has
412 thus far been missed.

413 The revised model of the germband does present some technical challenges for
414 future work on short/intermediate germ embryo. The flattened geometry of the germband
415 makes it difficult to image both the dorsal and ventral epithelium using bright-field
416 microscopy approaches. However, this problem can be overcome either by using
417 fluorescence based techniques and confocal microscopy or by mechanical sectioning of the
418 germband. Both approaches have been shown to work well in *Tribolium* (for examples see
419 [13,47] and the results in this manuscript). In the rest of this article, I discuss why the revised

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

420 fatemap and cell flow accord well with what we know about *Tribolium* development, and
421 outline the implications of this discovery on our understanding of the evolution of insect
422 development.
423

424 The cellular and molecular causes of tissue flow unify the blastoderm and the germband

425 The revised model of the *Tribolium* germband reconciles the blastoderm and germband
426 stages. The ectoderm of the germband is a continuous epithelium, which means that the
427 movement of cells from the dorsal epithelium to the ventral epithelium occurs as a tissue
428 level 'flow'. Such dorsal-to-ventral tissue flow also occurs during embryo condensation in
429 *Tribolium* [14], and I propose that the flow is caused by largely the same morphogenetic
430 processes at both stages. The evidence for this hypothesis is summarised here, but for an
431 extended discussion see S2 Text.

432 Three morphogenetic processes contribute to dorsal-to-ventral cell flow in *Tribolium*,
433 and at least two of the three occur at both the blastoderm and germband stages. First,
434 mediolateral cell intercalation occurs at both stages and causes tissue-wide convergence
435 (along the DV axis) and extension (along the AP axis). This process requires two *Toll* genes
436 that are expressed in rings around the entire blastoderm and germband epithelium [17].
437 Second, tissue specific cell shape changes occur at both stages such that ventral/lateral cells
438 become columnar and dorsal/dorsolateral cells become thinner (during condensation (S7 Fig
439)) or squamous (during GBE). The tissue level effect of these changes is contraction of the
440 ventral/lateral ectoderm and spreading of the dorsal tissue. The flattening of
441 dorsal/dorsolateral cells is likely regulated by BMP signalling, as not only does BMP activity
442 correlate with the cell shape changes (see S2 Text), but functional disruption of BMP
443 signalling components leads to uniform cell shape changes along the DV axis [25,48]. A third

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

444 major morphogenetic event is gastrulation of the mesoderm. This occurs along the ventral
445 midline, and as gastrulation occurs, the ectoderm moves ventrally to seal the gap left in the
446 epithelium [47]. At the stage when a complete germband has formed, gastrulation is
447 complete along most of the embryo. However, current data suggests mesoderm gastrulation
448 may be ongoing in the SAZ [47]. If true, the ongoing invagination would contribute to tissue
449 flow in this region.

450 It is important to note that while each of the events described here is involved in the
451 dorsal-to-ventral tissue flow, no single event is absolutely required for it. In the absence of
452 cell intercalation, embryo condensation and thinning of dorsal/dorsolateral ectoderm still
453 takes place, yielding abnormally wide and short germbands [17]. In the absence of tissue
454 specific cell shape changes, condensation occurs in a more radially symmetrical manner
455 yielding a tube-shaped germband that undergoes segment specification and convergent
456 extension [25,48]. Finally, both condensation and GBE are only mildly affected in the
457 absence of mesoderm specification [49]. This functional independence comes from each of
458 the three processes being specified by different pathways (intercalation via segment
459 specification, dorsal thinning via dorsal tissue specification, and gastrulation via ventral
460 tissue specification). There may also be further, as yet undiscovered, morphogenetic events
461 which also contribute to the dorsal-to-ventral tissue flow.

462

463 **Reconciling long and short germ development**

464 I propose that the dorsal-to-ventral tissue flow occurring during embryo condensation and
465 GBE in *Tribolium* is homologous to the dorsal-to-ventral tissue flow that occurs during
466 gastrulation and GBE in *Drosophila* (Fig 1). This conclusion is based on the flow being driven
467 by a conserved set of morphogenetic events.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

468 As described above, tissue flow in *Tribolium* is caused by (1) mediolateral cell
469 intercalation, (2) tissue specific cell shape changes along the DV axis, and (3) gastrulation at
470 the ventral side of the embryo. As described below, equivalent processes are all observed in
471 *Drosophila* as well.

472 In *Drosophila*, *Toll*-mediated mediolateral cell intercalation causes tissue-wide
473 convergence (along the DV axis) and extension (along the AP axis) of the ectoderm during
474 GBE [16]. As in *Tribolium*, the periodic expression of the *Toll* genes is regulated by the pair-
475 rule genes. Conservation at the level of tissue identity, morphogenetic process, and
476 molecular control strongly suggest *Toll*-mediated cell intercalation to be homologous.

477 Cell shape changes are harder to compare between *Drosophila* and *Tribolium*,
478 because unlike in most insects, cellularisation in *Drosophila* leads to the direct formation of
479 columnar cells [18,50]. However, tissue-specific cell shape changes along the DV axis do
480 occur in *Drosophila* and are dependent on BMP signalling ([51,52]; for a detailed description
481 see S3 Text). While the intracellular effectors of these cell shape changes are unknown, use
482 of BMP signalling for dorsal patterning is homologous in *Drosophila* and *Tribolium*, and many
483 dorsal cell specification genes are conserved between these two species [48].

484 Last, *Drosophila* mesoderm gastrulation also occurs along the ventral midline, and
485 causes lateral/dorsolateral ectoderm to move ventrally [51]. Similar to the tissue specific cell
486 shape changes described above, the intracellular effectors of *Tribolium* mesoderm
487 gastrulation are unknown, but the upstream patterning events and the tissue specification
488 genes are highly conserved [36,49]. Furthermore, mesoderm gastrulation at the ventral
489 region of the embryo is widely observed within the insects, and is undoubtedly a
490 homologous process in each species [53].

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

491 While I have focused on *Tribolium* and *Drosophila* here, evidence exists that
492 the new findings in *Tribolium* may also apply to other short/intermediate germ insects. For
493 example, in the intermediate germ bug *Oncopeltus fasciatus* (which forms a condensed,
494 multi-layered germband with tissue topology similar to that of *Tribolium* [26]), the dorsal
495 epithelium of the germband initially consists of a thick epithelium which progressively
496 becomes squamous late during GBE [54]. These tissue-specific cell shape changes are likely
497 the same as those occurring during *Tribolium* GBE. Furthermore, *Oncopeltus* pair-rule genes,
498 Loto *Toll* genes and even segment polarity genes are expressed in rings around the entire
499 germband prior to thinning of the dorsal epithelium [17,55,56]. The expression of these
500 genes in the dorsal epithelium provides additional evidence that much of the *Oncopeltus*
501 dorsal epithelium is made up of embryonic tissue. Future analyses of the molecular and
502 morphogenetic drivers of GBE must analyse the entire germband, rather than focusing on
503 the ventral half. In addition, further work will be needed to determine whether the new
504 findings in *Tribolium* also apply to more basally branching insects such as crickets.
505

506 **Materials and Methods**

507 *Tribolium* animal husbandry, egg collection, and RNA *in situ* hybridisation was performed as
508 previously described [17]. The *Tc-srp* ortholog was previously described [57] and was cloned
509 into pGEM-t (Promega Reference A1360) with primers TCCCGCTGCTTTGATCTAGT and
510 TGCGATGACTGTGACGTGTA. The *Tc-cad* ortholog was as previously used [14].
511 The *H2B-ven* fusion was created by fusing the *D. melanogaster* histone *H2B* coding
512 sequence (without the stop codon) from the published H2B-RFP [14] to the *venus*
513 fluorescent protein [58] and cloning into the pSP64 Poly(A) (Promega Reference P1241)
514 expression vector. The *NLS-tdEos* fusion was kindly provided by Matthias Pechmann.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

515 Additional details and both plasmids are available upon request to M. Pechmann or myself.
516 Capped mRNA synthesis was performed as previously described [14]. *H2B-ven* capped mRNA
517 was injected at 1 µg/µL, *NLS-tdEos* capped mRNA was injected at 2-3 µg/µL.

518 Embryo microinjection was performed as previously described [14], with the
519 following changes. Up to 100 dechorionated embryos were mounted on a rectangular
520 coverslip (24 mm by 50 mm) that rested on a microscope slide. Water was allowed to dry off
521 the embryos before they were covered in Voltalef 10S halocarbon oil and injected as usual.
522 The coverslip (still resting on the slide) was then placed in a petri-dish (92 mm) containing a
523 base layer of 1% agarose (dissolved in water) and placed at 30-32°C until the embryos were
524 at the appropriate stage for imaging. The coverslip was then removed from the slide,
525 inverted (so that embryos were face down), and quickly but gently placed on a lumox dish
526 (50 mm; Sarstedt Reference 94.6077.410) that was sitting upside down. The corners of the
527 coverslip rested on the raised plastic lip of the dish such that the membrane and embryos
528 were close to each other but not touching. To ensure lateral stability of the coverslip during
529 the timelapse recording, approximately 5-10 µL of heptane glue (made by soaking parcel
530 tape in heptane) was placed at each corner. Additional Voltalef 10S halocarbon oil was then
531 added to fill any remaining space between the coverslip and the oxygen permeable
532 membrane. This contraption was then stuck to a microscope slide (using double sided tape)
533 for imaging on an upright microscope. This last step may be unnecessary depending on the
534 microscope stage and orientation.

535 Live imaging was performed on an upright Zeiss SP8 confocal microscope equipped
536 with Hybrid detectors in the Biocentre Imaging facility (University of Cologne). Image stacks
537 of 15-50 focal planes with z-steps ranging from 2-10 µm were taken with a 10x/0.3NA dry
538 objective or a 20×/0.7NA multi-immersion objective at intervals of 5-45 minutes. The

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

539 temperature of the sample during imaging could not be carefully regulated, but was typically
540 between 25-28 degrees. While this lack of temperature control is not ideal, it does not affect
541 the findings presented in this manuscript.

542 Photoconversion of NLS-tdEos protein was performed by constantly scanning the
543 region of interest for 20-30 seconds with the 405 wavelength laser at low power (5%). These
544 settings were manually determined on the above microscope, and need to be determined
545 independently on different systems. Photoconversions were performed during the final
546 uniform blastoderm stage, as photoconversion prior to this resulted in substantial diffusion
547 of the photoconverted protein during nuclei division. The positions of the different regions
548 of the embryo (75% EL etc.) were determined by measuring the length of each embryo in the
549 LASX software and selecting the appropriate region. Photoconversions were performed on
550 all embryos on the coverslip before setting up the timelapse, which led to a 0.5-2 hour delay
551 between performing the photoconversion and beginning the timelapse. As such, the
552 positions of the photoconverted region at the first time point in the timelapses in this
553 manuscript do not reflect the original region of photoconversion.

554 Imaging of fixed material was performed on an upright Zeiss SP8 confocal, an upright
555 Zeiss SP5 confocal microscope and an inverted Zeiss SP5 confocal microscope. The
556 *Drosophila gooseberry* expression patterns were kindly provided by Erik Clark and acquired
557 as in [59]. Images and timelapses were analysed using FIJI [60] and Photoshop CS5. Manual
558 cell tracking was performed on confocal hyperstacks with MTrackJ [61]. The figures were
559 arranged and the schematics created using Inkscape.

560

561 **Acknowledgements**

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

562 I am very grateful to S. Roth for his generous support, and for the extensive and stimulating
563 discussions throughout this project. I am also thankful to E. Clark for in-depth discussions
564 and extensive feedback on the manuscript. I thank M. Pechmann for providing the *NLS-tdEos*
565 construct. In addition, I thank M. Akam, M. Pechmann and S. Roth for comments on the
566 manuscript, and B. Schmitt for comments on the schematics.

567

568 ## References

569 1. Grimaldi D, Engel M. Evolution of the Insects. Cambridge University Press; 2005.
570 2. Anderson DT. Embryology and Phylogeny in Annelids and Arthropod. Pergamon Press;
571 1973.
572 3. Jaeger J. The gap gene network. Cell Mol Life Sci. 2011;68: 243–74.
573 doi:10.1007/s00018-010-0536-y
574 4. Davis GK, Patel NH. SHORT, LONG, AND BEYOND: Molecular and Embryological
575 Approaches to. Annu Rev Entomol. 2002; 669–99.
576 5. Peel AD, Chipman AD, Akam M. Arthropod segmentation: beyond the Drosophila
577 paradigm. Nat Rev Genet. 2005;6: 905–16. doi:10.1038/nrg1724
578 6. Irvine KD, Wieschaus E. Cell intercalation during Drosophila germband extension and
579 its regulation by pair-rule segmentation genes. Development. 1994;120: 827–41.
580 Available: http://www.ncbi.nlm.nih.gov/pubmed/7600960
581 7. Bertet C, Sulak L, Lecuit T. Myosin-dependent junction remodelling controls planar cell
582 intercalation and axis elongation. Nature. 2004;429: 667–671. Available:
583 http://dx.doi.org/10.1038/nature02590
584 8. Collinet C, Rauzi M, Lenne P, Lecuit T. Local and tissue-scale forces drive oriented
585 junction growth during tissue extension. Nat Cell Biol. 2015;17: 1247–1258.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

586 doi:10.1038/ncb3226

587 9. Butler LC, Blanchard GB, Kabla AJ, Lawrence NJ, Welchman DP, Mahadevan L, et al.
588 Cell shape changes indicate a role for extrinsic tensile forces in Drosophila germ-band
589 extension. Nat Cell Biol. Nature Publishing Group; 2009;11: 859–64.
590 doi:10.1038/ncb1894

591 10. Firmino AAP, Fonseca FC de A, de Macedo LLP, Coelho RR, Antonino de Souza Jr JD,
592 Togawa RC, et al. Transcriptome Analysis in Cotton Boll Weevil (Anthonomus grandis)
593 and RNA Interference in Insect Pests. PLoS One. 2013;8: e85079.
594 doi:10.1371/journal.pone.0085079

595 11. da Silva SM, Vincent J-P. Oriented cell divisions in the extending germband of
596 Drosophila Development. 2007;134: 3049 LP-3054. Available:
597 http://dev.biologists.org/content/134/17/3049.abstract

598 12. Liu PZ, Kaufman TC. Short and long germ segmentation: unanswered questions in the
599 evolution of a developmental mode. Evol Dev. 2005;7: 629–46. doi:10.1111/j.1525-
600 142X.2005.05066.x

601 13. Sarrazin AF, Peel AD, Averof M. A segmentation clock with two-segment periodicity in
602 insects. Science. 2012;336: 338–41. doi:10.1126/science.1218256

603 14. Benton MA, Akam M, Pavlopoulos A. Cell and tissue dynamics during Tribolium
604 embryogenesis revealed by versatile fluorescence labeling approaches. Development.
605 2013;140: 3210–3220. doi:10.1242/dev.096271

606 15. Nakamoto A, Hester SD, Constantinou SJ, Blaine WG, Tewksbury AB, Matei MT, et al.
607 Changing cell behaviours during beetle embryogenesis correlates with slowing of
608 segmentation. Nat Commun. Nature Publishing Group; 2015;6: 6635.
609 doi:10.1038/ncomms7635

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

610 16. Paré AC, Vichas A, Fincher CT, Mirman Z, Farrell DL, Mainieri A, et al. A positional Toll
611 receptor code directs convergent extension in Drosophila. Nature. 2014;515: 523–
612 527. doi:10.1038/nature13953
613 17. Benton MA, Pechmann M, Frey N, Stappert D, Conrads KHKH, Chen Y-TY-T, et al. Toll
614 Genes Have an Ancestral Role in Axis Elongation. Curr Biol. Elsevier Ltd; 2016;26:
615 1609–1615. doi:10.1016/j.cub.2016.04.055
616 18. Campos-Ortega J a, Hartenstein V. The embryonic development of Drosophila
617 melanogaster. 2nd ed. Springer-Verlag Berlin Heidelberg; 1997.
618 19. Schönauer A, Paese CLB, Hilbrant M, Leite DJ, Schwager EE, Feitosa NM, et al. The Wnt
619 and Delta-Notch signalling pathways interact to direct pair-rule gene expression via
620 caudal during segment addition in the spider Parasteatoda tepidariorum.
621 Development. 2016; 2455–2463. doi:10.1242/dev.131656
622 20. Janssen R. Gene expression suggests double-segmental and single-segmental
623 patterning mechanisms during posterior segment addition in the beetle Tribolium
624 castaneum. Int J Dev Biol. 2014;58. doi:10.1387/ijdb.140058rj
625 21. Clark E. Dynamic patterning by the Drosophila pair-rule network reconciles long-germ
626 and short-germ segmentation. Desplan C, editor. PLOS Biol. 2017;15: e2002439.
627 doi:10.1371/journal.pbio.2002439
628 22. van der Zee M, Berns N, Roth S. Distinct Functions of the Tribolium zerknullt Genes in
629 Serosa Specification and Dorsal Closure. Curr Biol. 2005;15: 624–636.
630 doi:10.1016/j.cub.2005.02.057
631 23. Falciani F, Hausdorf B, Schröder R, Akam M, Tautz D, Denell R, et al. Class 3 Hox genes
632 in insects and the origin of zen. Proc Natl Acad Sci USA. 1996;93: 8479–8484.
633 24. Handel K, Grünfelder CG, Roth S, Sander K. Tribolium embryogenesis: a SEM study of

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

634 cell shapes and movements from blastoderm to serosal closure. Dev Genes. 2000;

635 25. Nunes da Fonseca R, von Levetzow C, Kalscheuer P, Basal A, van der Zee M, Roth S.
636 Self-Regulatory Circuits in Dorsoventral Axis Formation of the Short-Germ Beetle
637 Tribolium castaneum. Dev Cell. 2008;14: 605–615.
638 doi:https://doi.org/10.1016/j.devcel.2008.02.011

639 26. Schmidt-Ott U, Kwan CW. Morphogenetic functions of extraembryonic membranes in
640 insects. Curr Opin Insect Sci. Elsevier Inc; 2016;13: 86–92.
641 doi:10.1016/j.cois.2016.01.009

642 27. Panfilio K a. Extraembryonic development in insects and the acrobatics of
643 blastokinesis. Dev Biol. 2008;313: 471–91. doi:10.1016/j.ydbio.2007.11.004

644 28. Benton MA, Pavlopoulos A. Tribolium embryo morphogenesis: May the force be with
645 you. Bioarchitecture. 2014;4: 16–21. doi:10.4161/bioa.27815

646 29. Schmidt-Ott U. The amnioserosa is an apomorphic character of cyclorrhaphan flies.
647 Dev Genes Evol. 2000;210: 373–376. doi:10.1007/s004270000068

648 30. Rafiqi AM, Lemke S, Ferguson S, Stauber M, Schmidt-Ott U. Evolutionary origin of the
649 amnioserosa in cyclorrhaphan flies correlates with spatial and temporal expression
650 changes of zen. Proc Natl Acad Sci U S A. 2008;105: 234–9.
651 doi:10.1073/pnas.0709145105

652 31. Rafiqi AM, Park C, Kwan CW, Lemke S, Schmidt-ott U. BMP-dependent serosa and
653 amnion specification in the scuttle fly Megaselia abdita. 2012;3382: 3373–3382.
654 doi:10.1242/dev.083873

655 32. Kwan CW, Gavin-Smyth J, Ferguson EL, Schmidt-Ott U. Functional evolution of a
656 morphogenetic gradient. Bronner M, editor. Elife. eLife Sciences Publications, Ltd;
657 2016;5: e20894. doi:10.7554/eLife.20894

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

658 33. Caroti F, González Avalos E, González Avalos P, Kromm D, Noeske V, Wosch M, et al. In
659 toto live imaging in scuttle fly Megaselia abdita reveals transitions towards a novel
660 extraembryonic architecture. bioRxiv. 2018; Available:
661 http://biorxiv.org/content/early/2018/01/15/236364.abstract
662 34. Jacobs CGC, Spaink HP, van der Zee M. The extraembryonic serosa is a frontier
663 epithelium providing the insect egg with a full-range innate immune response. Elife.
664 2014;3: 1–21. doi:10.7554/eLife.04111
665 35. Jacobs CGC, Van Der Zee M. Immune competence in insect eggs depends on the
666 extraembryonic serosa. Dev Comp Immunol. 2013;41. doi:10.1016/j.dci.2013.05.017
667 36. Lynch JA, Roth S. The evolution of dorsal-ventral patterning mechanisms in insects.
668 Genes Dev. 2011;25: 107–118. doi:10.1101/gad.2010711
669 37. Horn T, Panfilio KA. Novel functions for Dorsocross in epithelial morphogenesis in the
670 beetle Tribolium castaneum. Development. 2016;143: 3002–3011.
671 doi:10.1242/dev.133280
672 38. Anderson DT. The Development of Holometabolous Insects. In: Counce SJ, Waddinton
673 CH, editors. Developmental systems Insects, Vol 1. New York: Academic Press; 1972.
674 pp. 165–242.
675 39. Anderson DT. The Development of Hemimetabolous Insects. In: Counce SJ,
676 Waddington CH, editors. Developmental systems Insects, Vol 1. New York: Academic
677 Press; 1972. pp. 95–163.
678 40. Aranda M, Marques-Souza H, Bayer T, Tautz D. The role of the segmentation gene
679 hairy in Tribolium. Dev Genes Evol. 2008;218: 465–77. doi:10.1007/s00427-008-0240-
680 1
681 41. Benton MA. Fluorescent live imaging of differentially labeled Tribolium embryos

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

682 [Internet]. Database: figshare [Internet]. 2018. Available:
683 https://figshare.com/authors/Matthew_Benton/4693354
684 42. Tomancak P, Berman BP, Beaton A, Weiszmann R, Kwan E, Hartenstein V, et al. Global
685 analysis of patterns of gene expression during Drosophila embryogenesis. Genome
686 Biol. England; 2007;8: R145. doi:10.1186/gb-2007-8-7-r145
687 43. Hammonds AS, Bristow CA, Fisher WW, Weiszmann R, Wu S, Hartenstein V, et al.
688 Spatial expression of transcription factors in Drosophila embryonic organ
689 development. Genome Biol. England; 2013;14: R140. doi:10.1186/gb-2013-14-12-r140
690 44. Tomancak P, Beaton A, Weiszmann R, Kwan E, Shu S, Lewis SE, et al. Systematic
691 determination of patterns of gene expression during Drosophila embryogenesis.
692 Genome Biol. England; 2002;3: RESEARCH0088.
693 45. Sam S, Leise W, Hoshizaki DK. The serpent gene is necessary for progression through
694 the early stages of fat-body development. Mech Dev. 1996;60: 197–205. Available:
695 http://www.ncbi.nlm.nih.gov/pubmed/9025072
696 46. Sachs L, Chen YT, Drechsler A, Lynch JA, Panfilio KA, Lässig M, et al. Dynamic BMP
697 signaling polarized by Toll patterns the dorsoventral axis in a hemimetabolous insect.
698 Elife. 2015;4. doi:10.7554/eLife.05502
699 47. Handel K, Basal A, Fan X, Roth S. Tribolium castaneum twist: gastrulation and
700 mesoderm formation in a short-germ beetle. Dev Genes Evol. 2005;215: 13–31.
701 48. van der Zee M, Stockhammer O, von Levetzow C, Nunes da Fonseca R, Roth S.
702 Sog/Chordin is required for ventral-to-dorsal Dpp/BMP transport and head formation
703 in a short germ insect. Proc Natl Acad Sci U S A. 2006;103: 16307–12.
704 doi:10.1073/pnas.0605154103
705 49. Stappert D, Frey N, von Levetzow C, Roth S. Genome-wide identification of Tribolium

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

706 dorsoventral patterning genes. Development. 2016;143: 2443–2454.
707 doi:10.1242/dev.130641
708 50. van der Zee M, Benton MA, Vazquez-Faci T, Lamers GEM, Jacobs CGC, Rabouille C.
709 Innexin7a forms junctions that stabilize the basal membrane during cellularization of
710 the blastoderm in Tribolium castaneum. Development. 2015;142: 2173–2183.
711 doi:10.1242/dev.097113
712 51. Rauzi M, Krzic U, Saunders TE, Krajnc M, Ziherl P, Hufnagel L, et al. Embryo-scale tissue
713 mechanics during Drosophila gastrulation movements. Nat Commun. The Author(s);
714 2015;6: 8677. Available: http://dx.doi.org/10.1038/ncomms9677
715 52. Leptin M, Grunewald B. Cell shape changes during gastrulation in Drosophila.
716 Development. 1990;110: 73 LP-84. Available:
717 http://dev.biologists.org/content/110/1/73.abstract
718 53. Roth S. Gastrulation in Other Insects. In: Stern CD, editor. Gastrulation From Cells to
719 Embryo. New York: Cold Spring Harbor Laboratory Press; 2004. pp. 105–121.
720 54. Ewen-Campen B, Jones TEM, Extavour CG. Evidence against a germ plasm in the
721 milkweed bug Oncopeltus fasciatus, a hemimetabolous insect. Biol Open. 2013;2:
722 556–68. doi:10.1242/bio.20134390
723 55. Erezyilmaz DF, Kelstrup HC, Riddiford LM. The nuclear receptor E75A has a novel pair-
724 rule-like function in patterning the milkweed bug, Oncopeltus fasciatus. Dev Biol.
725 Elsevier Inc.; 2009;334: 300–10. doi:10.1016/j.ydbio.2009.06.038
726 56. Liu PZ, Kaufman TC. even-skipped is not a pair-rule gene but has segmental and gap-
727 like functions in Oncopeltus fasciatus, an intermediate germband insect.
728 Development. 2005;132: 2081–92. doi:10.1242/dev.01807
729 57. Gillis WQ, Bowerman B a, Schneider SQ. The evolution of protostome GATA factors:

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

730 molecular phylogenetics, synteny, and intron/exon structure reveal orthologous
731 relationships. BMC Evol Biol. 2008;8: 112. doi:10.1186/1471-2148-8-112
732 58. Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. A variant of yellow
733 fluorescent protein with fast and efficient maturation for cell-biological applications.
734 Nat Biotechnol. Nature Publishing Group; 2002;20: 87. Available:
735 http://dx.doi.org/10.1038/nbt0102-87
736 59. Clark E, Akam M. Odd-paired controls frequency doubling in Drosophila segmentation
737 by altering the pair-rule gene regulatory network. Elife. England; 2016;5.
738 doi:10.7554/eLife.18215
739 60. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an
740 open-source platform for biological-image analysis. Nat Methods. 2012;9: 676–82.
741 doi:10.1038/nmeth.2019
742 61. Meijering E, Dzyubachyk O, Smal I. Methods for cell and particle tracking. [Internet].
743 1st ed. Methods in enzymology. United States: Elsevier Inc.; 2012. doi:10.1016/B978-
744 0-12-391857-4.00009-4

745

746

747 **S1 Fig. Results of photoconversions at 50% egg length.** NLS-tdEos labelled extended
748 germband stage *Tribolium* embryos in which a patch of blastoderm nuclei were
749 photoconverted at 50% egg length (from the posterior pole) at different DV positions. The
750 approximate DV position of the patch and the approximate DV width of the clone (in terms
751 of nuclei number) are shown. The dorsal labelled embryo is shown from both sides to
752 demonstrate the photoconverted nuclei cover the full DV extent of the amnion (arrows).
753 Unconverted protein is shown in magenta, converted protein is shown in cyan. Images are

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

754 maximum intensity projections of one egg hemisphere. All eggs are oriented with the
755 anterior to the left and ventral to the bottom. Scale bars are 100 µm.
756
757 **S2 Fig. Results of photoconversions at 25% egg length.** NLS-tdEos labelled extended
758 germband stage *Tribolium* embryos in which a patch of blastoderm nuclei were
759 photoconverted at 25% egg length (from the posterior pole) at different DV positions. The
760 approximate DV position of the patch and the approximate DV width of the clone (in terms
761 of nuclei number) are shown. Unconverted protein is shown in magenta, converted protein
762 is shown in cyan. Images are maximum intensity projections of one egg hemisphere. All eggs
763 are oriented with the anterior to the left and ventral to the bottom. Scale bars are 100 µm.
764
765 **S3 Fig. Results of photoconversions near the posterior pole.** NLS-tdEos labelled extended
766 germband stage *Tribolium* embryos in which a patch of blastoderm nuclei were
767 photoconverted near the posterior pole at different DV positions. The approximate DV
768 position of the patch and the approximate DV width of the clone (in terms of nuclei number)
769 are shown. The second dorsally labelled embryo is shown at high magnification at two
770 timepoints and with a transverse section (at the position of the dashed green line) to show
771 the movement of tissue from the dorsal epithelium into the hindgut. Unconverted protein is
772 shown in magenta, converted protein is shown in cyan. Images are maximum intensity
773 projections of one egg hemisphere except for the bottom three embryos, which are shown
774 as maximum intensity projects through the germband in order to better show the labelled
775 nuclei. All eggs are oriented with the anterior to the left and ventral to the bottom except
776 for the second timepoint of the second dorsal view, which is shown with the posterior of the
777 germband to the left. Scale bars are 100 µm.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

778

779 **S4 Fig. RNA expression of the *Tribolium* ortholog of the GATA factor *serpent.*** (A-F) whole
780 mount and (G-J) flatmount *Tribolium* embryos from the pre-blastoderm to the retracting
781 germband stage stained for *Tc-srp* mRNA (red) and nuclei (DAPI, blue). (G_1) and (G_2) show
782 the same embryo imaged from both sides. (H_1) and (H_2) show projections from the dorsal
783 epithelium (H_1) and the ventral epithelium (H_2) of the same embryo. *Tc-srp* mRNA is
784 maternally provided (A), and expression is ubiquitous until the late blastoderm stage (B-C)
785 when expression clears from the blastoderm but persists in the yolk nuclei (scattered spots
786 in (D-E)). During embryo condensation, *de novo* expression arises in a patch of blastoderm
787 cells at the anterior medial region (arrowhead in F). This patch of *Tc-srp* expressing cells
788 invaginates as part of the ventral furrow and becomes located beneath the ectoderm
789 (arrowhead in G_1-H_3). This expression domain is likely homologous to the anterior ventral
790 expression domain in *Drosophila* that marks the prohemocytes. During serosa window
791 closure, expression appears in a ring of dorsal epithelium cells (G_1). After serosa window
792 closure, expression persists in the dorsal epithelium (H_1) and (H_3). Unlike *Drosophila*, there is
793 no expression domain at the posterior of the blastoderm (E) or the early germband (G_2).
794 After germband elongation, a *de novo* expression domain appears at the posterior most
795 point of the embryo (I_1). Given the location of this domain at the base of the forming
796 hindgut, this is likely the posterior endoderm primordium. Expression can also be seen in a
797 patch of amnion that has remained attached to the germband (arrow in I_2), but most of the
798 rest of the amnion has been lost. Several other regions of expression can be seen, including
799 in the presumptive fat body (the segmental domains running down the body), in
800 presumptive hemocyte clusters (the two side-by-side domains in the anterior), and in an
801 anterior domain that may mark the anterior endoderm primordium. Expression also persists

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

802 in the yolk nuclei (visible in the remaining yolk fragments at the anterior of the germband in
803 I_1). All embryos are shown with anterior to the left. (E-E'') is oriented with the ventral to the
804 bottom, (F-F'') is oriented as a ventral view. (H_3-H_3'') is an optical sagittal section at
805 approximately the midline of the embryo in (H_2). (A-F) are maximum intensity projections of
806 one egg hemisphere. (G_1-G_2''), (I1-I1'') and (J-J'') are maximum intensity projections of
807 flatmounted germbands. (I_2-I_2'') is a maximum intensity projection of part of the germband
808 to better show the amnion/dorsal epithelium. Scale bars are 50 µm.
809 **S5 Fig. Schematics showing the photoconversion approach to determine the amnion**
810 **fatemap.** A patch of nuclei (of known dimensions) was photoconverted at the blastoderm
811 stage, then the same embryos were examined at the end of germband extension. In
812 embryos where all photoconverted nuclei were located in the amnion and these nuclei
813 spanned the entire DV width of the amnion (as shown here), the number of nuclei initially
814 photoconverted was used to determine the DV width of the blastoderm domain giving rise
815 to the amnion. Note that the precise number and distribution of nuclei shown here were
816 arbitrarily chosen. Blue shows photoconverted nuclei, yellow shows the yolk. The serosa is
817 omitted from the bottom panels.
818
819 **S6 Fig. Extended schematics showing the classic and revised *Tribolium* amnion fatemaps**
820 **and germband models.** Schematics drawn as in Fig 1 to show the classic and revised
821 fatemaps and germband models based on the results of this manuscript. The schematics of
822 the flatmounted germbands are drawn with the focus on the dorsal epithelium. See text for
823 additional details.
824

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

825 **S7 Fig. Tissue specific cell shape changes during *Tribolium* condensation**. Stills from
826 timelapses of two *Tribolium* embryos transiently expressing GAP43YFP to label membranes.
827 The second panel of each timepoint shows optical transverse sections at the position of the
828 dashed line in the related panel. Ventral and lateral ectoderm becomes columnar, while
829 dorsal ectoderm becomes flattened. The non-columnar cells at the bottom of the left hand
830 embryo are likely the presumptive mesoderm. The first frame of the timelapses was defined
831 as timepoint 0. Both embryos are oriented with the anterior to the left and ventral to the
832 bottom. Abbreviations are: Dorsal (Dor), Lateral (Lat), Ventral (Ven) and Ectoderm (Ect).
833 Scale bars are 100 µm.
834
835
836 **S1 Movie.** Confocal timelapse of a *Tribolium* embryo transiently expressing H2B-ven to mark
837 nuclei. A maximum intensity projection of one egg hemisphere is shown. Anterior is to the
838 left, the ventral side of the egg is to the bottom.
839
840 **S2 Movie.** Same timelapse as Supplementary Movie 1, but with nuclei of the dorsal
841 epithelium tracked until they join the ventral epithelium. Nuclei that join the ventral
842 epithelium are labelled magenta, nuclei that become located at the edge of the germband
843 are labelled yellow, nuclei that remain in the dorsal epithelium are labelled cyan. Anterior is
844 to the left, the ventral side of the egg is to the bottom. See Fig 2(A-C) for more details.
845
846 **S3 Movie.** Same timelapse as Supplementary Movie 1, but with a line of nuclei of the dorsal
847 epithelium tracked. Nuclei that remain in the dorsal epithelium are labelled cyan. Anterior is
848 to the left, the ventral side of the egg is to the bottom. See Fig 2(D-F) for more details.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

849

850 **S4 Movie.** Confocal timelapse of a *Tribolium* embryo transiently expressing NLS-tdEos
851 (magenta) with a line of blastoderm cells photoconverted (cyan). The brightness increases
852 approximately halfway through the movie due to a manual increase in laser power at this
853 point. A maximum intensity projection of one egg hemisphere is shown. Anterior is to the
854 left, the ventral side of the egg is to the bottom.

855

856 **S5 Movie.** Confocal timelapse of a *Tribolium* embryo transiently expressing NLS-tdEos
857 (magenta) with a patch of blastoderm cells photoconverted (cyan). (A) shows a maximum
858 intensity projection of one egg hemisphere is shown. (B) shows an optical transverse section.
859 (C) shows an average intensity projection of 10 optical transverse sections. Anterior is to the
860 left, the ventral side of the egg is to the bottom. See Fig 3(A-F) for more details.

861

862 **S6 Movie.** Confocal timelapse of a *Tribolium* embryo expressing NLS-tdEos showing the SAZ
863 during abdominal segment formation. Coloured points mark tracked nuclei. The embryo is
864 oriented based on polarity of the visible region of the germband with posterior to the right
865 and lateral to the top. Panels show maximum intensity projections of 15 µm to specifically
866 show the germband. See Fig 4 for more details.

867

868 **S7 Movie.** Confocal timelapse of a *Tribolium* embryo transiently expressing NLS-tdEos
869 (magenta) with a patch of blastoderm cells photoconverted (cyan). Towards the end of the
870 timelapse, the cyan nuclei in the dorsal epithelium appear to condense posteriorly to the
871 hindgut. A maximum intensity projection of one egg hemisphere is shown. Anterior is to the
872 left, the ventral side of the egg is to the bottom.

bioRxiv preprint doi: https://doi.org/10.1101/232751; this version posted June 6, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

873

874 **S1 Text. Extended discussion of topics from Discussion section 'A revised understanding of**

875 **the short germ embryo'.**

876 **S2 Text. Extended discussions of topics from Discussion section 'Cellular and molecular**

877 **causes of tissue flow'.**

878 **S3 Text. Extended discussions of topics from Discussion section 'Reconciling long and short**

879 **germ development'.**

880

Drosophila

Tribolium

Amnioserosa

Posterior gut

Serosa

Amnion

?

Cephalic furrow

?

?

?

?

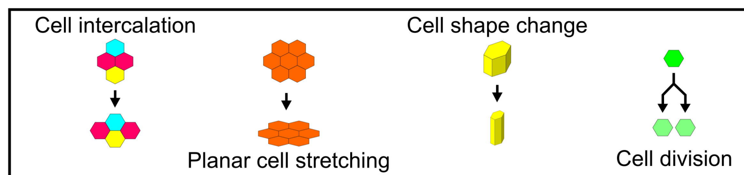
Surface view

Sagittal section

Surface view

Sagittal section

Flatmount



A 0 min A'

B 135 min B'

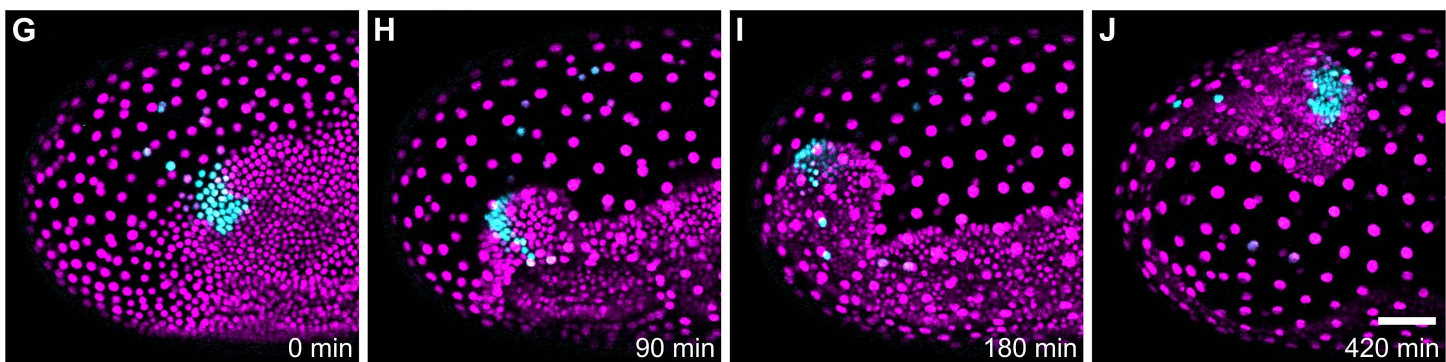
C 670 min C'

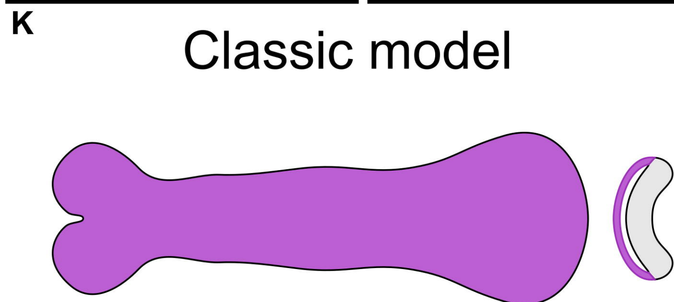
D 0 min

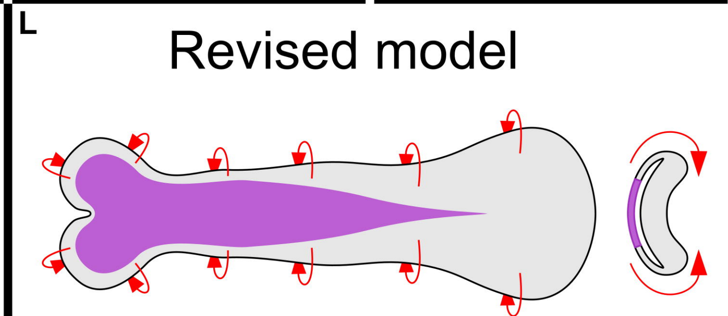
E 135 min

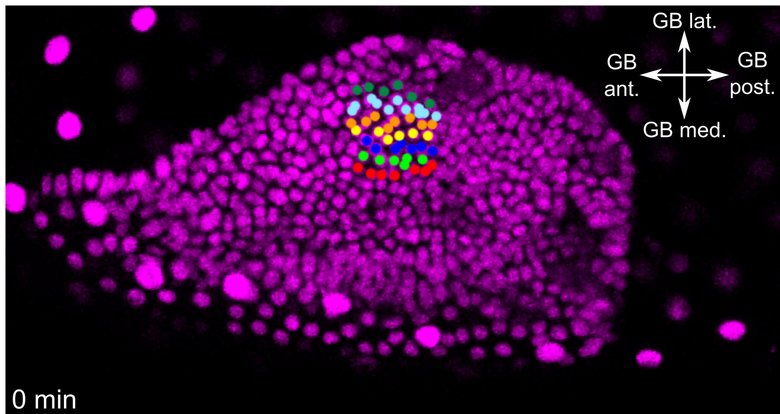
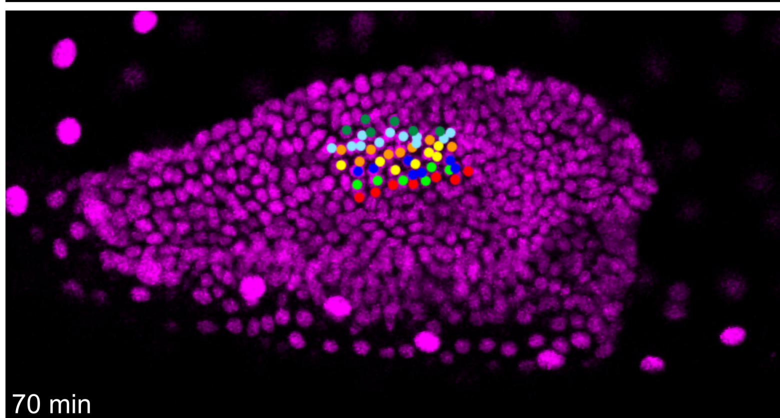
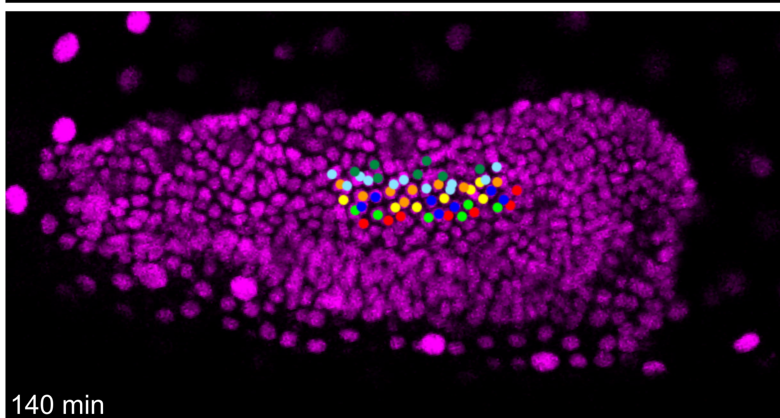
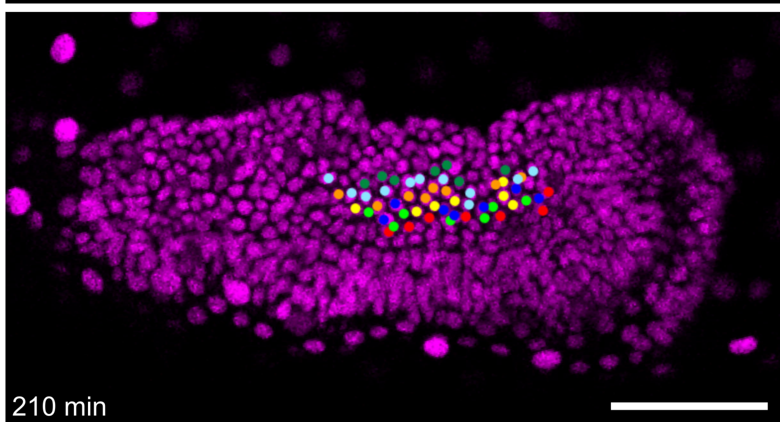
F 670 min

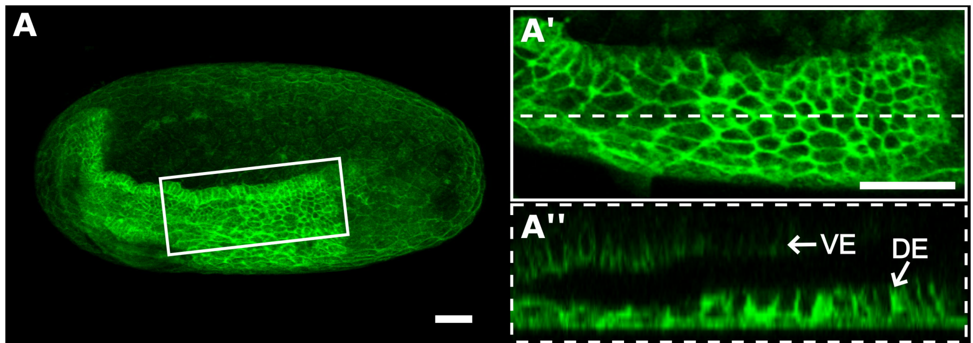
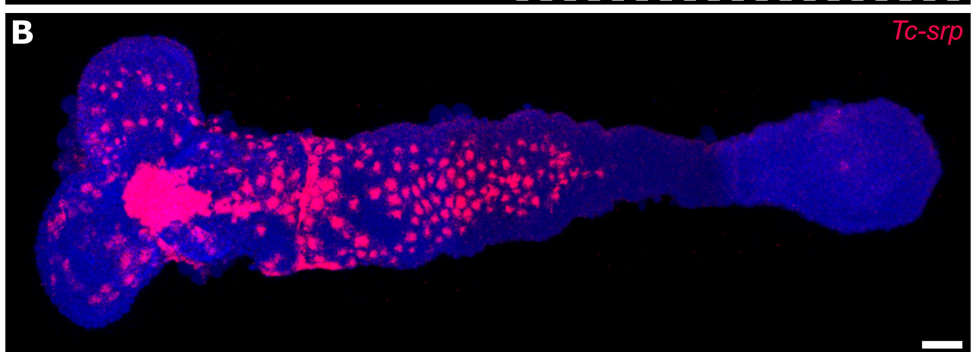
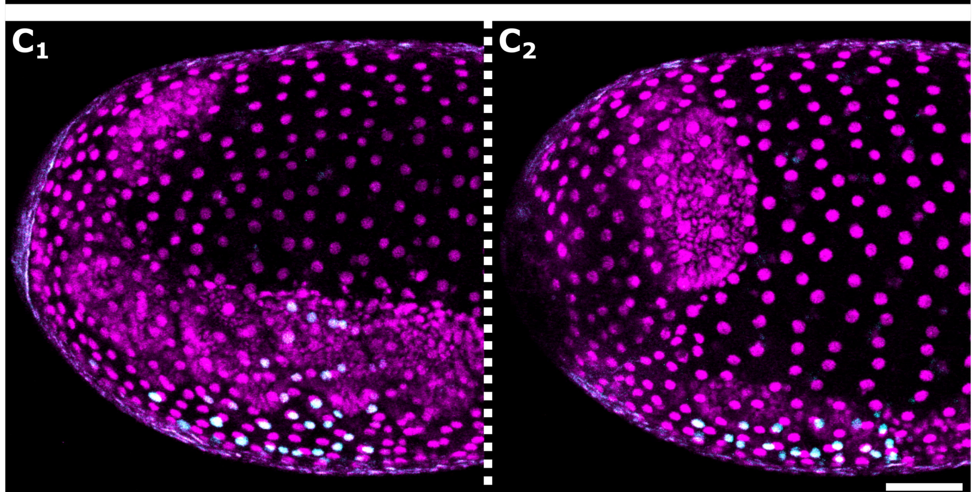
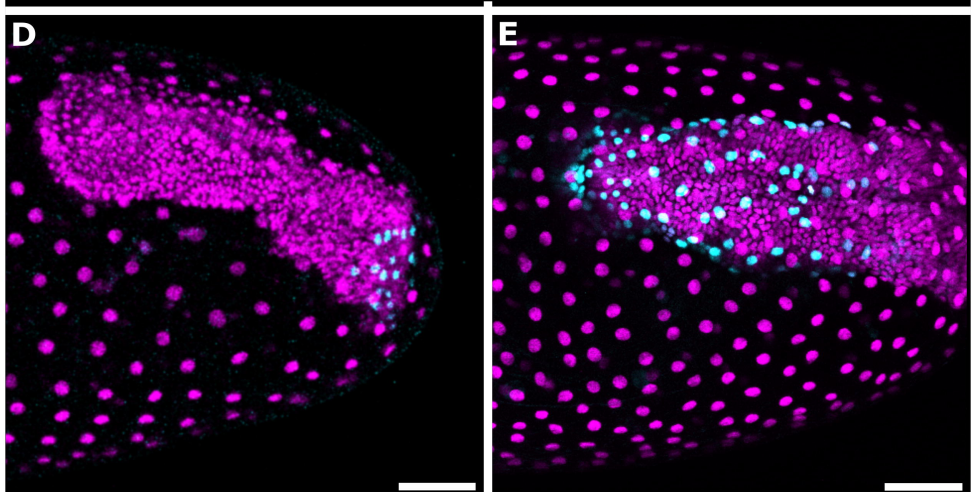
A 670 min A'
B 730 min B'
C 790 min C'
D 850 min D'
E 910 min E'
F 970 min F'

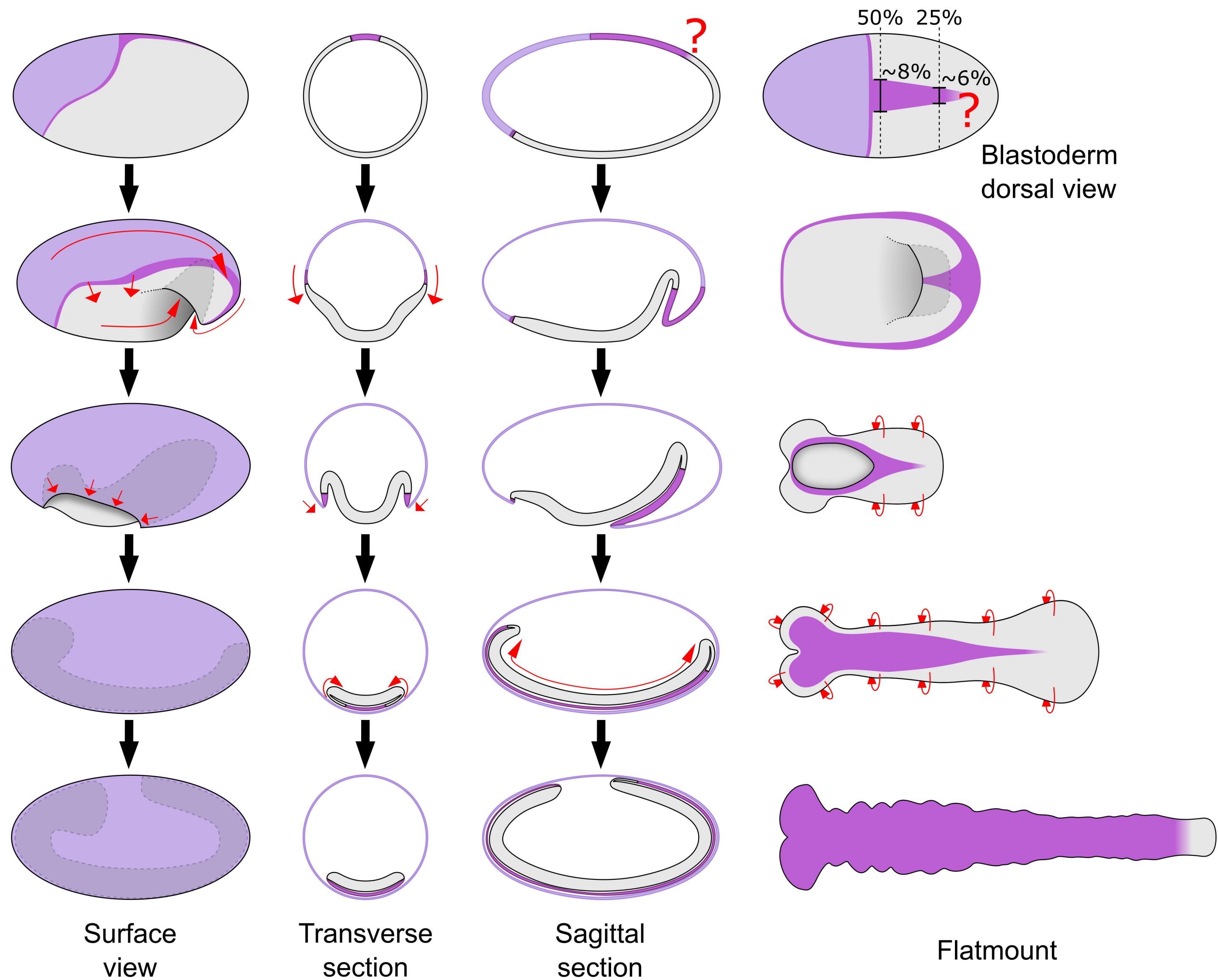







GB lat.
GB ant.
GB post.
GB med.
0 min

70 min

140 min

210 min


A
A'
A''
VE
DE

B
Tc-srp

C1
C2

D
E


50%
25%
~8%
~6%
?
?
Blastoderm
dorsal view
Surface
view
Transverse
section
Sagittal
section
Flatmount