Heterochronic shifts in germband movements contribute to the rapid embryonic development of the coffin fly *Megaselia scalaris*

Karl R. Wotton

EMBL/CRG Research Unit in Systems Biology, Centre for Genomic Regulation (CRG), and Universitat Pompeu Fabra (UPF), Dr. Aiguader 88, 08003 Barcelona, Spain

Abstract

The coffin fly, *Megaselia scalaris,* is a species of medical and forensic importance and is increasingly being used for the study of genetics. Postmortem interval can be estimated based on the life stage of *M. scalaris* recovered from corpses, therefore many studies have addressed the duration of each life stage. These studies demonstrate that embryogenesis completes significantly faster in *M. scalaris* than in the congener *Megaselia abdita* and faster even than the 24 hours needed for *Drosophila melanogaster* embryogenesis. However, until now it has been unclear if this increased speed is achieved by reducing developmental time across all embryonic stages or by the acceleration of individual stages and processes. Furthermore, the large difference in developmental time between the *Megaselia* species suggests that the staging scheme developed for *M. abdita* will not be directly applicable to *M. scalaris*. Here I use time-lapse imaging to create a staging scheme for *M. scalaris* embryogenesis. Comparison of stages between *D. melanogaster* and both *Megaselia* species reveals heterochronic shifts, increased coordination of morphogenetic movements and compression of individual stages all contribute to the rapid development of *M. scalaris*.

Abbreviations

TED: percentage of total embryonic development
AEL: time after egg laying

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1. Introduction

Megaselia scalaris (Loew, 1866) is a fly in the family Phoridae, also referred to as the hump-backed or the scuttle flies. Its ability to reach buried carrion has lead to its use in forensics and is reflected in another alternative name, the coffin fly. This name has the benefit of distinguishing it from *Megaselia abdita*, also referred to as the scuttle fly. In addition to its use in estimating postmortem interval in forsensics, *M. scalaris* is of medical important due to the ability of its larvae to invade living tissues causing myiasis (see Disney, 2008; Varney and Noor, 2010 for reviews of *M. scalaris* biology).

The genus *Megaselia* forms one of the largest groups among the phorids (one of the earliest branching lineage in the radiation of the cyclorrhaphan flies; see Jiménez-Guri et al., 2013; Wiegmann et al., 2011) and both *M. abdita* and *M. scalaris* have emerged as useful models for genetics. In the case of *M. scalaris,* this has mostly focused on sex determination (Sievert et al., 1997; Traut, 2010, 1994; Willhoeft and Traut, 1995, 1990), while the focus with *M. abdita* has been on embryonic development (Lemke et al., 2008; Rafiqi et al., 2008; Stauber et al., 2008, 2000, 1999; Wotton et al., 2014).

Genomic resources exist for both species, with a transcriptome available for *M. abdita* (Jiménez-Guri et al., 2013; http://diptex.crg.es/) and a genome available for *M. scalaris* (Rasmussen and Noor, 2009; http://metazoa.ensembl.org/Megaselia_scalaris). Additionally, techniques developed for *M. abdita*, including in situ hybridisation (Wotton et al., 2014, 2012) and gene knock-down (Rafiqi et al., 2013a, 2013b, 2013c) are likely to be directly applicable to *M. scalaris*.

Numerous publications have addressed the duration of *M. scalaris* development at different temperatures (see Table 1 in Disney, 2008) with egg to adult taking 17.2-

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18.4 days at 25°C, of which around 17 hours were needed for embryonic development (Prawirodisastro and Benjamin, 1979). Embryonic development in *M. abdita* lasts significantly longer than this, at least 24 hours (as in *D. Melanogaster*) and up to around 27.5 hours under oil (Rafiqi et al., 2013a; Wotton et al., 2014). However, no systematic characterisation and analysis of *M. scalaris* embryonic development has been carried out. To investigate whether this reduced developmental time is the result of a global decrease in developmental time at each embryonic stage or a reduction in individual stages or events, I carried out a detailed description of embryonic development. Stages were homologised to *D. melanogaster* and *M. abdita* development. Comparison of stages across all 3 species reveals a heterochronic shift in the stage in which the germband reaches its maximum extent that leads to the earlier completion of germband retraction in *M. scalaris*. Additionally, increased coordination of the morphogenetic movements of head involution and dorsal closure, as compared to *M. abdita*, combine with the altered germband dynamics to result in a compression of the time needed to complete stages 9 through 13 (from transient segmentation to the beginning of head involution). Additional heterochronic shifts of serosal rupture and contraction to an earlier stage, as compared to *M. abdita,* mean that *M. Scalaris* is enclosed in extraembryonic tissue for substantially less time than is *M. abdita.* A heterochronic shift is also found in the timing at which the ventral nervous system begins to shorten, from stage 16 in *D. melanogaster* and stage 15 in *M. abdita* to stage 14 in *M. scalaris*. Finally, both *Megaselia* species show a reduction in the time needed to complete stage 16 (from the appearance of intersegmental grooves at mid-dorsal levels until the dorsal ridge overgrows clypeolabrum).

2. Materials and Methods

2.1 Fly culture and embryo collection

M. scalaris embryos were collected after 5–10 min laying time, and dechorionated for 1 min 20 sec in 25% bleach. To image the embryos I brushed the dechorionated embryos onto a microscopy slide and covered them with a drop of 10S Voltalef oil ensuring that the embryos did not dry out.

2.2 Time-lapse imaging

Slides were placed on a temperature-controlled platform at 25^{o}C, and embryos were imaged with a Leica DM6000B upright compound microscope using a 20x objective, and time intervals between image acquisitions of every 1 min. Specifications of embryo orientation for each time-lapse are provided in Supporting File S1. Movies were processed using ImageJ (http://rsbweb.nih.gov/ij).

3. Results & Discussion

3.1 Embryonic staging scheme for *M. scalaris* development

Embryos were collected shortly after egg laying, dechorinated and placed on a microscope slide under Voltalef oil. Live imaging with differential interference contrast (DIC) was used to produce a series of movies covering all stages of embryonic development (see Supporting Movie S1). At 25^{o}C and under voltalef oil embryogenesis lasts approximately 22 hours (hrs) from oviposition until hatching, 2 hours shorter that in *D. melanogaster* and approximately 5 hrs 30 min shorter than in the congener *M. abdita*.

Development can be divided into 17 stages roughly corresponding to Bownes' stages in *D. melanogaster* and *M. abdita* (Campos-Ortega and Hartenstein, 1997; Wotton et al., 2014). Each stage can be distinguished by distinct morphological markers, as shown in Figure 1 (also see Supporting Movie S1). The similarity between

D. melanogaster, M. abdita and *M. scalaris* development allows a direct comparison between developmental stages, as discussed below and shown in Table 1 and Figure 2 (see section 3.2).

In this section, I provide an overview over all stages of development and provide a comparison to the well characterised embryology of *D. melanogaster* (Campos-Ortega and Hartenstein, 1997). All times are displayed as hrs:min unless otherwise indicated. Raw data for each event including the number of embryos examined *(n)* and standard deviations (SDs) are supplied in Supporting File S1. To assist identification of stages under different conditions (i.e. not under oil), and at different temperatures, a percentage of total embryonic development (TED) is supplied.

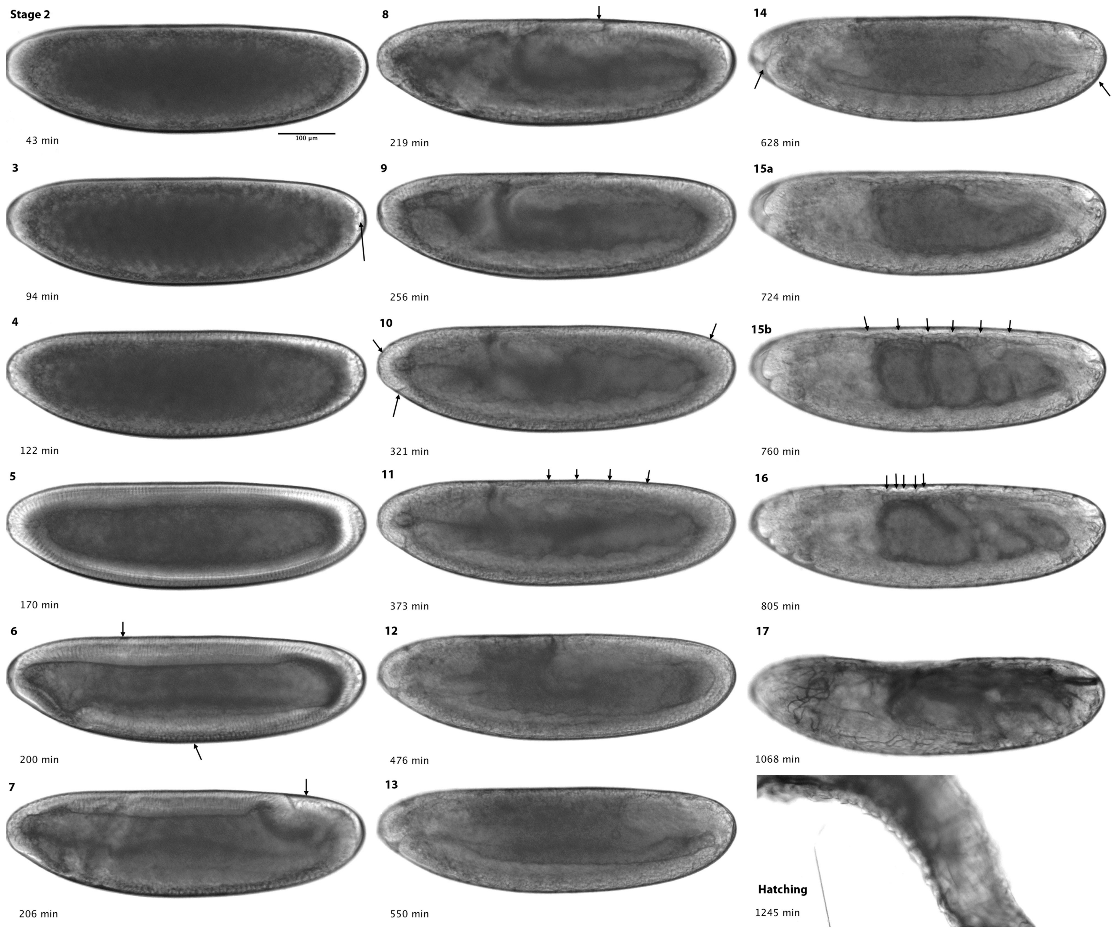

Figure 1. Embryonic staging and developmental events in *M. scalaris.* Embryos are shown as lateral views: anterior is to the left, dorsal is up. Stage numbers

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(roughly corresponding to Bownes' stages in *D. melanogaster*) are shown at the top left, and time after egg laying (AEL) in minutes in the bottom left corner of each panel. Black arrows indicate morphological landmarks. See main text for a detailed description, and Figure 2 and Table 1 for comparative timing of stages with reference to *D. melanogaster* and *M. abdita*.

Stage 1: 0:00–0:20 (duration: 0:20, 1.5% TED). This stage begins at egg laying, and lasts until the end of the first two cleavage divisions, at the beginning of cleavage cycle 3 (C3). The number of cleavage cycles appears to be conserved within the Diptera with *D. melanogaster*, *M. abdita* and *C. albipunctata* all undergoing 14 cleavage cycles before gastrulation (Foe and Alberts, 1983; Jimenez-Guri et al., 2014), I therefore base the timings for *M. scalaris* development on this observation. Since all cleavage cycles up to C12 are of a very similar duration (approximately 10 min), we infer stage 1 to last for at least 20 min. All 'times after egg laying (AEL)' below include a correction based on this estimate (see Supporting File S1 for raw timing data, and time adjustment values). In *D. melanogaster*, stage 1 occurs over a 25 min period (1.4% TED) (Campos-Ortega and Hartenstein, 1997 and references to *D. melanogaster* development hereafter unless stated).

Stage 2: 0:20–1:28 (duration: 1:08, 5.2% TED). Cleavage cycles C3 to C8 take place. During this time, an empty space appears between the vitelline membrane and the egg cytoplasm at the anterior and posterior poles. In *D. melanogaster*, stage 2 occurs from 0:25–1:05 and takes 0:40 (3% TED).

Stage 3: 1:28–1:38 (duration: 0:10, 0.8% TED). Stage 3 includes cleavage cycle C9 and the beginning of C10. At this stage, nuclei divide and migrate outwards, and the pole buds form (Figure 1, stage 3, black arrow). Stage 3 ends with the arrival of nuclei at the periphery of the embryo. In *D. melanogaster*, this stage occurs from

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1:05–1:20 and lasts for 0:15 (1% TED). During this stage, the empty space at the posterior of the embryo disappears in both species. The duration of blastoderm cleavage cycle 10 for *D. melanogaster*, *M. abdita* and *M. scalaris* is around 9, 13 and 13 min respectively.

Stage 4: 1:38–2:39 (duration: 0:61, 4.7% TED). At the onset of this stage, the nuclei have reached the periphery and form the syncytial blastoderm, cleavage cycles 11 to 13 take place. Stage 4 terminates at the beginning of cleavage cycle C14. In *D. melanogaster*, the syncytial blastoderm stage occurs from 1:20–2:10 and lasts for 0:50 (3.5% TED). The duration of blastoderm cleavage cycles 11-13 for *D. melanogaster*, are around 10, 12 and 21 mins, for *M. abdita*: 11, 14 and 23 mins, and for *M. scalaris*: 12, 12 and 20 mins respectively.

Stage 5: 2:39–3:19 (duration: 0:40, 3% TED). Similar to previous blastoderm cycles, cellular membranes begin to form at cleavage cycle C14, but these now progressively grow to engulf the elongating blastoderm nuclei forming the cellular blastoderm. Nuclear morphology changes from circular to elongated. Stage 5 ends just before the onset of gastrulation, and is marked by the wavy appearance of the ventral blastoderm cells (seen as uneven apical and basal surfaces), and the slight dorsal movement of the pole cells. In *D. melanogaster*, this stage occurs from 2:10–2:50 and lasts for 0:40 (3% TED).

Stage 6: 3:19–3:27 (duration: 0:08, 0.6% TED). Early gastrulation events occur: the ventral and cephalic furrows form (Figure 1, stage 6, black arrows), and the pole cells continue to shift dorsally. Stage 6 ends when the cell plate carrying the pole cells reaches a horizontal position. In *D. melanogaster*, this stage occurs from 2:50–3:00 and lasts for 0:10 (1% TED).

Stage 7: 3:27–3:32 (duration: 0:05, 0.4% TED). This stage begins with the pole cell plate in a horizontal position (parallel to the A–P axis; Figure 1, stage 7, black arrow).

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The plate continues to tilt, forming a pocket (the amnioproctodeal invagination). The beginning of cephalad (headwards) movement of this invagination marks the end of stage 7. The dorsal folds and amnioproctodeal invagination are less conspicuous in both *M. scalaris* and *M. abdita* movies. In *D. melanogaster*, this stage occurs from 3:00–3:10 and lasts for 0:10 (1% TED).

Stage 8: 3:32–4:39 (duration: 1:07, 5.1% TED). This stage starts with the cephalad movement of the amnioproctodeal invagination, marking the onset of the rapid phase of germband extension. The germband reaches approximately 40% A–P position (0% A–P position is at the anterior pole), and the amnioserosal lip forms (Figure 1, stage 8, black arrow). Originating from this lip, the serosa migrates to eventually engulf the entire embryo at stage 11 as is seen in *M. abdita.* Interestingly this stage lasts longer in both *Megaselia* species than in *D. melanogaster*. Stage 8 ends with the transient appearance of mesodermal segmentation. In *D. melanogaster*, this stage occurs from 3:10–3:40 and lasts for 0:30 (2% TED). During this time, the germband reaches beyond 40% A–P position. On the other hand, no serosal migration occurs in *D. melanogaster* since extraembryonic tissues are reduced to a dorsal amnioserosa that does not evaginate or migrate.

Stage 9: 4:39–5:07 (duration: 0:28, 2.1% TED). The germband continues to extend but at a slower rate (slow phase of germband extension), and the serosa continues to migrate ventrally. Also at this stage, and unlike in *D. melanogaster* and *M. abdita* where it occurs at stage 10, the germband reaches its maximum extent, around 30% A–P position. Stage 9 ends with the formation of the stomodeal invagination (seen more clearly in Figure 1, stage 10, ventral-anterior black arrow). In *D. melanogaster*, this stage occurs from 3:40–4:20 and lasts for 0:40 (3% TED).

Stage 10: 5:07–6:05 (duration: 0:58, 4.4% TED) During this stage, the stomodeum continues to form (Figure 1, stage 10, ventral-anterior black arrow). Stage 10 ends with the appearance of parasegmental furrows (seen more clearly in Figure 1, stage 11, dorsal black arrows). During this time, the serosa continues to migrate (Figure 1, stage 10, dorso-lateral black arrows). In *D. melanogaster*, this stage occurs from 4:20–5:20 and lasts for 1:00 (4% TED), during which the germband reaches its maximum extent at 25% A–P position.

Stage 11: 6:05–6:48 (duration: 0:43, 3.3% TED). Stage 11 begins with the appearance of parasegmental furrows (Figure 1, stage 11, dorsal black arrows), and ends with the beginning of germband retraction. During this time the serosa fuses forming a complete extraembryonic layer around the embryo. The serosa remains intact for around 4.5 hrs before finally breaking at stage 14. In contrast, this extra embryonic layer persists for around 7 hrs in *M. abdita* and consequently does not break until stage 15. In *D. melanogaster*, this stage occurs from 5:20–7:20 and lasts for 2:00 (8% TED).

Stage 12: 6:48–8:54 (duration: 2:06, 9.6% TED). During this stage, the germband retracts. Stage 12 ends with the completion of this process. In *D. melanogaster*, this stage occurs from 7:20–9:20 and lasts for 2:00 (8% TED).

Stage 13: 8:54–9:18 (duration: 0:24, 1.8% TED). Stage 13 lasts from the completion of germband retraction until the onset of head involution. During this time, the dorsal opening of the embryo remains covered by the amnion, and the serosa envelopes the entire embryo. Dorsal closure starts at the same time as the lengthening of the gut. In *D. melanogaster*, this stage occurs from 9:20–10:20 and lasts for 1:00 (4% TED), during which the dorsal egg surface remains open and the dorsal hole is covered by the amnioserosa.

Stage 14: 9:18–11:08 (duration: 1:50, 8.4% TED). Stage 14 starts at the beginning of head involution, and ends with closure of the midgut. During this time, the serosa ruptures (10:40) at a ventro-posterior position, slightly more pole-ward than in *M.*

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abdita and one stage earlier (Figure 1, stage 14, black arrow at posterior pole). During its retraction, the serosa first rounds the posterior pole before rounding the anterior pole, to be contracted into the dorsal hole 40 min after snapping (retraction also takes 40 min in *M. abdita*). Additionally during this stage, the ventral nerve cord (VNC) starts to shorten. In contrast, this occurs during stage 15 in *M. abdita* and at stage 16 in *D. melanogaster*. The head continues to involute beyond the end of this stage (Figure 1, stage 14, black arrow at anterior pole), and this process completes only by the time the serosa ruptures at stage 15. In *D. melanogaster*, stage 14 occurs from 10:20–11:20 and lasts for 1:00 (1.4% TED).

Stage 15: 11:08–13:25 (duration: 2:17, 10.4% TED). Stage 15 starts at the closure of the midgut (Figure 1, stage 15a), and covers the completion of dorsal closure and dorsal epidermal segmentation. This stage ends when intersegmental grooves can be distinguished at mid-dorsal levels (Figure 1, stage 15b, black arrows). Dorsal closure completes and dorsal epidermal segmentation is visible. Also during this stage, the gut constricts, and muscle contractions begin. In *D. melanogaster*, this stage occurs from 11:20–13:00 and lasts for 1:40 (7% TED).

Stage 16: 13.25–13:44 (duration: 0:19, 1.5% TED). Stage 16 begins with the appearance of the lateral intersegmental grooves (Figure 1, stage 16, black arrows), and ends when the dorsal ridge has completely overgrown the tip of the clypeolabrum (completion of head involution). The VNC continues to shorten; completion of this movement is not clearly detectable and probably continues into stage 17. In *D. melanogaster*, this stage occurs from 13:00–16:00 and lasts for 3:00 (13% TED).

Stage 17: 13:44–21:53 (duration: 8:09, 37% TED). During this stage, the retraction of the VNC is likely to continue and reach completion. The trachea fill with air at around

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18 hrs AEL. The first instar larva hatches at around 22 hrs AEL. In *D. melanogaster*, this stage occurs from 16:00–24:00 and lasts for 8:00 hrs (33% TED).

3.2 Comparative analysis of embryonic development in *M. scalaris, M. abdita* and *D. melanogaster*

M. scalaris embryonic development completes approximately 2 hrs faster than in *D. melanogaster* and 5.5 hrs faster than in the congener *M. abdita*. A comparative overview of embryonic development between *D. melanogaster, M. abdita* and *M. scalaris* is shown in Figure 2, while stage durations and %TED values are shown in Table 1. Using these resources I next discuss the features of *M. scalaris* that may account for the decreased time needed for embryonic development.

The timing of stages 1 to 7 is remarkably similar in all 3 species. At the start of stage 7 (when the pole cell plate reaches a horizontal position) only 30 min separates *D. melanogaster* (at 3 hrs AEL) from *M. scalaris,* and 45 min from *M. abdita*.

At stage 8 serosal migration begins in both *Megaselia* species but is absent from *D. melanogaster*. This may explain the shorter time needed to complete stage 8 in *D. melanogaster* (30 min), while this stage takes around 40 min longer in *M. scalaris* and 1 hr 10 min longer in *M. abdita*.

At stage 9 the germband of *M. scalaris* reaches its maximum extent, one stage earlier than in the other species (Campos-Ortega and Hartenstein, 1997; Wotton et al., 2014). This represents a large heterochronic shift and consequently the time at which the germband retracts (at stage 12) starts around 1 hr 20 min before it occurs in *M. abdita*. Additionally, germband retraction is completed as quickly in *M. scalaris* as in *D. melanogaster*, around 50 min quicker than in *M. abdita*. These features of germband extension create a relatively short stage 11 (30 min shorter than in *M.*

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abdita and 1 hr 17 min shorter than in *D. melanogaster*) and consequently *M. scalaris* reaches the end of stage 11 before *D. melanogaster*. Stage 12 lasts for around 2 hrs in both *D. melanogaster* and *M. scalaris* but continues for an additional 50 min in *M. abdita*. At stage 13, in *M. scalaris,* dorsal closure and head involution occur simultaneously (in *M. abdita*, the start of head involution occurs approximately 1 hour after dorsal closure begins) and this, together with the altered germband dynamics, forms a shorter stage 13 than in the other species. Therefore *M. scalaris* remains ahead in development until the end of stage 13.

A further heterochronic shift in *M. scalaris* sees the shortening of the VNS beginning at stage 14, a stage earlier than in *M. abdita* (stage 15), and 2 stages earlier than in *D. melanogaster* (stage 16). Additionally, at this stage the serosa ruptures and is contracted into the dorsal hole one stage earlier than in *M. abdita*. The disappearance of the serosa is correlated with a lengthened stage 14 in *M. scalaris* (50 min more than in *D. melanogaster* and 34 min more than in *M. abdita*) and a lengthened stage 15 in *M. abdita*.

Stage 16 is significantly shorter in both *Megaselia* species than in *D. melanogaster* (2 hrs 40 min shorter in *M. scalaris*) and allows *M. scalaris* to begin stage 17 around 2 hrs 16 min before *D. melanogaster,* and around 4 hrs before *M. abdita* starts. Finally, stage 17 ends embryonic development and takes around 8 hrs in both *M. scalaris* and *D. melanogaster* while *M. abdita* requires a further 2 hrs before hatching.

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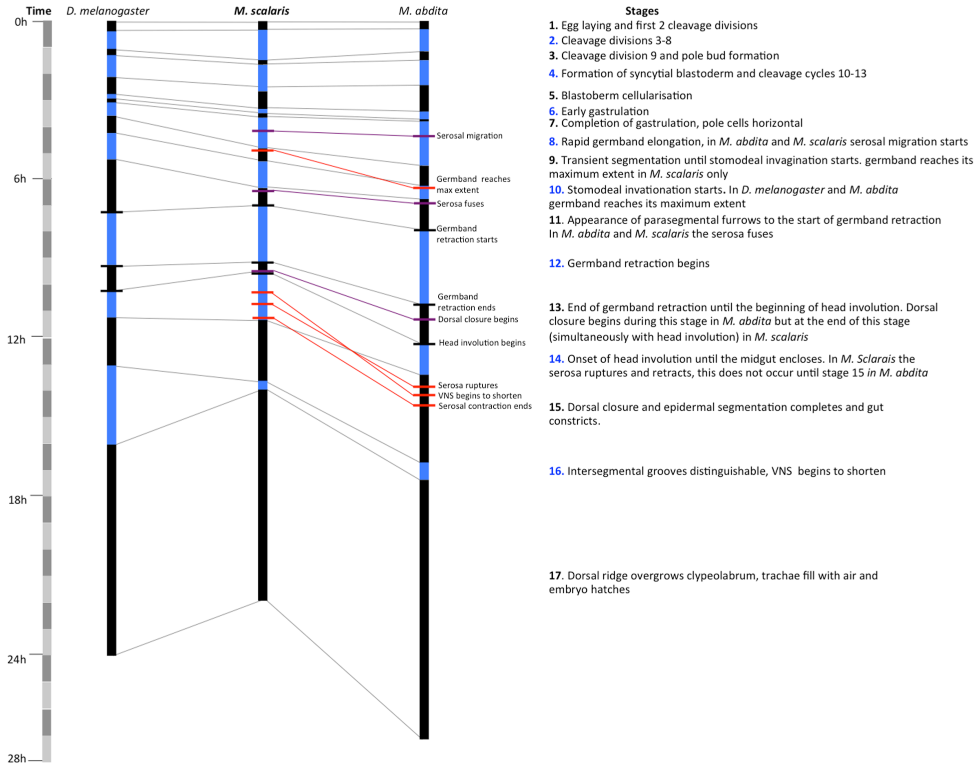

Figure 2. Comparative timing of embryonic developmental stages in *D. melanogaster, M. scalaris* and *M. abdita*. The duration of each stage is shown for each species in alternating black and blue bars. The time scale is divided into blocks of 1 hr on the far left hand side. A brief description of each stage is given on the right. Landmarks of development are indicated to the right of the *M. abdita* time scale and discussed in the text. Red horizontal bars indicate heterochronic shifts between *M. abdita* and *M. scalaris*, purple bars indicate landmark events occurring during a stage, while black bars indicate landmark events occurring at stage boundaries.

Stage	***D. melanogaster*** (Campos-Ortega and Hartenstein, 1997) Stage duration	%TED	***M. abdita*** (Wotton et al., 2014) Stage duration	%TED	***M. scalaris*** Stage duration	%TED
1	0:25	1.4	0:20	1.2	0:20	1.5
2	0:40	3	0:50	3	1:08	5.2
3	0:15	1	0:23	1.4	0:10	0.8
4	0:50	3.5	0:57	3.4	1:01	4.7
5	0:40	3	0:58	4	0:40	3.0
6	0:10	1	0:18	1	0:08	0.6
7	0:10	1	0:05	0.3	0:05	0.4
8	0:30	2	1:38	6	1:07	5.1
9	0:40	3	0:47	3	0:28	2.1
10	1:00	4	0:33	2	0:58	4.4
11	2:00	8	1:13	4	0:43	3.3
12	2:00	8	2:51	10	2:06	9.6
13	1:00	4	1:26	5.2	0:24	1.8
14	1:00	4	1:14	4	1:50	8.4
15	1:40	7	3:20	12	2:17	10.4
16	3:00	13	0:42	3	0:19	1.5
17	8:00	33	9:54	36	8:09	37.2
Total	24:00	100	27:36	100	21:53	100.0

Table 1. Stage durations in hours and minutes along with %TED values for stages 1 to 17 of embryonic development in *D. melanogaster, M. abdita* and *M. scalaris.*

Comparison of embryonic stages and developmental events across the 3 species indicates a number of features that contribute to the decreased developmental time needed to go from egg to larva *in M. scalaris*. First, I find that the germband reaches it maximum extent a stage earlier than in the other species and a consequence of this heterochronic shift is that the germband is able to retract sooner. Additionally, I find tightly coordinated movements of head involution and dorsal closure that are not present in *M. abdita* and result in a compression of the time need to progress through stages 9 to 13. A significantly compressed stage 16 in both *Megselia* species again decreases the developmental time of *M. scalaris* over *D. melanogaster*. Finally, heterochronic shifts in the stage at which the serosa ruptures and contracts results in a reduction in time during which *M. scalaris* is enclosed in extraembryonic membrane.

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4. Conclusions

In this paper I provide a detailed description of the embryonic development of the coffin fly *M. scalaris.* In agreement with previous results, *M. scalaris* embryogenesis is found to require less time to complete than *D. melanogaster,* and significantly less time than the congener *M. abdita*. Comparison of stages across all 3 species reveals heterochronic shifts, increased coordination of morphogenetic movements and compression of individual stages all contribute to this rapid development.

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