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

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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 h 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. 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 that heterochronic shifts, simultaneous morphogenetic movements and compression of individual stages all contribute to the rapid development of *M. scalaris*.

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 forensics, *M. scalaris* is of medical importance 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 lineages 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 (Crombach et al., 2012a, 2012b; Wotton et al., 2014) and gene knock-down (Rafiqi et al., 2011a, 2011b, 2011c) 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 17.2–18.4 days at 25 °C, of which around 17 h were needed for embryonic development (Prawirodisastro and Benjamin, 1979). Embryonic development in *M. abdita* (Sander strain; see Rafiqi et al., 2011d) lasts significantly longer than this, at least 24 h (as in *Drosophila melanogaster*) and up to around 27.5 h under oil (Rafiqi et al., 2011a; 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

Abbreviations: TED, Percentage of total embryonic development; AEL, Time after egg laying.

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stages across all 3 species reveals a number of features that contribute to the rapid embryonic development of *M. scalaris*.

2. Materials and methods

2.1. Fly culture and embryo collection

M. scalaris were sourced from the lab of Pat Simpson (University of Cambridge, UK). Embryos were collected after 5–10 minutes (min) laying time, and dechorionated for 1 min 20 s in 25% bleach (sodium hypochlorite 10%, Sigma–Aldrich; 2.5% active sodium hypochlorite). 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 °C, and embryos were imaged with a Leica DM6000B upright compound microscope using a 20× 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 °C and under Voltalef oil embryogenesis lasts approximately 22 hours (h) from oviposition until hatching, 2 h shorter than in *D. melanogaster* and approximately 5 h 30 min shorter than in the congener *M. abdita*.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.asd.2014.08.001

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 Fig. 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 Fig. 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 h:min unless indicated otherwise. Raw data for each event including the number of embryos examined (*n*) and standard deviations (SDs) are supplied in Supporting File S1. Measurements were taken from 4 individuals; standard deviation is generally below 10 min but increases around the hatching stage (see 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.

Stage 1: 0:00–0:20 (duration: 0:20, 1.5% TED). This stage begins at egg laying and includes the first two cleavage divisions. The number of cleavage cycles appears to be conserved within the Diptera with *D. melanogaster*, *M. abdita* and *Clogmia albipunctata* all undergoing 14 cleavage cycles before gastrulation (Foe and Alberts, 1983; Jiménez-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), I 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–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 to 1:05 and takes 0:40 (3% TED).

Stage 3: 1:28–1:38 (duration: 0:10, 0.8% TED). This stage includes cleavage cycle C9 and the beginning of C10. At this stage, nuclei divide and migrate outwards, and the pole buds form (Fig. 1, stage 3, black arrow). In *D. melanogaster*, this stage occurs from 1:05 to 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. Cleavage divisions during the blastoderm stages can be detected by the disappearance of nuclear envelopes, and their subsequent reappearance at the beginning of the interphase of each cycle. 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. In *D. melanogaster*, the syncytial blastoderm stage occurs from 1:20 to 2:10 and lasts for 0:50 (3.5% TED). The durations of blastoderm cleavage cycles 11–13 for *D. melanogaster*, are around 10, 12 and 21 min, for *M. abdita*: 11, 14 and 23 min, and for *M. scalaris*: 12, 12 and 20 min 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. In *D. melanogaster*, this stage occurs from 2:10 to 2:50 and lasts for 0:40 (3% TED).

Stage 6: 3:19–3:27 (duration: 0:08, 0.6% TED). This stage begins at 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. During this stage early gastrulation events occur: the ventral and cephalic furrows form (Fig. 1, stage 6, black arrows), and the pole cells continue to shift dorsally. In *D. melanogaster*, this stage occurs from 2:50 to 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; Fig. 1, stage 7, black arrow). The plate continues to tilt, forming a pocket (the amnioproctodeal invagination). 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 to 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 (headwards) 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) from a starting point of approximately 80% A–P position, and the amnioserosal lip forms (Fig. 1, stage 8, black arrow). Beginning from this lip, the serosa expands to eventually engulf the entire embryo at stage 11 as is also is seen in *M. abdita.* Interestingly this stage lasts longer in both *Megaselia* species than in *D. melanogaster.* In *D. melanogaster*, this stage occurs from 3:10 to 3:40 and lasts for 0:30 (2% TED). During this time, the germband reaches beyond 40% A–P position. On the