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Embryonic development of the cricket *Gryllus bimaculatus*

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ABSTRACT

Extensive research into *Drosophila melanogaster* embryogenesis has improved our understanding of insect developmental mechanisms. However, *Drosophila* development is thought to be highly divergent from that of the ancestral insect and arthropod in many respects. We therefore need alternative models for arthopod development that are likely to be more representative of basally-branching clades. The cricket *Gryllus bimaculatus* is such a model, and currently has the most sophisticated functional genetic toolkit of any hemimetabolous insect. The existing cricket embryonic staging system is fragmentary, and it is based on morphological landmarks that are not easily visible on a live, undissected egg. To address this problem, here we present a complementary set of "egg stages" that serve as a guide for identifying the developmental progress of a cricket embryo from fertilization to hatching, based solely on the external appearance of the egg. These stages were characterized using a combination of brightfield timelapse microscopy, timed brightfield micrographs, confocal microscopy, and measurements of egg dimensions. These egg stages are particularly useful in experiments that involve egg injection (including RNA interference, targeted genome modification, and transgenesis), as injection can alter the speed of development, even in control treatments. We also use 3D reconstructions of fixed embryo preparations to provide a comprehensive description of the morphogenesis and anatomy of the cricket embryo during embryonic rudiment assembly, germ band formation, elongation, segmentation, and appendage formation. Finally, we aggregate and schematize a variety of published developmental gene expression patterns. This work will facillitate further studies on *G. bimaculatus* development, and serve as a useful point of reference for other studies of wild type and experimentally manipulated insect development in fields from evo–devo to disease vector and pest management.

Introduction

The two-spotted field cricket, *Gryllus bimaculatus*, has long been used as a model for behavior, neurobiology, and physiology. More recently, it has emerged as a model for insect developmental genetics as well. *G. bimaculatus* is a member of the order orthoptera, a group that branches basally with respect to the most extensively studied insect species, including the fruit fly *Drosophila melanogaster*, the flour beetle *Tribolium castaneum*, and the silkmoth *Bombyx mori*. It thus serves as a useful comparator for inferring events in the early evolutionary history of Insecta, and potentially even in the shared ancestor of Arthropoda or Bilateria.

G. bimaculatus development (summarized in Fig. 1) is unlike that of *D. melanogaster* in several respects. First, in *D. melanogaster*, as in many long germ insects, nearly all cells of the blastoderm form the embryo proper. In this case, the embryo fills the entire space within the vitelline membrane and surrounds the yolk throughout embryogenesis, leaving a small proportion of blastoderm cells that are fated to become *D. melanogaster's* single extraembryonic membrane, called the amnioserosa. In contrast, typical of intermediate and short germ insects, only some of the cells of the *G. bimaculatus* blastoderm contribute to an embryonic rudiment that forms on the surface of the yolk. The remaining cells form two large and distinct extraembryonic membranes: the amnion, which lies on the ventral side of the embryo, and the serosa, which surrounds the embryo and yolk mass on the dorsal side (reviewed by Panfilio (2008)). Second, while *D. melanogaster* is a long germ insect that forms all of its body segments essentially simultaneously, *G. bimaculatus* is an intermediate germ insect, in

Abbreviations: A, anterior; A1, first abdominal segment; am, amnion; an, antenna/ae; cc, cercus/cerci; cho, chorion; co, coxopodite; D, dorsal; ect, ectoderm; ee, extra-embryonic region; end, endoderm; fe, femur; L, thoracic leg; la, labrum; LB, limb bud; me, mesectoderm; mes, mesoderm; mn, mandible; mx1, first maxilla; mx2, second maxilla; NB, neuroblast; ne, neuroectoderm; P, posterior; pp, pleuropodia; pr, proctodaeum; ser, serosa; st, stomodaeum; T1, first thoracic segment; ta, tarsus; te, telopodite; ti, tibia; V, ventral; VF, ventral furrow

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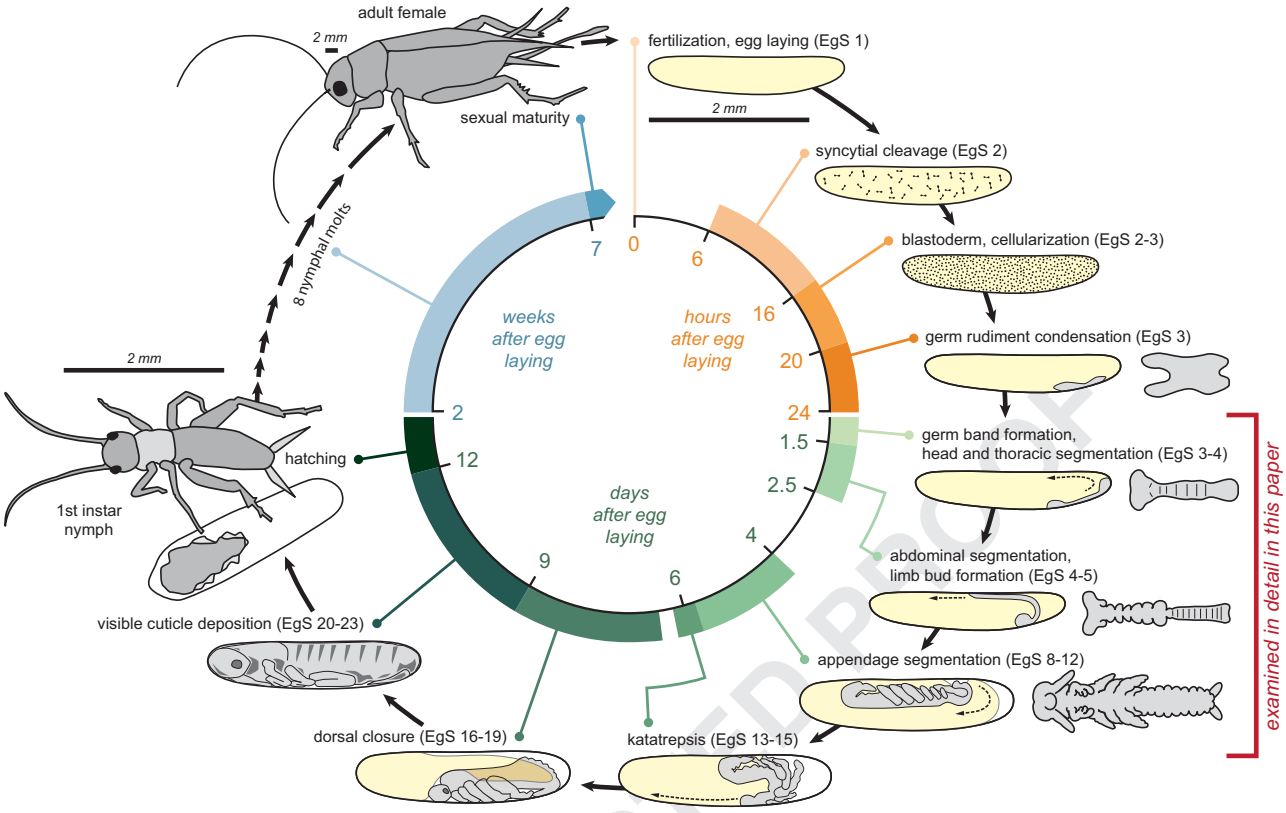

Fig. 1. Schematic of the *Gryllus bimaculatus* life cycle. Schematic drawings of selected embryonic and nymphal stages are shown, with the duration of each stage shown in hours (orange), days (green), or weeks (blue) after egg laying. Colored arcs indicate the window of time occupied by the indicated stage. Schematic drawings of embryogenesis show the position of the embryo (gray) within the egg relative to the yolk (yellow). Dotted lines with arrowheads indicate upcoming blastokinesis movements of the embryo. For stages examined in detail in this paper (bracketed in red), schematics on the left show morphologies of the embryo (gray) when dissected free from the yolk (yellow). "EgS" refers to egg stages defined in this paper. Live images of embryos at the stages corresponding to these schematics can be seen in time-lapse Movie S1 (EgS 1: 0 d 00:00 h–0 d 09:55 h; EgS 2: 0 d 10:00 h–0 d 15:15 h; EgS 3: 0 d 15:20 h–1 d 23:00 h; EgS 4: 1 d 23:05 h–2 d 12:10 h; EgS 5: 2 d 12:15 h–3 d 05:35 h) and Movie S2 (EgS 8–12: 4 d 01:45 h–6 d 11:50 h; EgS 13–15: 6 d 02:35 h–7 d 13:10 h; EgS 16–17: 7 d 11:15 h – end of Movie S2).

which head and thoracic segments are formed simultaneously and then abdominal segments are added sequentially as the anterior–posterior axis elongates. Third, unlike *D. melanogaster*, the cricket embryo changes its axial orientation while submerged within the yolk mass. This occurs in concert with morphogenetic movements of the two extraembryonic membranes in a two-part process termed blastokinesis (reviewed by Panfilio (2008)). The developing embryo completely reverses its anteroposterior orientation with respect to the eggshell once during anatrepsis (the first part of blastokinesis) and again during katatrepsis (the second part of blastokinesis) (Fig. 1). Fourth, crickets are hemimetabolous, which means that unlike the indirectly developing holometabolous insects, they do not pass through a larval stage nor go through total metamorphosis. Lastly, the *G. bimaculatus* ovary is panoistic. There are no germ line-derived nurse cells that provide cytoplasm to incipient oocytes; rather, each differentiating germ cell in females is thought to become an oocyte (Büning, 1994). Based on the phylogenetic distribution of these features, many of these characteristics are likely ancestral to insects (Sander, 1997).

G. bimaculatus is easily cultured in the lab, and there are well-established protocols for observing gene expression and protein localization in whole mount embryos and organ systems of juveniles and adults. Two recently published transcriptomes (Bando et al., 2013; Zeng et al., 2013) have made it straightforward to identify genes of interests and use RNA interference (RNAi) to knock down maternal or zygotic transcripts (techniques reviewed by Mito and Noji (2009)). It is also possible to generate stable transgenic lines (Nakamura et al., 2010). Thanks to these tools, our understanding of a diverse array of long-standing problems in arthropod and bilaterian evolution and development has improved, including limb development and regeneration (reviewed by Nakamura et al. (2008a)), segmentation (Kainz et al., 2011; Mito et al., 2011), anterior–posterior axis formation (Nakamura et al., 2010), novel gene evolution (Ewen-Campen et al., 2012), and germ cell specification (Donoughe et al., 2014). It is now also possible to perform site-directed genome editing (Watanabe et al., 2014, 2012), which opens up even more promising experimental possibilities.

All developmental studies, including those mentioned above, rely on a consistent embryonic staging system. A commonly used set of embryonic stages for *G. bimaculatus* was most recently updated by Mito and Noji (2009) and Kainz (2009), wherein embryonic stages are defined by morphological characteristics of the embryo. One drawback to the existing embryological staging system is that not all enumerated stages are defined by specific morphological features, and those that are well defined are limited to identification by examination of only a few organ systems. A second limitation of using these embryonic stages is that an embryo must be manually removed from the egg and examined in detail in order to identify its stage. This is because a large portion of cricket embryogenesis occurs when the embryo is submerged in yolk and the relevant morphological landmarks are not externally visible without this microdissection. Finally, although staging landmarks have been described for a number of other orthopterans (Bentley et al., 1979; Chapman and Whitham, 1968; Hägele et al., 2004; Harrat and Petit, 2009; Patel et al., 1989; Rakshpal, 1962; Riegert, 1961; Roonwal, 1936, 1937; Salzen, 1960; Sauer, 1961; Slifer and King, 1934; Slifer, 1932; Wheeler, 1893), it is not

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