

CHAPTER 10

Hox3/zen and the Evolution of Extraembryonic Epithelia in Insects

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Abstract

Insects have undergone dramatic evolutionary changes in extraembryonic development, which correlate with changes in the expression of the class-3 Hox gene *zen*. Here, we review the evolution of this gene in insects and point out how changes in *zen* expression may have affected extraembryonic development at the morphological and the genetic level.

Introduction

During the early radiation of insects, *Hox3* abandoned its ancestral role in specifying segmental identity along the anteroposterior axis of the embryo and acquired a new role in extraembryonic tissue.¹ This evolutionary transition may have occurred in the stem lineage of modern Pterygota (winged insects), as indicated by expression data and protein comparisons. Canonical Hox proteins share a hexapeptide or YPWM motif,¹ which enables them to interact with the Hox-cofactor Exd/Pbx.^{2,3} In the context of overlapping Hox gene expression patterns, e.g., during axis-specification, this interaction is important because it contributes indirectly to the DNA-binding specificity of individual Hox proteins.⁴ The *Hox3* gene of the apterygotan firebrat *Thermobia* is expressed in a nested arrangement with other Hox genes in the prospective gnathocephalon and in the growth zone and encodes a protein with the YPWM motif.^{5,6} In contrast, *Hox3* genes of Pterygota (named *zen* after their *Drosophila* prototype)⁷ are expressed in extraembryonic tissue, lack expression in the germband and encode proteins without YPWM motif.⁶ The present chapter is devoted to the evolutionary history of extraembryonic *zen* expression in insects. We cover data from five orders including the silverfish *Thermobia* (Thysanura), the grasshopper *Schistocerca* (Orthoptera), the bug *Oncopeltus* (Hemiptera), the beetle *Tribolium* (Coleoptera) and various fly species (Diptera). Yet, the focus of this review is on Diptera as this is the only insect order for which data on the expression and function of *zen* are available from multiple distantly related species. Even in this order, some relevant traits have been poorly sampled. Despite the ‘patchiness’ of the data matrix, we hope that as current working hypotheses our phylogenetic inferences will help to advance the field. To set the stage, we briefly review morphological aspects of extraembryonic development. In the following sections, we focus on variants of *zen* expression and their evolutionary significance. We propose that changes in *zen* expression underlie major reorganizations in extraembryonic development of pterygote insects.

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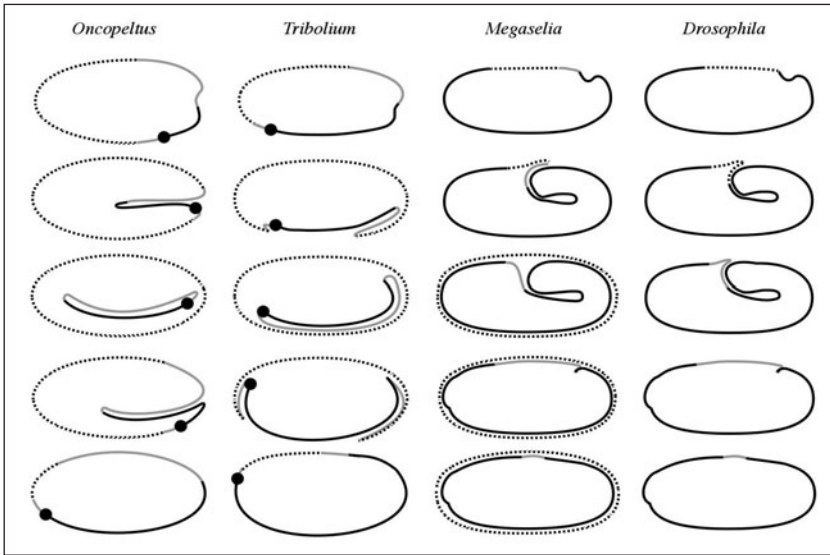


Figure 1. Schematic illustrations of extraembryonic developmental trajectories. Embryonic (black line), amniotic (grey) and serosal tissues (broken line) are indicated at consecutive developmental stages. Sketches are based on *Oncopeltus*,^{9,10} *Tribolium*,²³ *Megaselia*¹⁸ and *Drosophila*.¹⁹ A filled circle indicates the position of the head in *Oncopeltus* and *Tribolium* embryos.

Setting the Stage: Morphological Evolution of Extraembryonic Development

Extraembryonic development of insects begins at the blastoderm stage, when a single cell layer forms around the yolk. At this stage, a portion of anterior or dorsal blastoderm—depending on the species—is specified to become the serosa, an epithelium underneath the eggshell, which secretes a cuticle.⁸ Typically, serosa formation occurs by invagination of the posterior blastoderm (e.g., *Oncopeltus*, Fig. 1), or by closure over the ventral blastoderm (e.g., *Tribolium*, Fig. 1). In both cases, the internalized blastoderm pinches off from the serosa as a flattened hollow body, which is composed of a thin cell layer (amnion) and the gastrulating embryo. The fold through which part of the blastoderm is internalized is called ‘amnioserosal fold’ and the yolk-free space between the amnion and the embryo is called ‘amniotic cavity’. Later in development, while the flanks of the embryo close along the dorsal midline (dorsal closure), the process of serosa and amnion formation is reversed. The serosa and the amnion generate a continuous serosa-amnion epithelium (‘serosa-amnion fusion’), which retracts towards the anterior or dorsal side of the egg where both epithelia are resorbed by the yolk. This process ruptures the amniotic cavity and regenerates an opening in the extraembryonic epithelia (‘serosal window’). In hemimetabolous insects (lower Pterygota^a), serosa-amnion fusion seems to be widely conserved, as it is required for realigning the inverted anteroposterior (A-P) axis of the early embryo with the A-P axis of the egg (katatrepsis).^{9,10} In holometabolous insects (higher Pterygota), serosa-amnion fusion has been observed as well, but major variants of extraembryonic development have been found in all the large orders, including Hymenoptera, Coleoptera, Lepidoptera and Diptera.^{8,11–13} Here we limit the discussion to variants within the dipteran order. In lower Diptera (noncyclotriphaphan flies), serosa and amnion

^a Throughout this review, we use the term ‘lower’ in conjunction with a taxon name to designate basal branches of monophyletic taxa. Conversely, the term ‘higher’ is used when the intention is to exclude species on basal branches.