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Hedgehog signaling regulates imaginal cell differentiation in a basally branching holometabolous insect

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ABSTRACT

The evolution of imaginal cells, or stem cell-like cells, contributed to the spectacular diversification of holometabolous insects, which undergo complete metamorphosis. The proliferation and differentiation of these imaginal cells is under the control of juvenile hormone (JH), but which patterning genes respond to JH is currently unknown. Here, the role of Hedgehog (Hh) signaling in the development of imaginal cells was investigated. RNA interference-mediated knockdown of the components of the Hh signaling pathway showed that Hh is required for the proliferation of polymorphic and imaginal cells *in Tribolium castaneum*. Hh was also necessary for the regeneration of larval appendages. In contrast, knockdown of Hh signaling antagonists, *patched* and *costal 2* led to the overgrowth and precocious maturation of structures derived from imaginal cells and the occasional appearance of ectopic appendages from the head epidermis. In addition, JH suppressed the expression of *hh* both *in vivo* and *in vitro*. Our findings suggest that imaginal cells are created and maintained by modulating Hh signaling. Thus, Hh signaling may have played a critical role during the evolution of complete metamorphosis.

1. Introduction

The origin of insect metamorphosis has been debated for centuries and remains unresolved. Various theories have been proposed over the last century yet there is little consensus about how metamorphosis may have evolved (Belles and Santos, 2014; Erezyilmaz, 2006; Konopova et al., 2011; Truman and Riddiford, 1999). Specifically, two alternative views have been proposed to explain the origin of larval morphology. One posits that the larva is equivalent to the nymphal stages of hemimetabolous insects, insects that undergo incomplete metamorphosis (Belles and Santos, 2014; Hinton, 1963; Konopova et al., 2011; Konopova and Zrzavy, 2005). The other proposes that the larva is equivalent to the hemimetabolous embryo stage and that heterochronic shift in the timing of adult tissue maturation led to the origin of larval morphology (Berlese, 1913; Truman and Riddiford, 1999). To resolve these issues, the developmental genetic underpinnings of adult tissue maturation need to be understood. A distinct feature of larval development is the formation of imaginal cells or the delay of complete differentiation of larval cells (Truman and Riddiford, 2007). How these cells gained the ability to delay maturation remains unknown, but this issue is critical to our understanding of the origin of insect metamorphosis. In particular, while we know how the endocrine system influences the timing of metamorphosis (Nijhout, 1998), how the metamorphic hormones interact with the developmental genetic regulation of patterning remains unknown.

Imaginal discs, which give rise to adult structures, have been extensively studied, but this mode of development is in fact highly derived; the more ancestral mode of development involves the formation of imaginal primordia that do not form discs (Truman and Riddiford, 1999; Svácha, 1992). These imaginal primordia differ from imaginal discs in that they do not detach from the larval epidermis and do not proliferate until metamorphosis is initiated (Truman and Riddiford, 2002, 2007). These imaginal cells may be set aside in small clusters within the larval structures, or they may proliferate during metamorphosis from the larval epidermis, but they do not contribute to the larval structures (Tanaka and Truman, 2005; Truman and Riddiford, 2002; Fig. 1). In most insects, the wings and eyes develop from imaginal cells that proliferate and differentiate at the end of larval life. In some insects, such as *Manduca sexta*, imaginal cells are also found in legs (Tanaka and Truman, 2005). However, in the more ancestral state, holometabolous insects have simple larval legs and antennae

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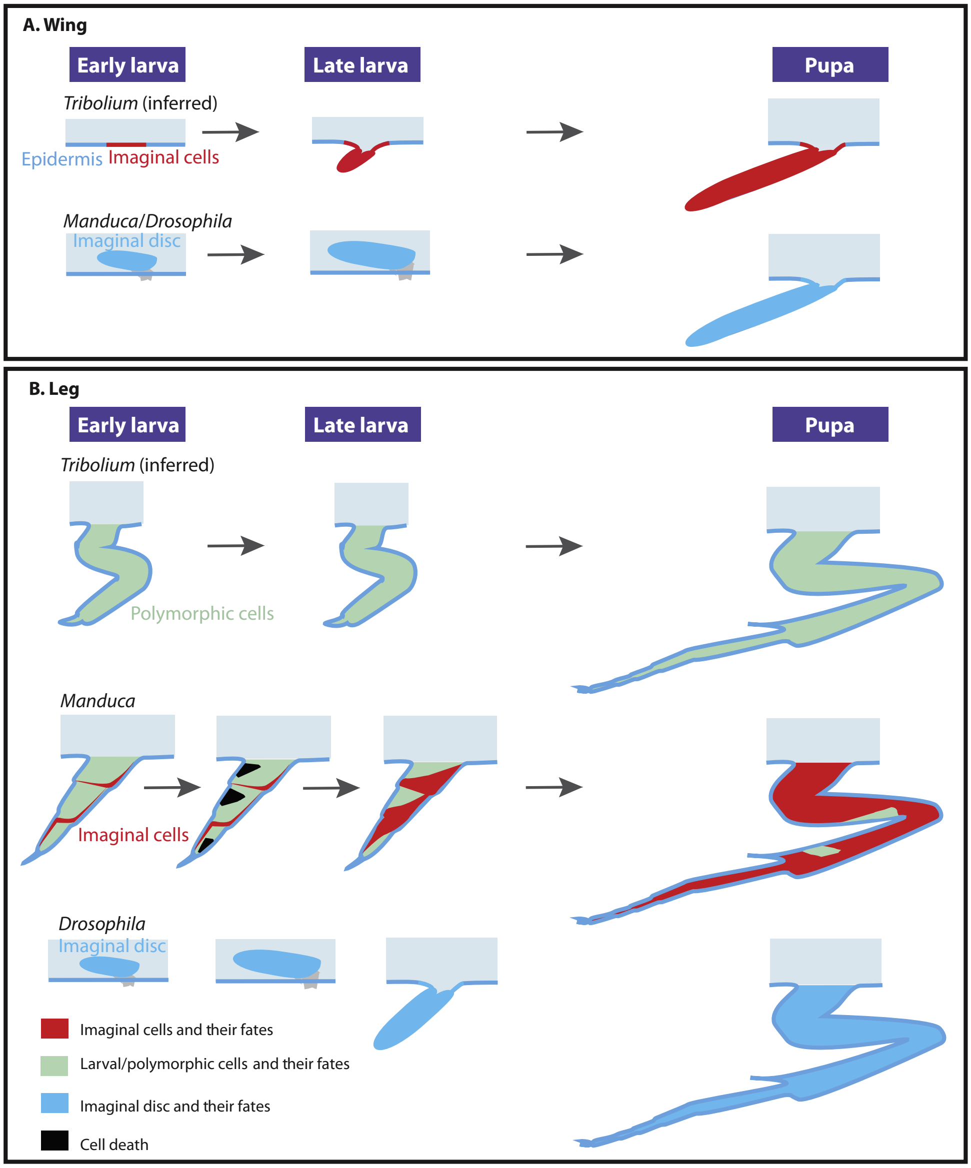

Fig. 1. Precursor cell types of various holometabolous insects. (A) Wing development *in Tribolium*, *Manduca* and *Drosophila*. *Tribolium* lack wing imaginal discs and instead form wings from imaginal cells during the final larval instar. *Manduca* and *Drosophila* develop wing imaginal discs during embryogenesis. (B) Leg development *in Tribolium*, *Manduca* and *Drosophila*. *Tribolium* legs appear to develop from polymorphic legs where most of the larval cells contribute to the adult legs. Much of the *Manduca* larval leg cells undergo apoptosis during the final instar. The adult leg derives largely from imaginal cells that begin proliferating during the last larval instar; a small portion of adult leg derives from polymorphic cells (Tanaka and Truman, 2005). *Drosophila* develop leg imaginal discs that develop during embryogenesis.

whose cells are thought to create adult appendages (Truman and Riddiford, 2002). These cells are called polymorphic cells as they contribute to both the larval and adult appendages (Fig. 1). Alterations to patterning in the larval legs are carried over to the adult legs, indicating that there is developmental continuity between the larval and adult structures, unlike imaginal cells that can give rise to several different structures (Lee et al., 2013). The nature of these polymorphic cells remains unclear, however, and the cell fates of these polymorphic cells can be re-specified during metamorphosis (Angelini et al., 2009; Shippy et al., 2009), indicating that they retain some level of potency throughout their larval development. During metamorphosis, differentiation is completed and adult structures form. Thus, both imaginal cells and polymorphic cells represent undifferentiated or incompletely differentiated precursor cells. Insects, such as Megaloptera and Neuroptera as well as the hymenopteran suborder Symphyta all lack imaginal discs (Svácha, 1992). Thus, imaginal cells and polymorphic cells represent the more ancestral mode of adult development, and studying the development and evolutionary origin of these cells holds the key to understanding the origin of metamorphosis (Truman and Riddiford, 2002). For the remainder of this paper, we will group them together under the broad term of precursor cells.

The sesquiterpenoid lipid hormone, juvenile hormone (JH), has been shown to act as a morphostatic hormone, which suppresses the morphogenetic growth of these precursor cells (Truman et al., 2006). JH is a key developmental hormone in insects, and it has been linked to the regulation of life history transition by acting as a *status quo* hormone (Riddiford, 1996; Truman et al., 2006). This action of JH is typically conferred through JH modifying the effects of the molting hormone ecdysteroids (Jindra et al., 2013). A much less understood action of JH is the morphostatic action of JH, which inhibits imaginal cell morphogenesis independent of the action of ecdysteroids (Truman et al., 2006; Truman and Riddiford,