

The stages between these larval molts are called **instars**. The number of molts before becoming an adult is characteristic for the species, although environmental factors can increase or decrease the number. The instar stages grow in a stepwise fashion, each being qualitatively larger than the previous one. Finally, there is a dramatic and sudden transformation between the larval and adult stages. After the last instar stage, the larva undergoes a **metamorphic molt** to become a **pupa**. The pupa does not feed, and its energy must come from those foods it ingested while a larva. During pupation, the adult structures are formed and replace the larval structures. Eventually, an **imaginal molt** enables the adult ("imago") to shed the pupal case and emerge. While the larva is said to *hatch* from an egg, adults are said to **eclose** from the pupa.

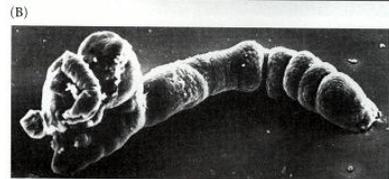
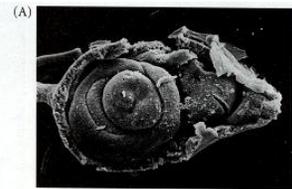
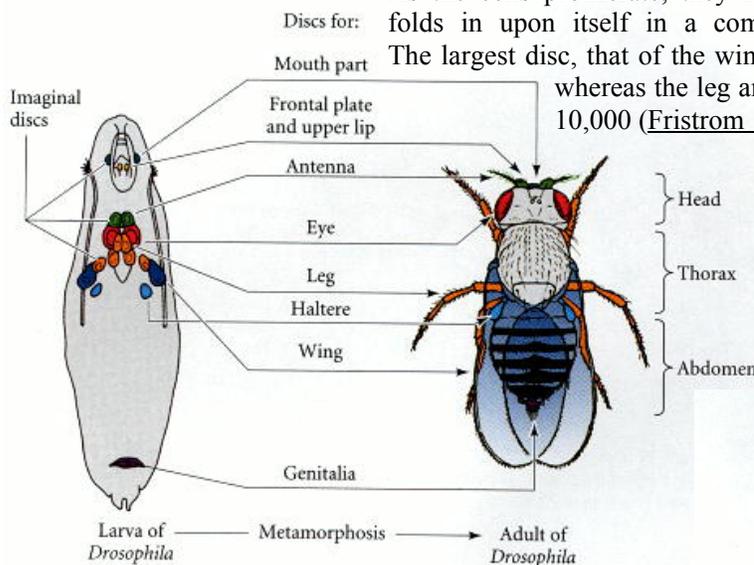
Eversion and differentiation of the imaginal discs

In holometabolous insects, the transformation from juvenile into adult occurs within the pupal cuticle. Most of the old body of the larva is systematically destroyed by apoptosis, while new adult organs develop from undifferentiated nests of cells, the **imaginal discs**. Thus, within any larva, there are two distinct populations of cells: the larval cells, which are used for the functions of the juvenile insect, and the thousands of imaginal cells, which lie within the larva in clusters, awaiting the signal to differentiate.

In *Drosophila*, there are ten major pairs of imaginal discs, which construct many of the adult organs, and an unpaired genital disc, which forms the reproductive structures (Figure 18.12). The abdominal epidermis forms from a small group of imaginal cells called **histoblasts**, which lie in the region of the larval gut. Other nests of histoblasts located throughout the larva form the internal organs of the adult. The imaginal discs can be seen in the newly hatched larva as local thickenings of the epidermis. Whereas most of the larval cells have a very limited mitotic capacity, the imaginal discs divide rapidly at specific characteristic times.

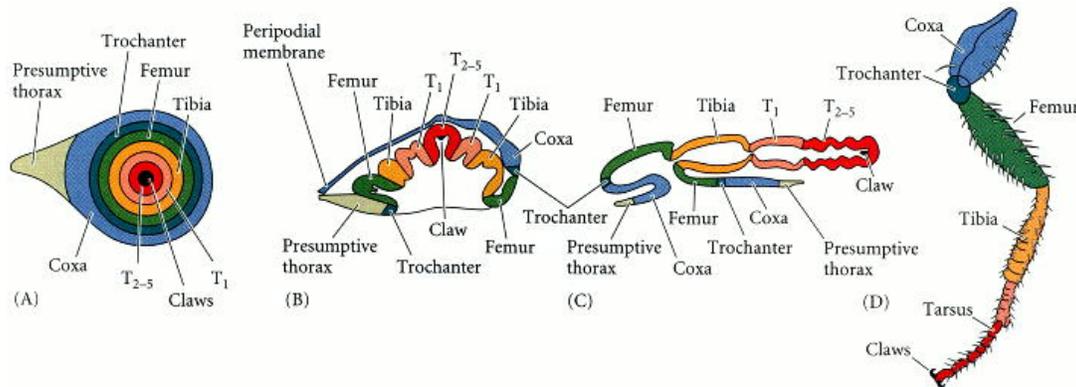
As the cells proliferate, they form a tubular epithelium that folds in upon itself in a compact spiral (Figure 18.13A). The largest disc, that of the wing, contains some 60,000 cells, whereas the leg and haltere discs contain around 10,000 (Fristrom 1972).

At metamorphosis, these cells proliferate, differentiate, and elongate (Figure 18.13B).

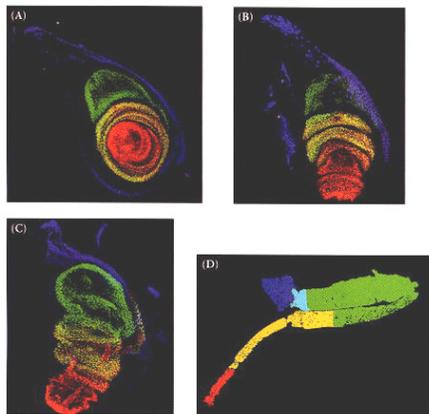


The fate map and elongation sequence of the leg disc are shown in Figure 18.14. At the end of the third instar, just before pupation, the leg disc is an epithelial sac connected by a thin stalk to the larval epidermis.

On one side of the sac, the epithelium is coiled into a series of concentric folds "reminiscent of a Danish pastry" (Kalm et al. 1995). As pupation begins, the cells at the center of the disc telescope out to become the most distal portions of the leg—the claws and the tarsus. The outer cells become the proximal structures—the coxa and the adjoining epidermis (Schubiger 1968). After differentiating, the cells of the appendages and epidermis secrete a cuticle appropriate for the specific region. Although the disc is composed primarily of epidermal cells, a small number of **adepithelial cells** migrate into the disc early in development. During the pupal period, these cells give rise to the muscles and nerves that serve that structure.



Studies by Condic and her colleagues (1990) have demonstrated that the elongation of imaginal discs is due primarily to cell shape change within the disc epithelium. Using fluorescently labeled phalloidin to stain the peripheral microfilaments of leg disc cells, they showed that the cells of early third-instar discs are tightly compressed along the proximal-distal axis. This compression is maintained through several rounds of cell division. Then, when the tissue begins elongating, the compression is removed, and the cells "spring" into their rounder state. This conversion of an epithelium of compressed cells into a longer epithelium of noncompressed cells represents a novel mechanism for the extension of an organ during development.



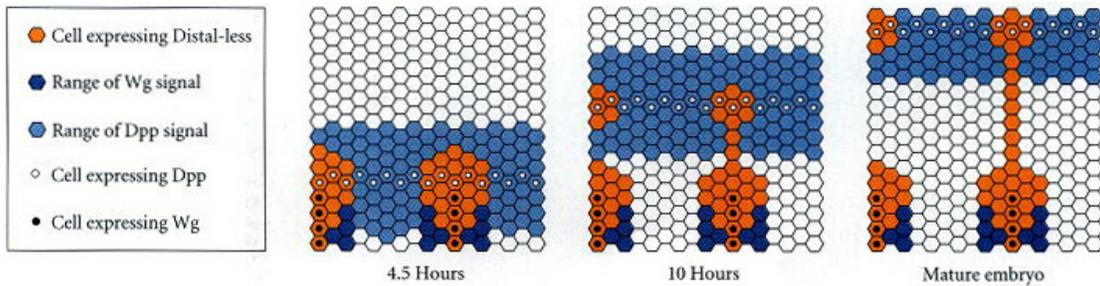
The type of leg structure generated is determined by the interactions between several genes in the imaginal disc. Figure 18.15 shows the expression of three genes involved in determining the proximal-distal axis of the fly leg. In the third-instar leg disc, the center of the disc secretes the highest concentration of two morphogens, Wingless (Wg) and Decapentaplegic (Dpp). High concentrations of these paracrine factors cause the expression of the *Distal-less* gene. Moderate concentrations cause the expression of the *dachshund* gene, and lower concentrations cause the expression of the *homothorax* gene. Those cells expressing *Distal-less* telescope out to become the most distal structures of the leg—the claw and distal tarsal segments. Those expressing *homothorax* become the most proximal structure, the coxa. Cells expressing *dachshund* become the femur and proximal tibia.

Areas of overlap produce the trochanter and distal tibia (Abu-Shaar and Mann 1998). These regions of gene expression are stabilized by inhibitory interactions between the protein products of these genes and of the neighboring genes. In this manner, the gradient of Wg and Dpp proteins is converted into discrete domains of gene expression that specify the different regions of the *Drosophila* leg.

Determination of the Wing Imaginal Discs

Determination of discs from ectoderm: distal-less protein

The molecular biology of insect metamorphosis begins with the specification of certain epidermal cells to become imaginal disc precursors. As we discussed in [Chapter 9](#), the organ rudiments in *Drosophila* are specified on an orthogonal grid by intersecting anterior-posterior and dorsal-ventral signals. In most segments, Hox gene products prevent *Distal-less* gene expression and the establishment of limb primordia; but in those segments that are specified to be thoracic, limb formation is permitted. Cohen and his colleagues (1993) have demonstrated that the leg and wing originate from the same set of imaginal precursors, specified at the intersections between the anterior-posterior stripes of Wingless (Wg) protein expression and the horizontal band of cells expressing the Decapentaplegic (Dpp) protein. Both proteins are soluble and have a limited range of diffusion. In the early *Drosophila* embryo (at germ band extension about 4.5 hours after fertilization), a single group of cells at these intersections forms the imaginal disc precursors in each thoracic segment. These cells (and only these cells) express the Distal-less protein. As the cells expressing Dpp are moved dorsally, some of these Distal-less-expressing cells move with them to establish a secondary cluster of imaginal cells (derived from the original ventral cluster). The initial clusters form the leg imaginal disc, while the secondary clusters form the wing or haltere disc. Thus, the leg and wing discs have a common origin ([Figure 18.16](#)).



Determination of disc identity: vestigial protein

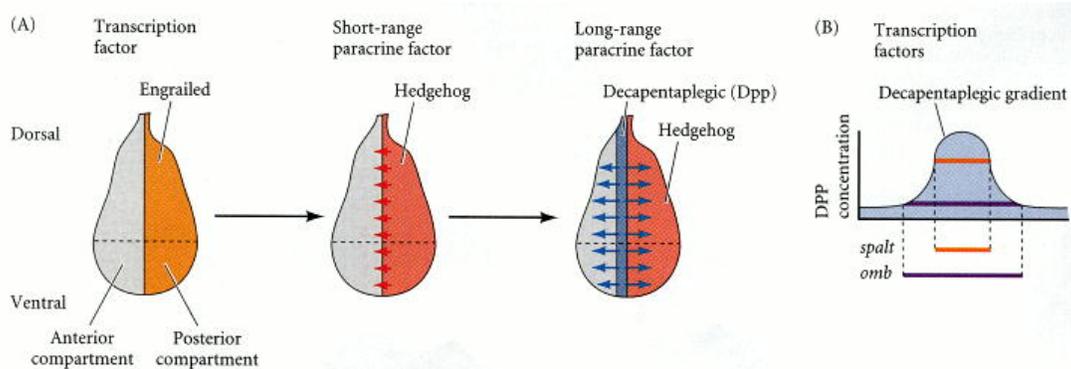


Despite their common origin, it is obvious that the leg and wing discs are determined to become different structures. The determination of the wing disc appears to be regulated by the *vestigial* gene. Using a targeted gene expression system, [Kim and colleagues \(1996\)](#) have caused the *vestigial* gene to be expressed in eye, antenna, and leg discs ([Figure 18.17](#)). When this happens, regions of the normal structure are converted into wing tissue.

Determination of the anterior-posterior axis: engrailed and decapentaplegic proteins

The axes of the wing are specified by interactions at their compartmental boundaries ([Meinhardt 1980](#); [Causo et al. 1993](#); [Tabata et al. 1995](#)). After these initial interactions, a polar coordinate system may subdivide the wing regions more finely ([Held 1995](#)).

During the first larval instar, the leg and wing imaginal discs acquire their anterior-posterior axis. The discs become split into two compartments representing the future anterior and posterior regions of the appendage (i.e., the front of the wing and the rear of the wing). Based on the position of its cells in the segment, the posterior compartment of the wing disc expresses the *engrailed* gene (Figure 18.18; Garcia-Bellido et al. 1973; Lawrence and Morata 1976). If *engrailed* function is absent, all the disc cells become anteriorized. The boundary between the posterior and anterior compartments is strictly observed. Cells from one side cannot produce descendants that cross over the boundary to the other.



The Engrailed protein is a transcription factor, and it activates the *hedgehog* gene in the cells of the posterior compartment. The posterior wing disc cells express the Hedgehog protein, which acts as a short-range signal to induce the expression of Dpp in adjacent *anterior* cells, while the expression of *engrailed* in the posterior cells renders them nonresponsive to the Hedgehog they secrete (preventing them from expressing Dpp). Dpp is a TGF- β family paracrine factor that acts as a long-range signal to establish the anterior-posterior axis of the wing (Guillen et al. 1995; Tabata et al. 1995; Nellen et al. 1996). Cells in both compartments close to the area of Dpp expression are exposed to relatively high concentrations of this protein and activate the *spalt* and *oculomotor-blind* (*omb*) genes. Those cells farther away receive lower concentrations of Dpp and activate only the *omb* gene. These two genes encode transcription factors that specify the region of the wing from the center (where Dpp is expressed) to the periphery.

Dorsal-ventral axis: wingless and apterous proteins

During the second larval instar, the dorsal-ventral axis of the wing disc is determined. The dorsal-ventral boundary lies at the future margin of the wing blade, separating the upper surface of the wing from the lower (Bryant 1970; Garcia-Bellido et al. 1973). The gene involved in this compartmentalization event is *apterous*. Cells expressing *apterous* become the dorsal cells (Figure 18.19A; Blair 1993; Diaz-Benjumea and Cohen 1993). When *apterous* is deleted, all cells in the disc acquire ventral fates. The Apterous protein is a transcription factor that activates the genes for the Serrate and Fringe proteins. Serrate is a ligand for the Notch receptor, and Fringe is involved in regulating Notch ligand binding (Irvine and Wieschaus 1994; Williams et al. 1994; Kim et al. 1995). The Notch receptor is found in the ventral cells, so the binding of Notch on the ventral side with Serrate on the dorsal side stabilizes the wing margin.