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The aim of this laboratory is to observe the variety of morphogenetic processes that occur during the ontogenesis of multicellular organisms and to analyze the mechanisms of such processes through mainly morphological methods. The accumulation of such analyses of the embryogenetic processes of related species is expected to provide an insight into the evolution of morphogenetic processes. This laboratory uses the wings of lepidopteran insects (butterflies and moths) as the main subject of morphological studies.

I. Wing outline shape formed by cell death

The wings of lepidopteran insects develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. The outline shape of an adult wing often differs from that of a pupal wing. This difference is brought about by the programmed cell death of the marginal area of the pupal wing. The marginal dying area is called “the degeneration region” and the internal area which develops into the adult wing is called “the differentiation region”.

Cell death in the degeneration region proceeds very rapidly and finishes in a half-day to one-day period in *Pieris rapae* and in several other examined species. It has been shown that the dying cells in the degeneration region have characteristics in common with apoptotic cell death in mammalian cells, such as fragmented and condensed nuclei containing short DNA fragments detected by TUNEL staining. The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. The macrophages are excluded from the differentiation region by a close adherence of dorsal and ventral epithelia and concentrated in the degeneration region, thus accelerating the removal of dead cells and the shrinkage of the degeneration region.

A possible physiological role of cell degeneration at the wing margin is to make space for the growth of the marginal scales. These scales are remarkably elongated and grow densely on the edge of the wing. These scales are considered to be important in stabilizing the turbulence occurring posterior to the wing. The movements of the marginal scales are closely monitored by sensory scales and the bristles growing among them.

II. Wing morphogenesis and the growth of marginal scales in small moths

In small moths, which have very long scales along their wing margins, the cuticle of the pupal wing does not appear to be large enough to house these scales. We examined the developmental processes of the pupal wings of three species of small gelechiid moths and found that a shrinkage of the differentiation region occurs which has not been observed in large winged butterflies and moths. This phenomenon is

concomitant with programmed cell death at the wing margin, and gives rise to the space between the pupal wing and the cuticle. This space appears to allow the growth of long marginal scales.

Microscopic observation of the long marginal scales of *Phthorimaea operculella* have revealed that they have a novel branching morphology and the branches were ubiquitously and densely distributed within the scale array to form a mesh-like architecture similar to a nonwoven fabric. The marginal scales maintain a coherent sheet-like structure during wingbeat (Yoshida, A. *et al.* *Sci. Nat.* 104, 27, 2017).

III. Transparent wing formation through scale removal

Butterfly and moth wings are usually fully covered with scales. However, in some species, the adult wing lacks scales in some parts, thus giving the wing a transparent appearance.

We studied the emergence process of the *Cephonodes hylas* moth and found that:

- 1) the future transparent part of the wing immediately after emergence is covered with scales that are morphologically different from the scales on the other part
- 2) young adults detach numerous scales on the future transparent part at the initial take-off after emergence, and consequently the transparent part appears.

We also studied the scale morphology and found that the detaching scale is remarkably large and its proximal pedicel, through which the scale attaches to the wing, is tapered (Figure 1). We concluded that these morphological features facilitate the scale detachment through fluttering. (Yoshida, A. *et al.*, Programmed scale detachment in the wing of the pellucid hawk moth, *Cephonodes hylas*: Novel scale morphology, scale detachment mechanisms, and wing transparency. *Zool. Sci.* 38, 427-435, 2021. doi: 10.2108/zs210031).

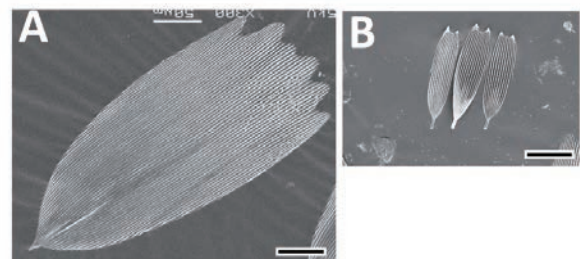


Figure 1. Scanning electron microscopy of the wing scales in the future transparent part (A) and the other part left covered with scales (B) at the same magnification. The scale in A is much larger than that in B, and the pedicel, the protrusion seen at the lower left corner of A is tapered while pedicels in B are columnar. Scale bars: 50 μ m.