LABORATORY OF MORPHODIVERSITY

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The aim of this laboratory is to observe the variety of morphogenetic processes that occur in the course of the ontogenesis of multicellular organisms and to analyze the mechanisms of such processes, mainly by 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 as the main subject of morphological studies.

I. Wing outline shape formed by cell death

The wings of the lepidopteran insects (butterflies and moths) develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. Due to its simple construction, this tissue is a good material for studying cellular interactions during the course of morphogenesis.

The outline shape of an adult wing is often different 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. At that time, the macrophages are excluded from the differentiation region because the basal surfaces of the dorsal and ventral epithelium strongly adhere to each other in the differentiation region. The concentration of macrophages to the degeneration region seems to accelerate the removal of the 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. Marginal scales are remarkably elongated scales that 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 (Yoshida and Emoto, Zool. Sci. *28*, 430-437, 2011).

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 the programmed cell deaths at the wing margin, and causes the space between the pupal wing and the cuticle to extend, which appears to contribute to 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 (Figure 1).

III. Transparent wing formation by scale removal

The wings of butterflies and moths are usually fully covered with scales but 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 pupal wing is covered with scales that are morphologically different from the scales found on the other part and 2) the young adult moth just after emergence strongly vibrates its wing which loosens the scales of the future transparent part until the moth flies away. This results in the transparent wing leaving scales behind. The large size and the shape containing a small peg, which stabs the socket on the wing plane holding the scale in place, found on the scales of the future transparent part are key feature in enabling the formation of the transparent wing (Yoshida *et al*., in preparation).

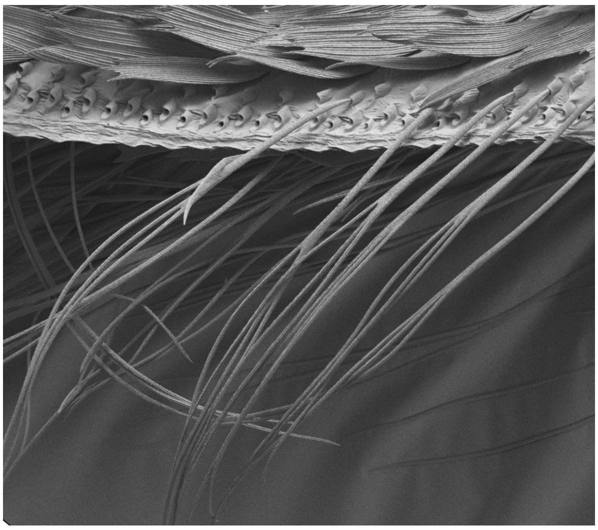

Figure 1. Scanning electron microscopy of the wing margin of *P. operculella*. Long marginal scales show branching morphology.