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Title	Real-Time In Vivo Imaging and Scale Distribution Analysis in Nymphalid Butterfly Wings(Abstract_論文要旨)
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論 文 要 旨

論 文 題 目

• **Title:** Real-Time *In Vivo* Imaging and Scale Distribution Analysis in Nymphalid Butterfly Wings

• **Abstract:** Butterfly wings are covered with regularly arranged single-colored scales that are formed at the pupal stage. Understanding pupal wing development is therefore crucial to understand wing color pattern formation. Chapter 1 in this thesis describes a new method for observing living pupal wings for a long time in real time, revealing the dynamics of wing development. I successfully applied the real-time *in vivo* imaging technique to pupal hindwings of the blue pansy butterfly, *Junonia orithya*. A transparent sheet of epithelial cells that were not yet regularly arranged was observed immediately after pupation. Bright-field imaging and autofluorescent imaging revealed free-moving hemocytes and tracheal branches of a crinoid-like structure underneath the epithelium. The wing tissue gradually became gray-white, epithelial cells were arranged regularly, and hemocytes disappeared, after which scales grew. The wing tissue exhibited slow and low-frequency contraction pulses with a cycle of approximately 10 to 20 minutes, mainly occurring at 2 to 3 days postpupation. The wing tissue area became larger after contraction. These results of live *in vivo* imaging that covered wide wing area for a long time can serve as a foundation for studying the cellular dynamics of living wing tissues in butterflies.

Chapter 2 in this thesis describes spatial patterns of scale size in relation to color pattern elements in butterfly wings. Scale size measurements on butterfly hindwings were made using three species of nymphalid butterflies: *Junonia almana* (Nymphalinae), *Vanessa indica* (Nymphalinae), and *Danaus chrysippus* (Danainae). In these species, there were a reasonable level of correspondence between the color pattern element and scale size, and a general decrease in scale size from the basal to the distal areas. The results correspond with our previous finding for other two species of nymphalid butterflies, *Junonia orithya* and *Junonia oenoe*. In addition, we observed that the *J. almana* ectopic eyespots that were induced by physical damage on the background area had larger scales than in the surrounding area. This size-color relationship supports a possibility that single morphogenic signal contains positional information for both the color and size of scales.

Finally, in Chapter 3 in this thesis, to evaluate the feasibility of an assumption that a white spot in adult butterfly wings corresponds to an organizing center and that the size of the white spot in butterfly wings indicates how active that organizing center was, we studied the unique color patterns of *Calisto tasajera* (Nymphalidae, Satyrinae). The anterior hindwing contained two adjacent white spots not associated with eyespots, one of which showed a sparse pattern. The posterior hindwing contained two adjacent pear-shaped eyespots, and the white spots were located at the proximal side or even outside the eyespot bodies (defined as all the eyespot portions except a white focal area). These results argue for the uncoupling of white spots from eyespot bodies, suggesting that an eyespot organizing center does not necessarily differentiate into a white spot and that a prospective white spot does not necessarily signify organizing activity for an eyespot.