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Editorial overview: Development and regulation: The diverse traits that have facilitated the successful radiation of insects

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Insects are the most abundant and diverse group of organisms on earth. Insects have conquered almost every corner of the world (except for the marine environment) through a diverse array of adaptive strategies at morphological, physiological, and behavioral levels. In addition, competition among insects (including inter-, intra-, and sexual competition) have further promoted the evolution of diverse traits. Examples of diverse traits can be found in their appendages (antennae, mouthparts, legs, flight appendages and more), which have been evolutionarily modified quite extensively both within, and across species in adaptation to various selective pressures. The diverse colors and textures of insect cuticles are also the result of adaptations used for mimicry, defense, sexual competition, and physiological processes. In addition, the evolution of novel structures, such as beetle horns, has helped insects explore new adaptive strategies. The evolution of eusocialism is another fascinating example of the diverse traits found in insects, which has been achieved by the combination of morphological, physiological, and behavioral evolution.

Although the diverse traits found in insects have fascinated scientists for centuries, until recently, the molecular and developmental underpinnings of these traits have been inaccessible in most species due to the lack of genetic and genomic tools applicable to many insects. However, advances in molecular biology techniques have begun to liberate scientists from this limitation. Twenty years since the first report of RNA interference (RNAi) in an animal species, RNAi-based gene knockdown techniques have now become established approaches in many insects. More recently, the application of CRISPR/Cas9 genome editing technologies in insects has caused quite a stir in the insect community, and many scientists are currently rushing to apply this technique to their insects. Furthermore, as the cost of next generation sequencing continues to drop, comprehensive transcriptomic and genomic analyses can now be performed in various insects, making gene network analyses possible even in non-model species. Leveraging these technical advances, some researchers have begun to explore diverse traits in various insect species.

There is no argument that the fruit fly (*Drosophila melanogaster*) is the most advanced insect model system, and studies in *D. melanogaster* have established our understanding of insect biology at all levels, including genetic, cellular, developmental, behavioral, and physiological processes. Application of some genetic and genomic tools in other insects has made these species genetically amenable model systems, however, that does not mean

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that there is not still much to be learned from *Drosophila* studies. On the contrary, studies in *D. melanogaster* continue to be at the forefront of genetic and developmental studies, spearheading new fields in insect biology.

In this issue, we highlight both *Drosophila* studies that explore detailed developmental mechanisms and studies in non-traditional model insects that investigate diverse traits. *Drosophila* research allows for the elucidation of the intricate genetic and developmental mechanisms responsible for the production of complex morphological traits, while studies outside of *Drosophila* will take us deeper into the molecular mechanisms underlying the evolution and development of many intriguing traits found in various species.

For the articles that feature *Drosophila* studies, first, [Tajiri](#) highlights recent reports investigating how pre-existing cuticles and the material property of the newly formed cuticles influence the body shape during ecdysis and metamorphosis. The cuticle of insects plays a pivotal role in maintaining the shape and integrity of insect bodies. Recent studies in *D. melanogaster* have revealed a surprising, novel function of the insect cuticle, where the cuticle is involved in ‘determining’ the shape of insects (instead of the traditional view where the cuticle shape is the outcome of prior patterning events). This cuticle-driven regulation of morphology provides another step that can be modified during morphological evolution, and thus will inspire investigation of the importance of cuticles in the evolution of diverse morphological traits in other insects.

Next, [Kojima](#) reviews our current understanding of the leg formation mechanism, with a particular focus on the tarsus (the most distal leg component). Ventral appendages of insects (including legs) display a dazzling array of diversity in their shape and size. The molecular and development basis for the formation of ventral appendages has been studied quite extensively in *D. melanogaster*, establishing the ‘leg gene regulatory network’. The tarsal segments are quite diverged among insects as they have adapted to their specific habitats and life styles. Therefore, the detailed genetic interactions learned from *Drosophila* studies summarized in this article will be used as a paradigm to explore the molecular basis underlying the diversity of tarsal structures.

Two articles in this issue highlight studies that use *D. melanogaster* as a starting point to explore morphological diversity. [Rebeiz and Williams](#) provide a comprehensive overview of research that investigates the changes in *cis*-regulatory mechanisms that have facilitated the evolution of pigmentation patterns among *Drosophila* species. With the available genomic resources, the *Drosophila* species group offers powerful opportunities to explore detailed molecular mechanisms underlying a diversity of traits. Pigmentation patterns vary significantly among the *Drosophila* species, and also frequently between males and females. Therefore, investigation into the evolution of pigmentation patterns allows for the pinpointing of the changes in their genomes that are responsible for the phenotypic changes resulting from natural and sexual selection.

Insect wings present one of the chief examples of morphological diversity. The evolutionary modifications on the wing occur not only between species, but also between the two pairs of wings in the same species (*i.e.*, forewing and hindwing), producing a beautiful array of diverse wings. The Hox gene, *Ultrabithorax* (*Ubx*), has been identified as the key factor in differentiating the hindwing from the forewing in *Drosophila*. The function of *Ubx* as the

hindwing selector gene has since been extended to other insect orders, such as butterflies and beetles. However, it is still elusive how Ubx has come to control the hindwing identity, and if the *Drosophila* paradigm is applicable to a broader taxonomy. Tomoyasu reviews the recent progress in our understanding of Ubx function obtained from studies in non-*Drosophila* insects as well as from genomic studies in *D. melanogaster*, and discusses how these new findings impact our current view of the role of Ubx in the evolution of insect flight structures.

The rest of the articles in this issue highlights research that investigate various intriguing traits in non-traditional model insects, from morphological traits such as butterfly eyespots and beetle horns to physiological and behavioral traits like desiccation tolerance and eusocialism. With varying numbers, shapes, colors, and their wide-spread occurrence among lepidopteran insects, eyespots on butterfly wings have been a powerful model to study various aspects of evolutionary processes, including evolution of novel structures, co-option, and production of phenotypic variation at intra- and inter- species levels. Beldade and Peralta provide a comprehensive overview of our current understanding of butterfly eyespot formation from an ecological, developmental, and evolutionary perspective. They also highlight some unsolved mysteries in the development and evolution of the butterfly eyespot and discuss how the application of new techniques, such as transcriptomic and genomic analyses, as well as CRISPR/Cas9-based genome editing (no more struggles with RNAi-based gene knockdown in Lepidoptera!), can help researchers approach those mysteries.

Beetle horns are an iconic example of morphological novelty. Horns are formed in the head and/or prothoracic segments in several lineages of beetles, and are mainly used for sexual competition. The horn of the *Onthophagus* beetles shows an interesting nutrition-dependent polyphenism, allowing them to be either “small horn sneakers” or “big horn machos”, both of which are advantageous over beetles with a “mediocre horn”. The complex molecular underpinnings of this type of environment-dependent allometry regulation (or scaling relationship) has only recently begun to be revealed. Casasa et al. review the recent findings from the Moczek Lab and other groups investigating the evolution of scaling relationships in beetle horns. With comprehensive intra- and inter- species transcriptome comparisons as a starting point, many factors have been identified to be involved in the beetle horn development (e.g., *doublesex*, Hedgehog, Insulin, and Serotonin signals). The authors provide a detailed summary on how these identified factors contribute to establishing a nutrition-dependent sigmoidal allometry that facilitated the evolution of horn polyphenism.

Most of the traits featured in this issue are related to morphology. However, other types of traits, such as

physiological traits, are equally important for adapting to various selective pressures and expanding into diverse niches. Extreme desiccation tolerance is one such trait, which allows insects to explore arid environments. A sub-Saharan insect, the sleeping chironomid (*Polypedilum vanderplanki*), is a truly amazing insect, exhibiting an incredible tolerance to complete desiccation (i.e., anhydrobiosis). Sogame and Kikawada review the recent progress of the research on the anhydrobiosis of the sleeping chironomid, and provide an overview of the factors involved in anhydrobiosis. The authors also describe the changes in genomic structures that may have enhanced the ability of chironomids to survive extremely dry conditions. Similar to other studies featured in this issue, many of these new insights were obtained through the application of genomic techniques, such as transcriptome and genome comparisons.

Each ant is an independent organism. But at the same time, a colony of ants, with their elaborate division of labor (i.e., the caste system), can be considered as one functional biological unit, namely a superorganism. Evolution of superorganisms (or eusocialism) has fascinated many scientists, and the regulation of the ant caste system has been investigated at various levels, including developmental, physiological, behavioral, ecological, and even at the theoretical system level. Lillico-Ouachour and Abouheif review over a century of accumulated studies investigating this complex trait, and provide a comprehensive summary of the factors involved in the formation and evolution of the ant caste system. This article will serve as a foundation to formulate new ways of studying the evolution of complex traits.

There are several recurring themes found in the articles presented in this issue. First, the application of genomic techniques (such as RNA-sequencing and genome sequence comparison) allows for the unbiased identification of the genes and genetic changes involved in the context of interest without relying on the *Drosophila* paradigm. Second, the incorporation of interdisciplinary knowledge provides a more ‘natural’ context to evo-devo research. Many studies featured in this issue beautifully incorporate ecological and physiological knowledge into their research, allowing researchers to study the traits of interest in ecologically relevant settings (i.e., eco-evo-devo). Along this direction, we would like to see more contributions from entomology in the future. The rich knowledge of the life of insects accumulated in entomology-based studies will be a strong asset when investigating the evolution of diverse traits. Third, we should continue to learn from *Drosophila* studies. Maturation and sophistication of genetic and genomic techniques in *Drosophila* now allows us to elucidate molecular and cellular mechanisms with an unprecedented resolution. Therefore, *Drosophila* studies will continue to reveal new molecular and cellular mechanisms, which will be a

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powerful source of inspiration when studying other insects. In addition, genome editing techniques may now allow the establishment of ‘*Drosophila*-type’ genetic tools in various species, which will significantly increase the resolution of molecular studies in non-*Drosophila* insects.

In conclusion, we hope that the eight articles presented here will give the reader a glimpse of the excitement and enthusiasm of the evo-devo research that has begun to unveil the molecular and developmental mechanisms underlying the evolution of the diverse traits, and also provide many inspirations for future evo-devo research. There are so many unique and intriguing traits that have

emerged during the evolution of insects, which have resulted in the endless and beautiful diversity you now see in this clade. Through the study of these diverse insect traits, we can gain a deeper understanding of the history and the inner workings of life.

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