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論文審査委員	主査　准教授　髙橋　文 委員　教授　田村　浩一郎 委員　教授　坂井　貴臣

【論文の内容の要旨】

Many organisms, including insects, have variations in body color traits such as the hue, darkness, and pattern of pigmentation. *Drosophila melanogaster* has a wide range of variation in the intensity of dark pigmentation in many natural populations from different continents. Previous studies have shown that *ebony* is the major causative gene for body color variation. Its expression level in the epidermis immediately after the eclosion is strongly associated with the phenotype. Furthermore, the genes involved in pigment biosynthesis are often pleiotropic. Thus, I predicted that *ebony* might have pleiotropic effects on some physiological traits. In this study, the mechanism of the transcription regulation of *ebony* in the developing epidermis was investigated by genome editing and the physiological traits associated with the *ebony* expression level were analyzed.

The majority of the expression level variation of *ebony* in the developing epidermis can be attributed to the variation in its *cis*-regulatory region. An epidermis enhancer (the primary epidermis enhancer, priEE) of *ebony* was located in its upstream intergenic region by a reporter assay in a previous study. However, no common nucleotide polymorphisms across worldwide populations were found within or around the priEE that could explain the variation in the *ebony* expression, the body darkness, or both.

Therefore, the sequence variation within the priEE is insufficient to explain the variations in the expression level of *ebony* and the associated body pigmentation.

To detect unknown *cis*-regulatory elements of *ebony*, first, I knocked out the priEE region by genome editing. There was no significant change in the body color. This result suggested that there are similar enhancer activities in the sequences outside the priEE. Also, the dark line along the dorsal midline on the abdomen disappeared by the priEE deletion. Next, the expression pattern of *ebony* was visualized by knocking in a fluorescent tag at the 3-prime end of this gene. While the wild-type control showed an absence of the fluorescence signal in the dorsal midline of the abdomen, the signal was present in the same region in the priEE-deleted individuals. Therefore, the priEE fragment includes a silencer that represses *ebony* expression at the abdominal midline. Furthermore, the silencer region was narrowed down to 351 bp by generating additional strains with partially deleted priEE. These results suggested that the interactions of multiple *cis*-regulatory elements act to form the spatially restricted expression patterns of *ebony* in the epidermis. Also, I demonstrated in this study that silencers can be effectively identified by combining reporter assays with deletion assays.

ebony encodes an enzyme that catalyzes dopamine to N-beta-alanyl dopamine. Previous studies have suggested that dopamine levels affect the composition of the cuticular hydrocarbons (CHCs), which are lipids secreted on the surface of the epidermal cuticle. CHCs are known to act as sex pheromones and are thought to be involved in determining the surface properties of the cuticle related to functions such as the protection against desiccation.

To find the physiological traits associated with *ebony*, the relationship between its expression level and the composition of CHCs was investigated using the Drosophila Genetic Reference Panel (DGRP), a natural population that originated from North America. The CHC profiles of the strains with low *ebony* expression were biased toward CHCs with long carbon chain lengths, while those with high *ebony* expression were biased toward short carbon chain lengths. The results indicated that the expression level of *ebony* is associated with the CHC profile. Furthermore, the dehydration speed was increased by knocking down and over-expressing *ebony* in the epidermis. However, the role of this gene in determining the variation of desiccation tolerance in natural populations was not apparent since no relationship was found between the dehydration speed and the expression level of *ebony* in the DGRP strains.

Taken together, the results from my study have advanced the understanding of the genetic basis of how the body color variation is controlled at the transcriptional level of *ebony* and the features of its pleiotropic effects on ecologically relevant physiological

traits.