Juvenile Hormone Regulates Butterfly Larval Pattern Switches

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The spectacular diversity of adult insect color patterns can also extend to differences among sequential larval instars within some species. The swallowtail butterfly, *Papilio xuthus*, represents such an example: Young caterpillars (from the first to the fourth instars) are mimics of bird droppings, whereas the larger, final larval instar (the fifth) has a completely different pattern that is well camouflaged among the leaves of the host plant (Fig. 1A). Here, we show that this developmental switch is regulated by juvenile hormone (JH), which is known to regulate the overall black or green forms caused by differences in environmental conditions in some larvae of Lepidoptera and some adults of Orthoptera (1, 2). We applied a JH analog (JHA) to the larval body pattern (table S1). Gray box indicates a JH-sensitive period. HCS indicates head capsule slippage, the clear sign of molting period. 20E, 20-hydroxyecdysone. Scale bars indicate 1 mm. (C) Model of JH effect on expression of genes associated with mimetic and cryptic pattern (fig. S1).

The major differences between the mimetic and the cryptic larval color patterns include the green coloration of the cryptic pattern, specific tubercle structures (arrowheads in fig. S1D) as components of the mimetic pattern, and the distribution of black pigment. We examined the effects of JHA treatment on gene expression associated with these three differences. By using cDNA subtraction methods, we cloned the hard cuticle protein genes (*HCP1* and *HCP2*) associated with the specific tubercle structures and the bilin-binding protein gene (*BBP*) that is only expressed at the final molt [supporting online material (SOM) text]. JH treatment induced the expression of tubercle-associated cuticle protein genes and inhibited BBP expression at the fourth molt, as is the case for the normal third molt (fig. S1, B and C). Larval black patterning is regulated by co-localization of melanin synthesis genes tyrosine hydroxylase (TH) and dopa decarboxylase (DDC) (3). The spatial expression patterns of these genes are changed to the mimetic pattern in the JH-treated specimen (fig. S1D). These results suggest that JH regulates the stage-specific gene expression pattern in *P. xuthus* (Fig. 1C) that is required to modulate the larval pattern from mimetic to cryptic.

Our results suggest that a high titer of JH induces the expression of genes associated with the mimetic pattern and that a decrease in JH titer causes a switch to the cryptic pattern. In addition to coloration, JH also regulates exoskeletal structures and pigment distribution at specific markings. Because progressive changes of green and black coloration and exoskeletal structures are frequently found in lepidopteran larvae, our findings imply that JH regulation on progressive larval pattern switches may commonly exist.

References and Notes
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Supporting Online Material
www.sciencemag.org/cgi/content/full/319/5866/1061/DC1
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SOM Text
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![Fig. 1. (A) Mimetic and cryptic pattern of *P. xuthus* larva. (B) Model of JH titer and its determination of larval body pattern (table S1). Gray box indicates a JH-sensitive period. HCS indicates head capsule slippage, the clear sign of molting period. 20E, 20-hydroxyecdysone. Scale bars indicate 1 mm. (C) Model of JH effect on expression of genes associated with mimetic and cryptic pattern (fig. S1).](image-url)