Evolution of a Polyphenism by Genetic Accommodation

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Polyphenisms are adaptations in which a genome is associated with discrete alternative phenotypes in different environments. Little is known about the mechanism by which polyphenisms originate. We show that a mutation in the juvenile hormone-regulatory pathway in *Manduca sexta* enables heat stress to reveal a hidden reaction norm of larval coloration. Selection for increased color change in response to heat stress resulted in the evolution of a larval color polyphenism and a corresponding change in hormonal titers through genetic accommodation. Evidently, mechanisms that regulate developmental hormones can mask genetic variation and act as evolutionary capacitors, facilitating the origin of novel adaptive phenotypes.

Polyphenisms, such as the castes of social insects, the solitary and gregarious phases of migratory locusts, and the winged and wingless forms of aphids, are evolved adaptations to a varying environment (*1*–*3*). The adaptive importance of polyphenisms has been demonstrated in many cases, and many studies have shown that the threshold for the switch between alternative phenotypes can evolve in response to external selective pressures (*4*–*9*). Although much work has been done on the evolutionary maintenance of polyphenisms and the evolutionary shifts of polyphenic thresholds (*10*, *11*), little is known about the evolutionary and developmental mechanism behind the origin of these threshold traits.

We tested the hypothesis that a polyphenism can evolve through genetic stabilization of a stress-induced phenotype, a process known as genetic assimilation (*2*). Because related species are likely to share genetic and developmental backgrounds, we reasoned that exposing hidden genetic variation by stress (*12*) may allow us to evolve a polyphenic regulatory mechanism in a monophenic species that shares a recent common ancestor with a polyphenic species. We studied this possibility by evolving a larval color polyphenism in the tobacco hornworm, *Manduca sexta*, a monophenic species with green larvae (*13*); a related species, *M. quinquemaculata,* exhibits a larval color polyphenism, developing a black phenotype at 20°C and a green phenotype at 28°C (*14*). Because thermal stress is commonly encountered in the wild (*15*), we chose to use temperature stress to obtain phenocopies (*16*).

Wild-type larval coloration was robust to thermal stress, with the fifth instar larva remaining green after heat shock during the mid and late fourth larval instar. We also examined the effect of thermal stress in the *black* mutant line of *M. sexta*. The *black* mutation is a sex-linked recessive allele that reduces juvenile hormone (JH) secretion (*17*), which results in an increased melanization of the larval epidermis. The black mutant phenotype can be rescued by treatment with JH (*17*), yielding a normal green-colored larva. Larvae of the *black* mutant are black at physiologically tolerable temperatures ranging from 20°C to 28°C (fig. S2). Heat shocks during the sensitive period of the fourth larval instar generated fifth instar larvae with colors that ranged from normal black to nearly normal green, with the majority showing a slight color change (Fig. 1 and fig. S2). The *black* strain was most sensitive to a 6-hour heat shock applied less than 8 hours before apolysis (the detachment of the epidermis from the cuticle, which is the first step in the molting process), at the molt from the fourth to the fifth larval instar (fig. S1).

The diversity of heat shock–induced phenotypes provided us with a range of phenotypic variants upon which we could artificially select. We established two lines: one selected for increased greenness upon heat treatment (polyphenic line), the other for decreased color change upon heat treatment (monophenic line). About 300 larvae were reared and heat-shocked every generation, and approximately 60 with the most desirable phenotypic response were selected to establish the subsequent generation. An unselected control line was heat-shocked every generation to monitor any change that was not a direct result of selection. The response to selection (Fig. 2A) shows that the induced color change is heritable. The variation in the phenotype is continuous rather than discrete, which indicates that the induced color change is under polygenic control. The monophenic line lost its response to temperature shock after about the seventh generation of selection and remained black thereafter, with little phenotypic response to heat shock.

The reaction norms of the three lines in the 13th generation are shown in Fig. 2B. The unselected control line has a narrow threshold between 30°C and 33°C, with the inflection point at 32.7°C. As a result of selection, two major evolutionary changes have taken place in the polyphenic line: (i) completely green coloration at lower temperatures of 28°C, not seen in the control line (fig. S2), and (ii) a threshold shift to a lower temperature with the inflection point at 28.5°C. The monophenic line remained black at all temperatures. Thus, selection resulted in the evolution of different phenotypes at different constant environmental temperatures (fig. S2) and changed the shape of the reaction norm (Fig. 2B) so that the response to a small temperature change in the transition region became more discrete, or switchlike.

The time of the sensitive period for heat shock corresponds to the time of the JH-sensitive period for epidermal color determination (*17*, *18*). Topical application of JH to unselected black mutant during this sensitive period reverses the black phenotype to the green wild-type color. Dopa decarboxylase (DDC), the enzyme that converts dopa to dopamine in the melanin synthesis pathway, is first synthesized about 16 hours after this sensitive period (*19*), which indicates that heat shock

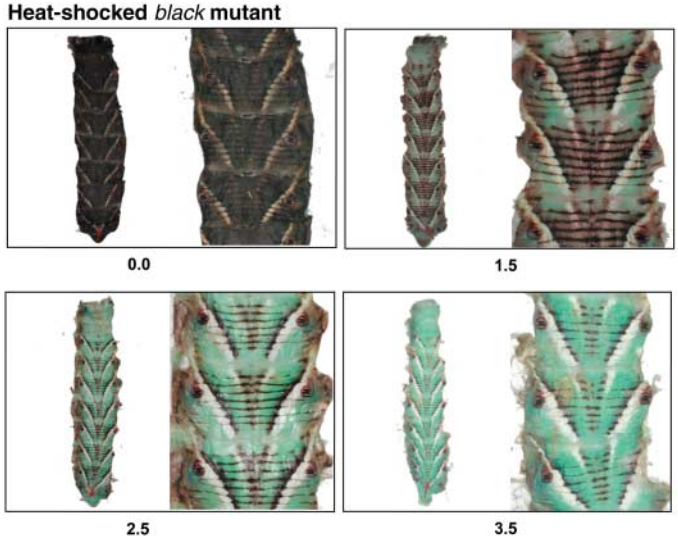

Fig. 1. The range of larval coloration observed in the heat-shocked larvae of the *black* mutant. The numbers below represent the scoring system used to quantify the color change: 0 is completely black, and 4 is completely green. Non–heat-shocked *black* mutant and non–heat-shocked wild-type larvae of *M. sexta* have the phenotypic scores of 0 and 4, respectively.

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affected an upstream regulatory control of melanin synthesis. Because JH and ecdysteroids have been implicated in the regulation of melanization (*19*) and because these hormones play a major role in the control of most insect polyphenisms (*18*), we determined whether the polyphenic and monophenic lines differed in the hormonal regulation of melanin synthesis. Hormones are secreted from either the prothoracic glands in the thorax (e.g., ecdysone) or the brain and corpora allata in the head (e.g., JH). A blood-tight ligature across the body of the larvae allows us to investigate which hormones might be involved (Fig. 3A). When the ligature was placed behind the first abdominal segment, the anterior compartment of larvae from the polyphenic line changed color upon heat shock, but the posterior compartment did not (Fig. 3B and fig. S3). The monophenic line remained black both anterior and posterior to the ligature (Fig. 3C and fig. S3). When the ligature was placed behind the neck, no color change response to heat shock was observed in either line, indicating that a cue from the brain/corpora allata was required for the color change (fig. S4).

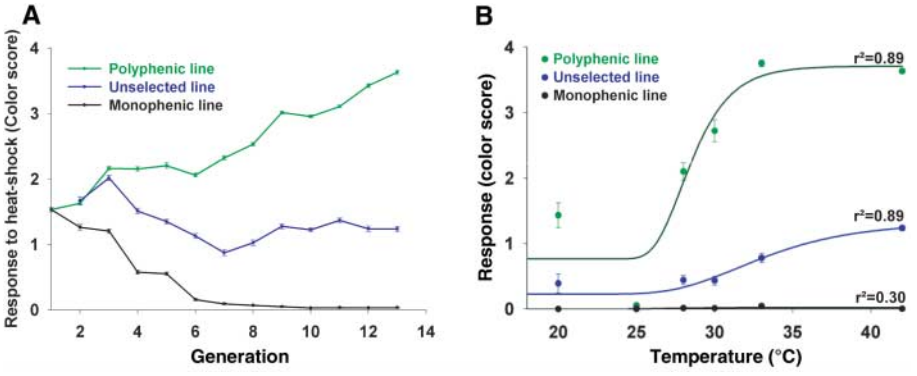

Fig. 2. Effect of selection on temperature-mediated larval color change. (**A**) Changes in the mean coloration of heat-shocked larvae in response to selection for increased (green) and decreased (black) color response to heat-shock treatments, and no selection (blue). (**B**) The reaction norm of generation 13 lines reared at constant temperatures between 20°C and 33°C, and heat-shocked at 42°C. The curves are sigmoidal regressions on the mean data points. Error bars represent 1 SE.

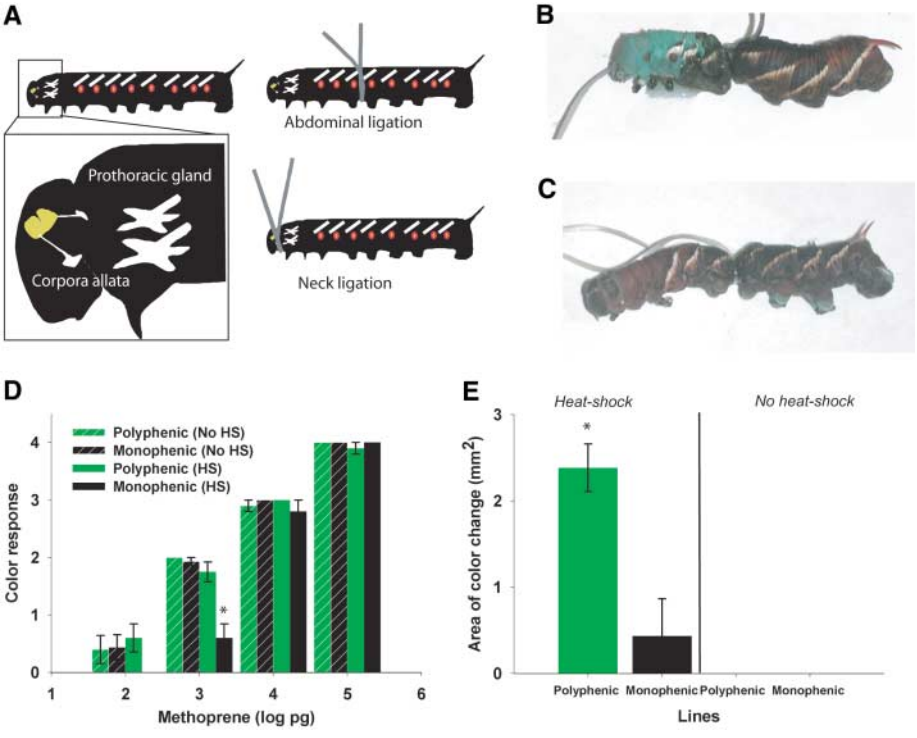

Fig. 3. The hormonal control in larvae of the polyphenic and monophenic lines. (**A**) Abdominal and thoracic ligations result in the exclusion of both corpora allata (secretes JH) and prothoracic gland (secretes ecdysone) posterior to the ligation. Neck ligation results in the exclusion of only the corpora allata posterior to the ligation. (**B** and **C**) Heat-shocked, ligated larvae from the polyphenic (B) and monophenic (C) lines. (**D**) Effect of methoprene treatment on thoracically ligated larvae of the polyphenic (green bars) and monophenic (black bars) lines with (filled) and without (diagonal) heat shock (HS). (*$P < 0.0001$ compared with non–heat-shocked monophenic line). (See table S2 for raw data.) (**E**) Results from the JH bioassay. The area of color change reflects the dose of hemolymph JH in polyphenic (green bars) and monophenic (black bars) larvae (*$P < 0.001$ compared with heat-shocked monophenic line). Error bars represent 1 SE. Statistical significance is based on a two-tailed Student's *t* test.

The polyphenic and the monophenic lines may therefore differ in secretion or degradation of JH, sensitivity to JH, or molecular interactions downstream of JH. To distinguish between these mechanisms, we topically applied the JH analog, methoprene, to larvae ligated behind the mesothorax. These larvae were either maintained at 25°C or heat shocked. No difference in the response to JH application was observed between the heat-shocked and non–heat-shocked polyphenic lines and the non–heat-shocked monophenic line. The monophenic line exhibited reduced sensitivity to methoprene when heat-shocked (Fig. 3D). Thus, the ability to change in the polyphenic line is in part due to a change in JH secretion or degradation, not the sensitivity to JH, and the monophenic line evolved to be less sensitive to JH.

We compared the JH titers of the two lines during the sensitive period using a JH bioassay (*20*). The results show that polyphenic larvae, when heat-shocked, had higher JH titer during the critical period than did monophenic larvae (Fig. 3E). Thus, selection for increased color change was accompanied by an increased JH titer during the heat shock.

Thus, changes in hormonal regulation may underlie the evolution of a larval color polyphenism. Our results provide an example of the quantitative genetic model for genetic accommodation (*3*). Genetic accommodation is a mechanism of evolution wherein a novel phenotype introduced through a mutation or environmental change is molded into an adaptive phenotype through quantitative genetic changes. Genetic accommodation differs from genetic assimilation in that the latter results in canalization of the new phenotype so that it is no longer affected by environmental variation, whereas genetic accommodation can result in an increased environmental sensitivity of a plastic phenotype (*3*). Because the *black* mutation was necessary to predispose the population to reveal genetic variants through heat shock, and the final result was an enhanced response to the environment, with alternative canalizations in different environments, genetic accommoda-

tion is the correct description of the observed results.

We present here a mechanistic view of the evolution of polyphenisms by genetic accommodation: First, a mutation in the hormonal regulatory pathway (the *black* mutation in the current study) lowers the hormonal titer in such a way that environmental variation can expose genetic variants. This is followed by selection on modifier genes, which shift hormonal titers or hormonal response (the JH titer in this study). This results in the evolution of either the threshold, or the population distribution about the threshold (*21*), in such a way that the population crosses the phenotypic threshold in response to temperature changes (Fig. 4). The genetic accommodation step results in both lowering of the threshold temperature and an increase in the steepness of the threshold, so the traits become more discrete.

The wild-type population is too far from the threshold, and no temperature fluctuation is sufficient to cross the threshold. Thus, we can think of the *black* mutation as a sensitizing mutation that brings the JH titer of the population closer to the threshold. Given the robustness of most traits to environmental perturbations, it is likely that many traits have thresholds that cannot be crossed without an initial sensitizing mutation that alters a homeostatic mechanism. After plasticity is exposed through the sensitizing mutation, selection can act to genetically accommodate the novel alternative phenotype.

Previous studies have shown that heat-shock proteins (e.g., *hsp90*) can act as capacitors for genetic variation (*22*, *23*). Our results show that mechanisms that control developmental hormones may also act as capacitors for genetic variation. Genes that maintain the titer of a developmental hormone far above its threshold of activity can mask mutations in genes that alter the secretion of, or response to, the hormone. Such genes can therefore allow the accumulation of silent mutations whose effects only become evident when normal regulation of the hormone is disrupted. Hormones have critical and widespread roles in the regulation of postembryonic development (*18*, *22*), and genetic variation in hormonal regulation likely plays an important role in the evolution of postembryonic developmental processes (*3*).

Our results suggest that the evolution of highly nonlinear reaction norms, such as polyphenisms, does not depend on the origin of novel favorable mutations that produce a threshold response (*3*). Rather, mutations in the mechanism that controls hormone titer can shift the phenotypic threshold and reveal previously covert genetic variation. Subsequent small-scale changes in hormone titer, or in the timing of hormone secretion, can reveal progressively more genetic variation upon which selection can act to cause a gradual heritable shift in the threshold. Thus, although the loss of condition-dependence can occur by a single mutation (*24*), drift (*25*), or selection (this study), the evolutionary origin of discontinuous reaction norms involves a complex interplay of sensitizing mutations, environmental fluctuations, and quantitative genetic variation.

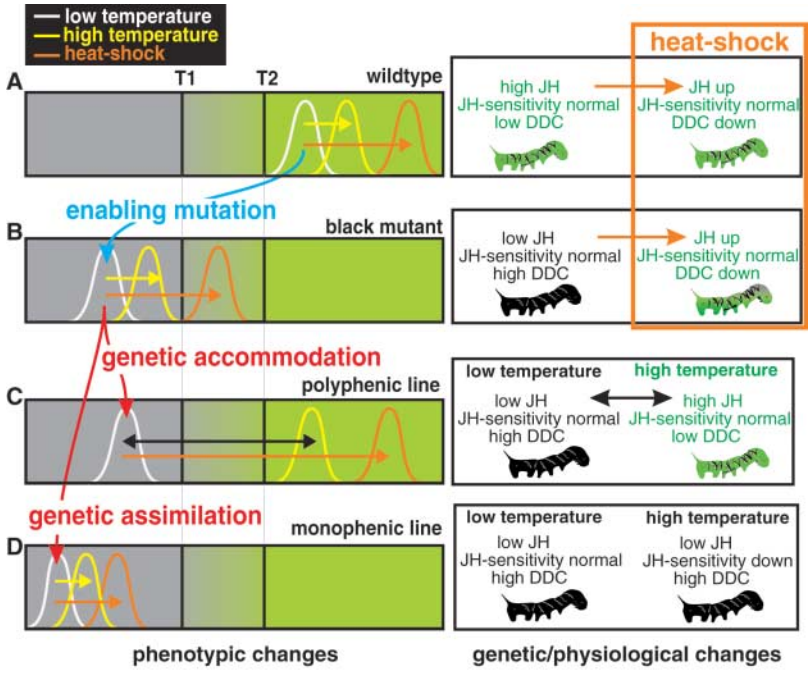

Fig. 4. (Left) Model for the evolution of a threshold trait at the phenotypic level. The evolutionary process required for the evolution of a threshold trait depends on the proximity of the population to the two thresholds (T1 and T2). Below T1, the phenotype is all black. Above T2, the phenotype is all green. Between T1 and T2, individuals express some intermediate phenotype. If the physiological control lies far from the phenotypic threshold (**A**), a mutation of larger effect or a sensitizing mutation is required to bring the population closer to the threshold (**B**). Once the population is closer to the threshold, the population can evolve a threshold response through genetic accommodation (**C**) or become canalized through genetic assimilation (**D**). (Right) The corresponding changes at the genetic/physiological level observed in this study. Unidirectional arrows indicate high-temperature–induced (yellow) and heat-shock–induced (orange) shifts. Bidirectional arrows indicate polyphenic shifts induced by temperature shifts.

References and Notes

1. A. D. Bradshaw, *Adv. Genet.* **13**, 115 (1965).
2. A. M. Shapiro, *Evol. Biol.* **9**, 259 (1976).
3. M. J. West-Eberhard, *Developmental Plasticity and Evolution* (Oxford Univ. Press, New York, 2003).
4. N. A. Moran, *Am. Nat.* **139**, 971 (1992).
5. D. A. Roff, *Genetics* **136**, 395 (1994).
6. D. J. Emlen, *Evolution Int. J. Org. Evolution* **50**, 1219 (1996).
7. A. P. Moczek, H. F. Nijhout, *Evol. Dev.* **4**, 252 (2002).
8. A. P. Moczek, *Behav. Ecol.* **14**, 841 (2003).
9. J. L. Tomkins, G. S. Brown, *Nature* **431**, 1099 (2004).
10. C. D. Schlichting, M. Pigliucci, *Phenotypic Evolution: A Reaction Norm Perspective* (Sinauer, Sunderland, MA, 1998).
11. A. P. Moczek, H. F. Nijhout, *Evol. Dev.* **5**, 259 (2003).
12. G. Gibson, I. Dworkin, *Nat. Rev. Genet.* **5**, 681 (2004).
13. I. J. Kitching, *Zool. J. Linn. Soc.* **135**, 471 (2002).
14. A. Hudson, *Can. J. Zool.* **44**, 541 (1966).
15. T. M. Casey, *Ecology* **57**, 485 (1976).
16. Mortality rate increases above 30°C in the last larval instar (*26*), but short-term heat stress has no discernible effect on mortality.
17. L. Safranek, L. M. Riddiford, *J. Insect Physiol.* **21**, 1931 (1975).
18. H. F. Nijhout, *Insect Hormones* (Princeton Univ. Press, Princeton, NJ, 1994).
19. K. Hiruma, L. M. Riddiford, *Int. J. Insect Morphol.* **22**, 103 (1993).
20. M. J. Fain, L. M. Riddiford, *Biol. Bull.* **149**, 506 (1975).
21. J. M. Rendel, *Canalization and Gene Control* (Logos Press, Academic Press, New York, 1967).
22. S. L. Rutherford, S. Lindquist, *Nature* **396**, 336 (1998).
23. A. Bergman, M. L. Siegal, *Nature* **424**, 549 (2003).
24. D. B. Rountree, H. F. Nijhout, *J. Insect Physiol.* **41**, 1141 (1995).
25. E. Abouheif, G. A. Wray, *Science* **297**, 249 (2002).
26. C. Petersen, H. A. Woods, J. G. Kingsolver, *Physiol. Entomol.* **25**, 35 (2000).

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Supporting Online Material

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Figs. S1 to S4
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