Evolution of polyphenism in the Lepidoptera *Manduca sexta*

A polyphenic trait is a trait for which several, discrete phenotypes can arise from a single genotype as a result of differing environmental conditions. It is therefore a special case of phenotypic plasticity.

Wild-type larvae of the lepidopteran species *Manduca sexta* are green, irrespective of their breeding temperature. This article investigates the mechanisms underlying a larval color polyphenism that appeared in the laboratory in *M. sexta*, through genetic stabilization of a stress-induced phenotype.

The color of larvae raised at two different temperatures (20°C and 28°C) was assessed for *M. sexta* and for it closely related species *Manduca quinquemaculata*.

	20°C	28°C
Wild-type *M. sexta*	green	green
Wild-type *M. quinquemaculata*	black	green
M. sexta black mutant	black	black
F1 hybrid female between *M. sexta black* mutant females and wild-type males	green	green
F1 hybrid female between *M. sexta black* mutant males and wild-type females	black	black
F1 hybrid male between *M. sexta black* mutant females and wild-type males	green	green
F1 hybrid male between *M. sexta black* mutant males and wild-type females	green	green

Table 1. Larval color of various genotypes raised at various temperatures.

What is the observed difference between M. sexta and M. quinquemaculata?
What can you conclude about the black mutation?

Juvenile hormone (JH) secretion is reduced in the *black* mutant compared to the wild-type in developing larvae. Treatment with Juvenile hormone (JH) of the black mutant rescues the green phenotype.

What can you conclude about the effect of the black mutation?

Black mutant animals were raised at 28°C and 6-h heatshock pulses at 48°C were applied during the fourth instar larval stage. The color of fifth instar larvae were recorded as a color score.

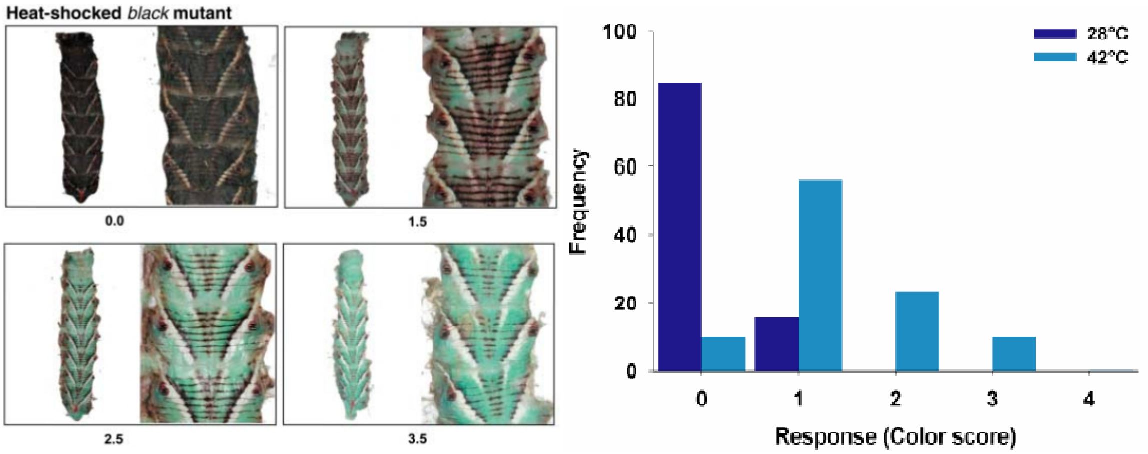

Figure 1. Larval coloration observed in heat-shocked *black* larvae. Numbers below correspond to color score (0=completely black, 4=completely green). Wild-type larvae have a score of 4. Distribution of color scores for *black* animals raised at 28°C and subjected to a 6-h heatshock pulse (light blue) or not (dark blue).

What can you conclude from this experiment?

The phenotypic variants were used to perform an artificial selection experiment. Two lines were established: one selected for increased greenness upon heat-shock (polyphenic line), one selected for decreased color change upon heat-shock (monophenic line). About 300 larvae were heat-shocked at each generation and approximately 60 with the most desirable phenotypic response were selected to establish the subsequent generation. An unselected line was also heat-shocked at each generation.

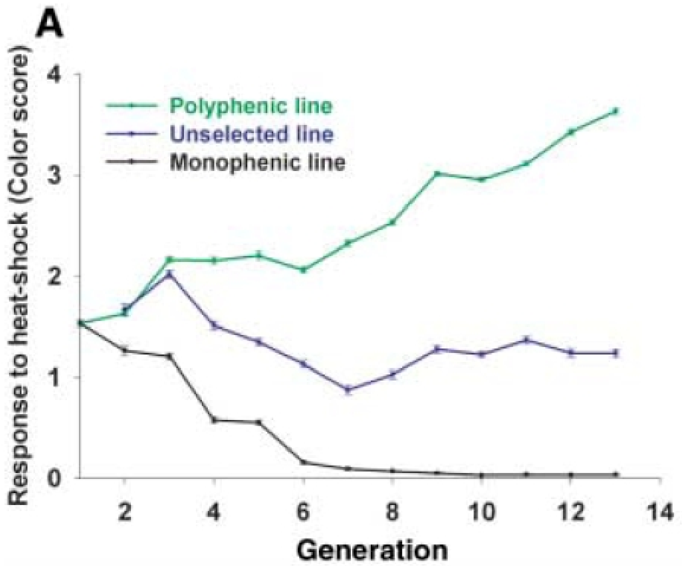

Figure 2. Changes in the mean coloration of heat-shocked larvae in response to selection for increased (green) and decreased (black) color response to heat-shock treatments and no selection (blue).

Why did the researchers analyze an unselected line?
What can you conclude from this selection experiment?
What can you conclude from the shape of the curves for the polyphenic line and for the monophenic line?

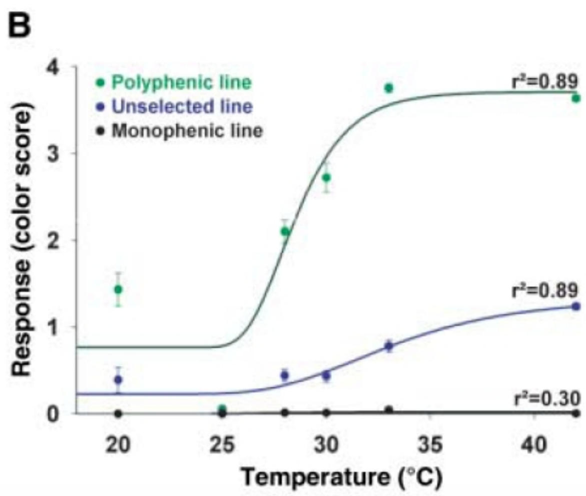

Figure 3. Reaction norms of generation 13 lines reared at constant temperature between 20°C and 33°C and heat-shocked at 42°C. The curves are sigmoid regressions on the mean data points. Error bars represent 1 SE.

What can you conclude from this graph?
The unselected line can now survive at raising temperatures higher than 30°C, whereas wild-type and *black* mutants cannot.
How can you explain this change?

The time of sensitive period for the heat-shock corresponds to the time of the JH-sensitive period for epidermal color determination.
Which experiments were performed to draw such a conclusion?

JH and ecdysone are known to be involved in many insect polyphenisms. Ecdysone is secreted from the prothoracic glands in the thorax, whereas JH is secreted by the corpora allata in the head. This allows the role of these hormones to be tested by tight ligation across the body of the larvae.

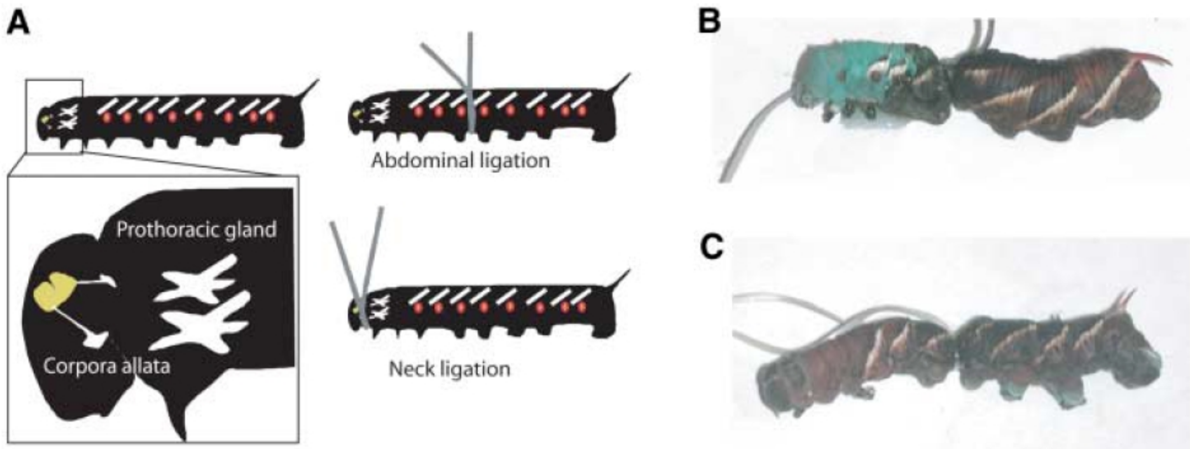

Figure 4. A: scheme of the two types of ligations that were performed. B and C: Heat-shocked, ligated larvae from the polyphenic (B) and the monophenic (C) lines. When the ligature was placed behind the neck, no color change response to heat-shock was observed in either line (not shown).

What is the consequence of neck ligation posterior to the ligation? And of abdominal ligation?
What can you conclude from Fig. 4? (check images and legends)

Methoprene, a JH analogue, was applied to heat-shocked and thoracically ligated larvae of the polyphenic or monophenic lines.

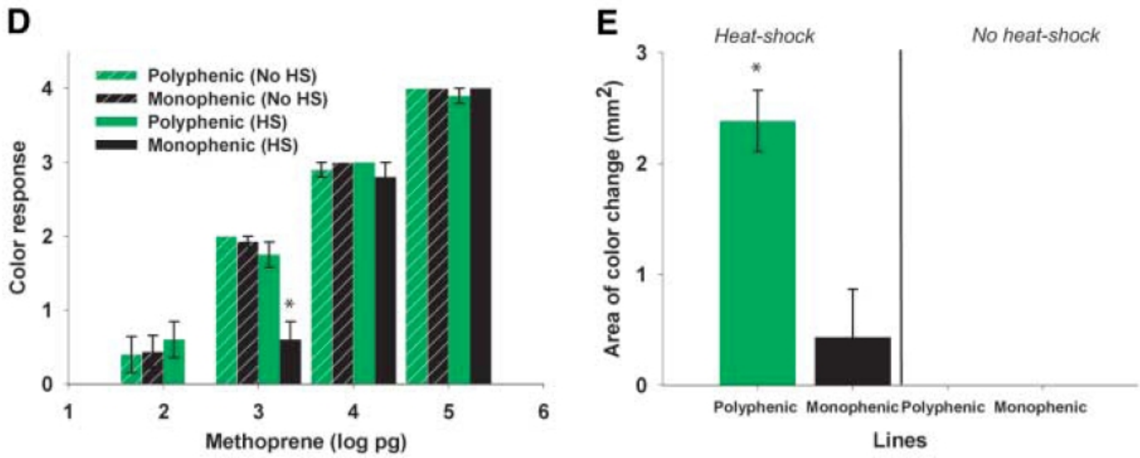

Figure 5. D: Effect of methoprene treatment on thoracically ligated larvae of the polyphenic (green bars) and monophenic (black bars) lines with (filled) and without (diagonals) heat-shock. * $p<0.0001$ compared with non heat-shocked monophenic line.

E: JH titers of the two lines were compared during the sensitive period using a JH colorimetric bioassay. The area of color change reflects the dose of hemolymph JH in polyphenic (green bars) and monophenic (black bars) larvae. * $p<0.001$. Error bars: 1 SE.

What can you conclude from Fig. 5D?

What can you conclude from Fig. 5E?

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.

Would you qualify the presented results as genetic accommodation or genetic assimilation?

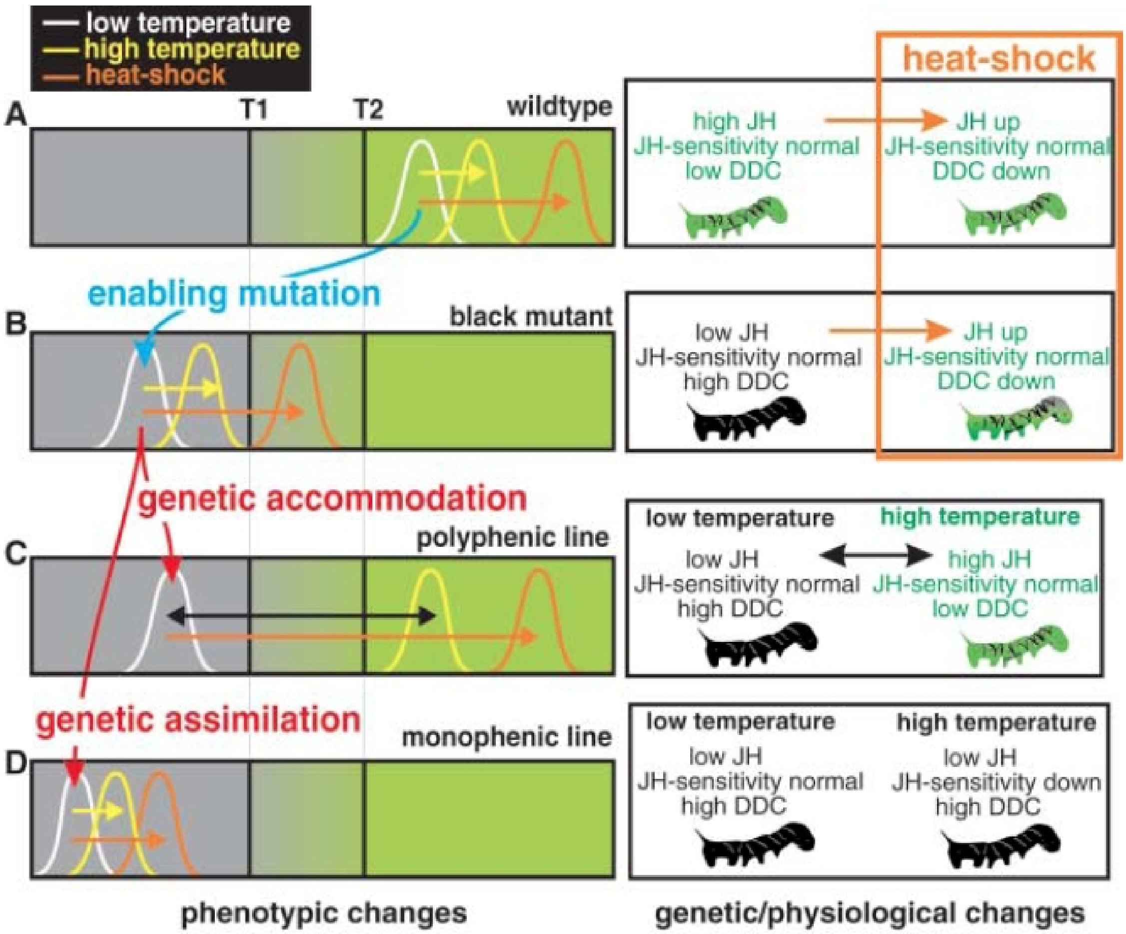

Figure 6. Model proposed by the authors. T1 and T2 represent two thresholds. The position of the populations relative to the thresholds condition the pigmentation phenotype.

Describe the model. What is known about the "enabling mutation"?

What kind of experiment can you suggest to identify the genetic differences associated with larval color between the polyphenic and the monophenic lines?

The genetic basis of the polyphenism was investigated using crosses between the polyphenic line and the monophenic line. Larvae were reared at 25°C followed by heat-shock at the temperature sensitive period. Pigmentation of fifth-instar larvae was scored and compared to that of the parental strains.

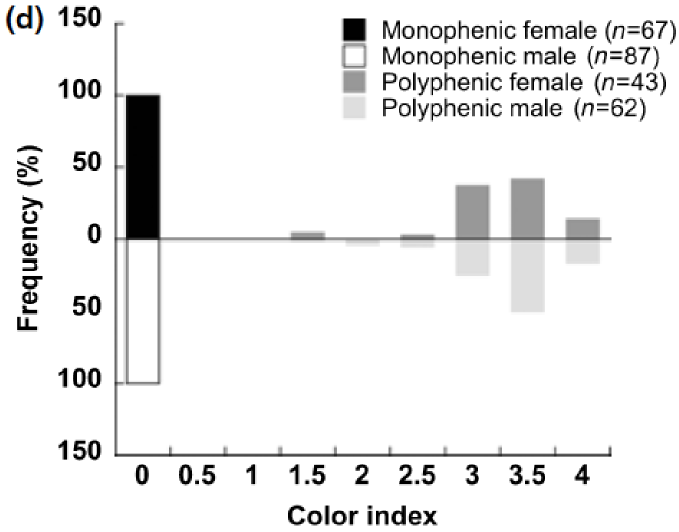

Figure 7. Pigmentation of the parental lines.

How do the new experimental conditions compare with the reaction norm of Fig. 3? Why did the authors choose these new experimental conditions rather than raising all individuals at 35°C?

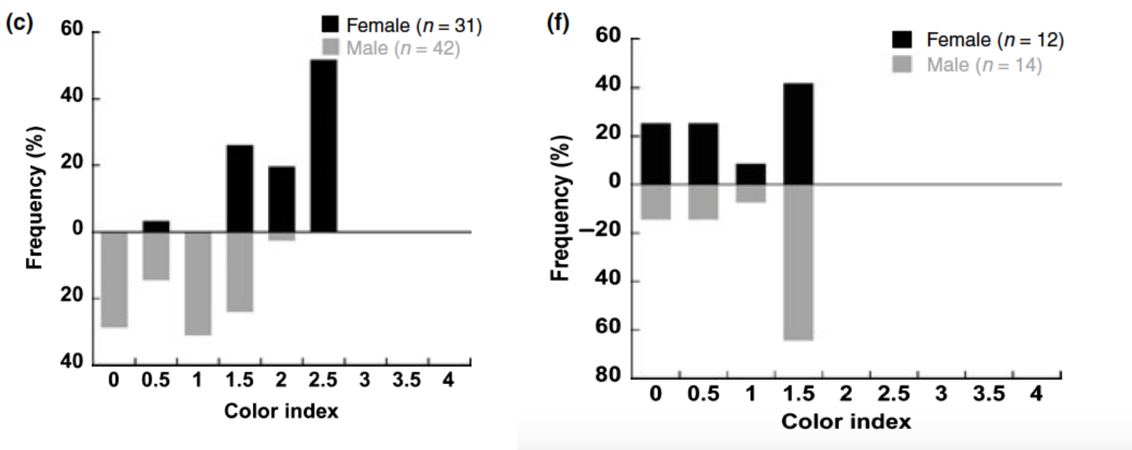

Figure 8. Pigmentation of F1 larvae obtained from a cross between (c) monophenic females and polyphenic males or (f) polyphenic females and monophenic males.

What can you conclude from Fig. 7-8?

Which experiment would you suggest to map the gene(s) underlying larval color between the polyphenic and the monophenic lines?

Further reading

Yuichiro Suzuki and H. Frederik Nijhout, 2006. *Evolution of a Polyphenism by Genetic Accommodation.* Science 311, 650-652.
Yuichiro Suzuki and H. Frederik Nijhout, 2008. Genetic basis of adaptive evolution of a polyphenism by genetic accommodation. Journal of evolutionary Biology. 21, 57-66.