

H. melpomene crosses carried out by James Mallet at University of Texas, Austin, with help from Debbie Ferrara of Flushing, New York (a high-school intern) and Barbara Green, a UT undergraduate, 1979-1980

Introduction

A total of 962 offspring were produced in 19 broods. For pictures of broods, see: <http://www.ucl.ac.uk/taxome/texasbroods/> (unfortunately, there are a few errors in annotation, so any queries welcome!). Broods shown are: C2-C17, C19. (C1 – the first F1 is uniform so not shown online. Some other small or uninformative broods are also not shown).

This document was written up as a brief explanation from memory, without careful reference to the brood data itself. It should help to explain the result to anyone interested in these data, but may contain some errors. To understand this document, you should know something about *Heliconius* genetics; I suggest Mallet, J. (1989. "The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*." Proceedings of the Royal Society of London Series B: Biological Sciences 236: 163-185) is a useful introduction to N, B, and Yb. Feel free to send specific queries to Jim Mallet at Harvard or UCL.

The dried specimens are now I believe in Chris Jiggins' laboratory in Cambridge.

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Aims

The purpose was to investigate the true linkage distance between N-Yb, and to determine if this varied between broods as Sheppard, P. M., et al. (1985. "Genetics and the evolution of muellerian mimicry in *Heliconius* butterflies." Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 308: 433-613) had suggested, see pp. 472-474. I was also interested in testing using much larger broods than hitherto the possibility that there might be crossing over between linked genes in females (thought to be absent in Lepidoptera).

Sheppard et al. found in most broods that N and Yb were tightly linked with recombination rate $c \sim 1-2\%$, although in other broods it was as high as 27%. It was also of interest to try to see if any crossing over occurred in females.

The parents of the original F1 cross (brood C1) were:

A. Females from Costa Rica $N^B N^B ybyb BBdd$, red forewing postman pattern with yellow hindwing bar and...

B. Males from "Venezuela" (actually from the border region, near San Jose de Guaviare, Colombia) $N^N N^N YbYb BB-dd$. This stock was from a hybrid zone between postman and rayed races, and consisted of forewing yellow bands, sometimes fringed on the outside with red (the effect of the B gene), with no hindwing bar, or dennis, or rays.

You can see from the discussion in Sheppard et al. that it became very complicated in part because they'd proceeded in their crosses with no fixed plan. However, if you're interested in linkage, a "test-cross" of doubly heterozygous F1s with a doubly homozygous recessive is the most useful strategy (because all recombinants are revealed).

I planned to make use of the fact that both the Costa Rican N^B and y^b alleles are the recessives, so the Costa Rica stock is a useful double-recessive test-crossing strain. (By the way, $N^N N^B$ heterozygotes are difficult to distinguish from $N^N N^N$ homozygotes whatever Sheppard or Turner said!, and the shadow band of $Y^b y^b$ heterozygotes is also very hard distinguish in some individuals).

Results

Briefly the results were as follows:

1) Backcrossing the F1s to Costa Rica-like phenotypes did sometimes produce some apparent $N^N N^B y^b y^b$ recombinants in rather too high amounts, although the expected reciprocal genotype: $N^B N^B y^b y^b$ was much rarer (the reciprocals are expected to be equally common). Furthermore, the recombination was found even when the females were the doubly heterozygous parent – this should not occur if there was recombination in females. Therefore these "recombinants" were suspect.

2) In one case, I was able to show this was a recessive effect, because apparent $N^N N^B y^b y^b$ bred "true." In other words, the parents could not have been heterozygous. I also showed that apparent $N^N N^B y^b y^b$ showed up at a rate of around 25% in a brood where both parents were putatively $N^B N^B y^b y^b$; this is as expected if there is a third, recessive gene that is heterozygous in both parents that causes the effect.

3) I postulated another gene, M/m , causes this recessive effect of apparently making $N^B N^B$ homozygotes appear like $N^N N^B$ heterozygotes. Thus the newly inferred genotypes of the original parent races are:

Costa Rica $N^B N^B y^b y^b BB dd MM$, red forewing postman pattern with yellow hindwing bar and

Colombia $N^N N^N Y^b Y^b B-dd mm$ with yellow forewing bands, no yellow hindwing bar and variable presence of thin red streaking in the outer part of the fw band (caused by polymorphism at B/b).

The hypothesis of the M gene can explain (2) above. The double recessive mm presumably enhances the action of N^N in some way, or it may be required for yellow pigment expression in N^N genotypes perhaps in a single dose. But it has not been investigated further.

4) I was able to show that M is unlinked to Y^b because even doubly heterozygous female parents produce 50% recombinant gametes. But I didn't have suitable B/D containing strains, so whether it was linked to the other major colour pattern locus was not investigated further. However, when I gave my brood specimens to Simon Baxter in Jiggins' group, he was able to extract DNA and showed that the M effect indeed appears to be linked to B/D , using a small number of individuals.

5) In conclusion, N is indeed tightly linked to Y^b with $c \sim 1-2\%$, and the recombinant chromosomes only appear in male parents (so there is no crossing over in females for this locus – I've got pretty big broods showing this). The apparent recombinants were much commoner than expected under that model, and in spite of the rarity of the reciprocal. The apparent recombinants are simplest to explain as follows: apparent heterozygous $N^N N^B$ phenotypes are produced in $N^B N^B$ homozygote genotypes because that individual is also homozygous for alleles at an unlinked locus M , that presumably originated from an Amazonian yellow-banded race.