

Genomes and Phenotypes Exam

17 November 2022

You can answer in English or French. Documents are not allowed.

I. Oakleaf butterflies

Doleschallia bisaltide is a butterfly species from Asia. They resemble dead leaves when their wings are closed.



Fig. 1. *Doleschallia bisaltide* butterfly. Left: wings open (dorsal side), right: wings closed (ventral side).

1) What may be the fitness advantage of mimicking dead leaves? Propose an experiment to test your hypothesis. (2 pts)

The wings of all *Doleschallia bisaltide* individuals look similar. In contrast, 10 categories of wings mimicking dead leaves can be found in the butterfly species *Kallima inachus* (Fig. 2A, next page).

2) In your opinion, for which reasons have the wing patterns shown in Fig. 1 been considered by taxonomists to belong to the same species? Same question for the wing patterns shown in Fig. 2A. (2 pts)

The genome of 78 *Kallima inachus* specimens collected in China was sequenced. Genome-wide association mapping was performed to compare the different forms.

3) Interpret the genome-wide association mapping results presented in Fig. 2C-D. (2 pts)

4) Based on Fig. 2B, what are the dominance relationships between the five alleles? (2 pts)

Butterfly wings are covered with scales and the gene *cortex* is known to regulate scale development in *Heliconius* butterflies. To test this candidate gene, CRISPR-Cas9-mediated genome editing was used to obtain mutations in *cortex* in *Kallima* butterflies.

5) Explain briefly (<10 lines) the protocol for CRISPR-Cas9-mediated genome editing in butterflies **and** the genotype of the resulting animals. (3 pts)

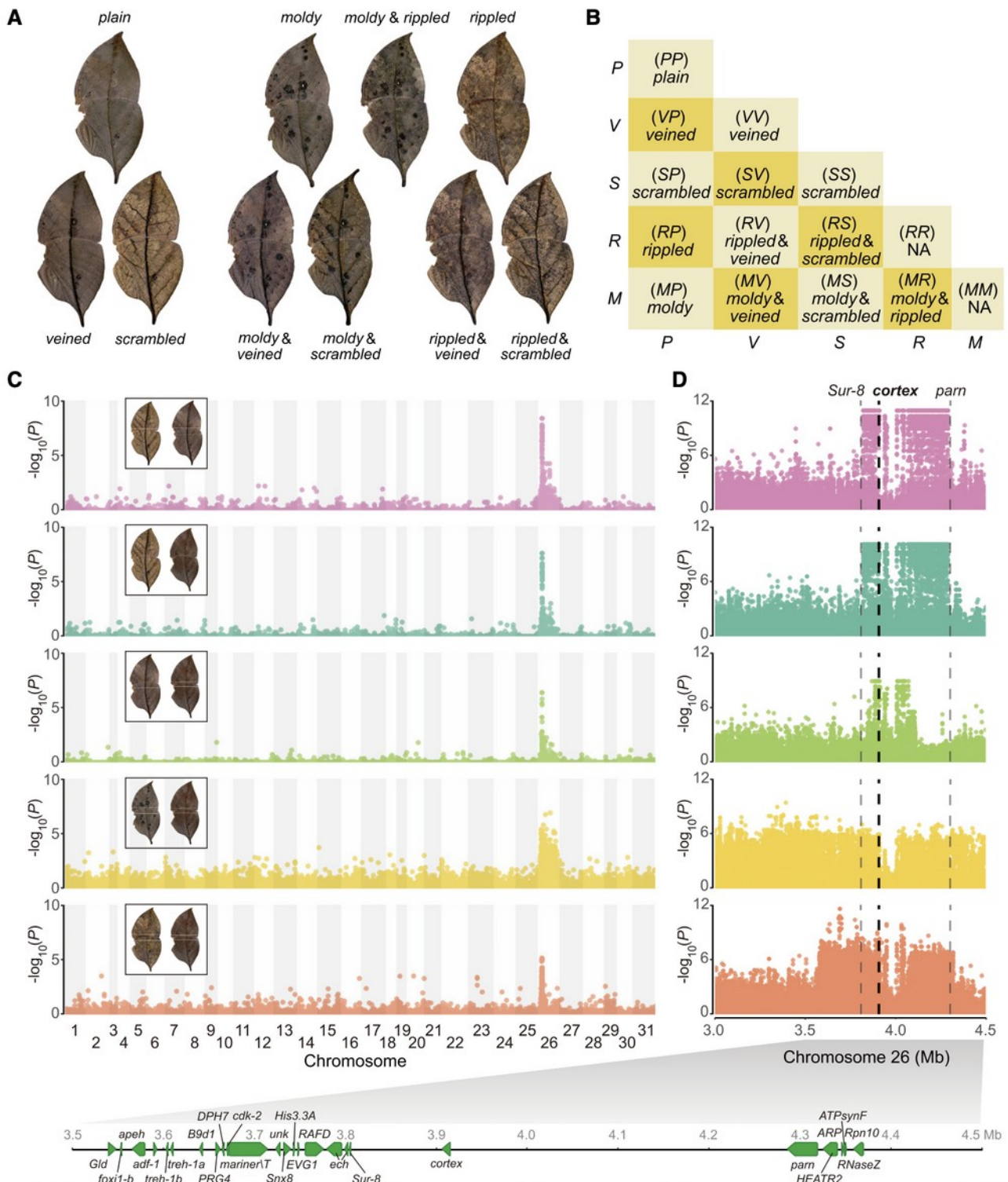


Fig. 2. Genetics of leaf wing polymorphism in *K. inachus*. (A) Ten ventral wing forms (ventral side). (B) Five alleles and their phenotypic effect on leaf wing patterns. NA: not obtained. (C) Multiple genome-wide association studies performed on *veined* versus *scrambled* forms, *plain* versus *scrambled* forms, *plain* versus *veined* forms, *moldy* versus *plain* forms, and *rippled* versus *plain* forms. (D) Magnification of part of chromosome 26.

Butterflies subjected to CRISPR-Cas9-mediated genome editing displayed wings with aberrant phenotypes. Wing tissues showing mutant phenotypes were photographed and then dissected to extract DNA. A PCR was performed using primers flanking the CRISPR-Cas9 target region. In wild-type individuals, a PCR fragment of 301 bp was obtained.

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WT      GTGTCACCAGACGCCCCGCTACGTCGCTGTCACTACTGTTAACTCAGCTG
deletion 1  GTGTCACCAGACGCC-----CGCTGTCACTACTGTTAACTCAGCTG
deletion 2  GTGTCACCAGACGCCCGCTA----GCTGTCACTACTGTTAACTCAGCTG
deletion 3  GTGTCACCAGACGCC-----CGCTGTCACTACTGTTAACTCAGCTG
deletion 4  GTGTCACCAGACG-----TCGCTGTCACTACTGTTAACTCAGCTG
  
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Fig. 3. Partial PCR sequences of the *cortex* locus obtained after CRISPR-Cas9-mediated genome editing in *K. inachus*.

6) Based on Fig. 3, draw schematically one electrophoresis gel obtained after the PCR experiment with DNA extracted from:

- wing tissue from a wild-type individual
 - wing tissue from a CRISPR-Cas9 individual carrying deletion 1 (Fig. 3)
 - wing tissue from a CRISPR-Cas9 individual carrying deletion 2 (Fig. 3)
- (2 pts)

7) The mutations presented in Fig. 3 are located in a coding region of *cortex*. What is their expected effect on the protein? (1 pt)

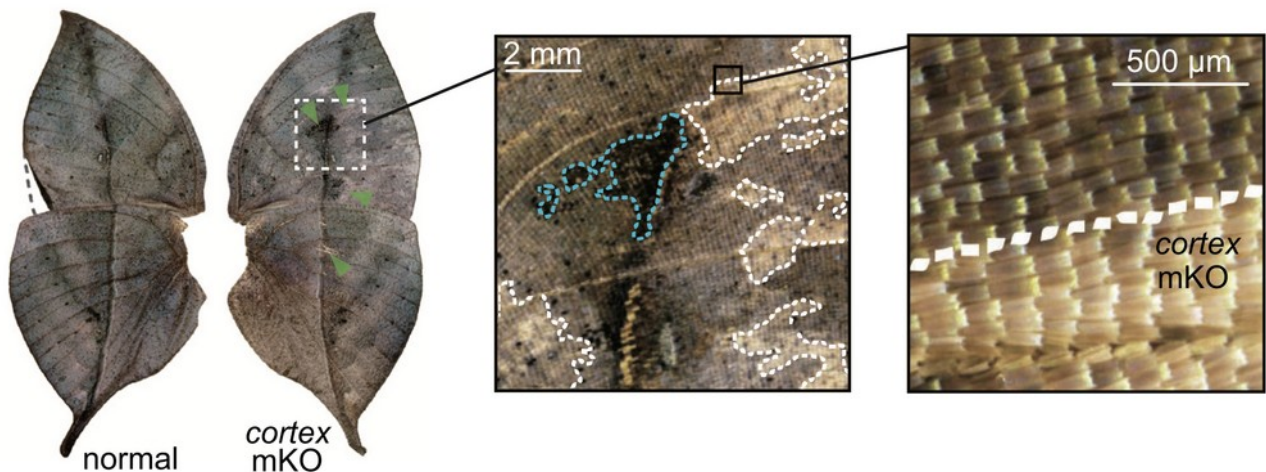


Fig. 4. Whole wing (ventral side) of a veined individual with no treatment (left) or obtained after CRISPR-Cas9 genome editing at the *cortex* locus (right). Blue and white dashed lines indicate the border of the *cortex* loss-of-function mutant clones. *Cortex* loss-of-function results in faded scale pigmentation around the lateral vein region and in a blurred and darkened pigmentation around the main leaf midrib vein. Green arrows point to various *cortex* knock-out (KO) clones.

8) Based on Fig. 4, does *cortex* regulate scale cell pigmentation in *K. inachus* butterflies? What is the effect of *cortex* on *Kallima inachus* scales? (1 pt)

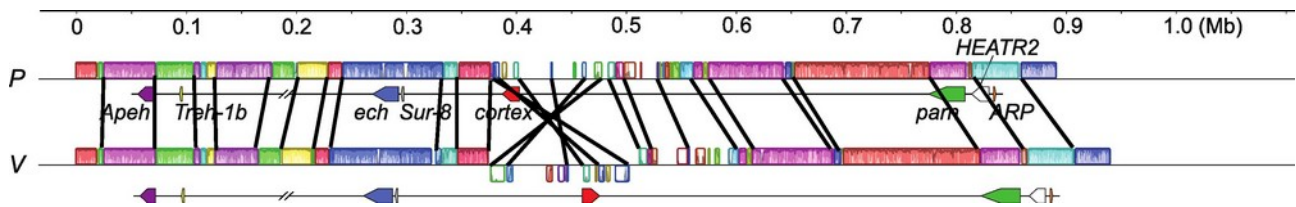


Fig. 5. Comparison of the chromosomal structure around the *cortex* coding region between the plain (P) and the veined (V) allele. The diagonal lines indicate homologous DNA sequence blocks.

9) What can you infer from the black diagonal lines in Fig. 5? (0.5 pt)

10) Explain why such genomic alterations prevent recombinations. A scheme is welcome. (1.5 pt)

11) The researchers performed additional experiments and concluded that the *cortex* gene appears to be functional and to lead to the production of a protein with a similar wild-type activity in the plain and in the veined forms. What experiment have they possibly done to conclude that? (1 pt)

12) Fill up the Gephbase entry corresponding to the difference in wing pattern between the plain and the veined forms. (2 pts)

Gene name	<i>cortex</i>
Trait Category	Morphology/Physiology/Behavior
Trait	
Trait state in Taxon A	
Trait state in Taxon B	
Taxon A species name	
Taxon B species name	
Taxonomic Status	Intergeneric or Higher /Interspecific /Intraspecific/ Domestication/ Experimental Evolution
Presumptive Null	Yes/No
Molecular Type	Coding/Cis-regulatory/Gene Loss/Gene Amplification/Other/Unknown
Aberration Type	SNP/Indel/Inversion/Translocation/Complex Change/Epigenetic Change/Unknown
Experimental Evidence	Linkage mapping/Association mapping/Candidate gene