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)

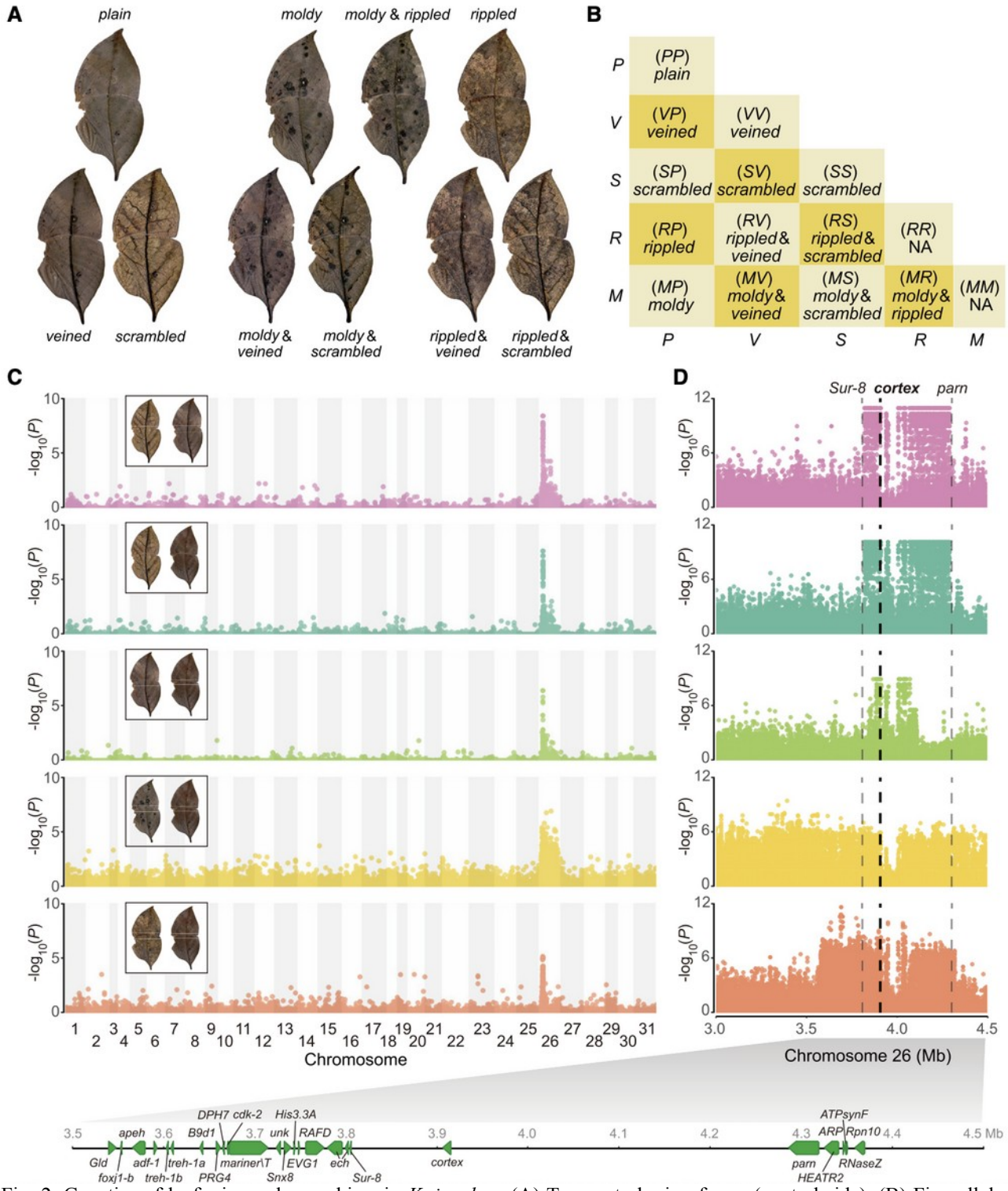

Fig. 2. Genetics of leaf wing polymorphism in *K. inachus*. (A) Ten ventral wing forms (ventral side). (B) Five alleles and their phenotypic effect on leaf wing patterns. NA: not obtained. (C) Multiple genome-wide association studies performed on *veined* versus *scrambled* forms, *plain* versus *scrambled*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

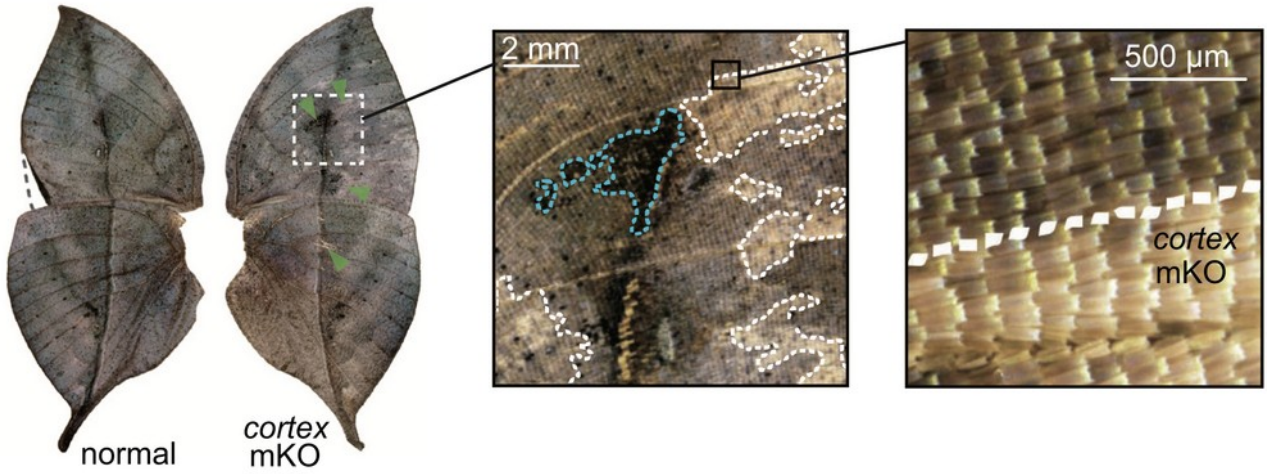

Fig. 4. Whole wing (ventral side) of a veined individual with no treatment (left) or obtained after CRISPR-Cas9 genome editing at the *cortex* locus (right). Blue and white dashed lines indicate the border of the cortex loss-of-function mutant clones. *Cortex* loss-of-function results in faded scale 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)

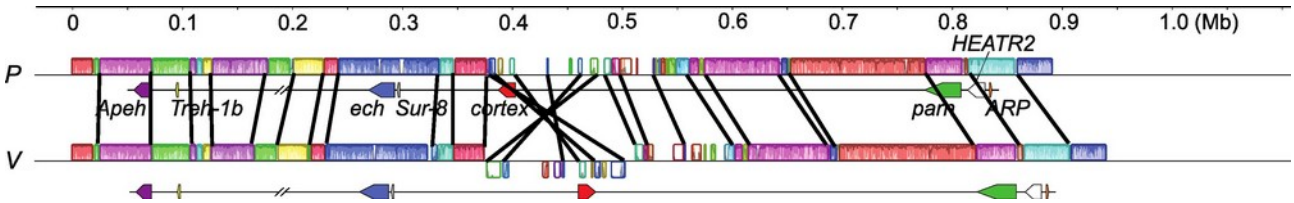

Fig. 5. Comparison of the chromosomal structure around the *cortex* coding region between the plain (P) and the veined (V) allele. The diagonal lines indicate homologous DNA sequence blocks.

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 Gephebase entry corresponding to the difference in wing pattern between the plain and the veined forms. (2 pts)

Gene name	*cortex*
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

Correction

1) Mimicking dead leaves allows the individuals to escape predation (from birds or from other vertebrates) as they are better camouflaged in the environment.
Suggested experiments:
- examine these butterflies in nature and try to find if they are more predated when their wings are open
- remove dead leaves, put orange paper leaves on the ground and then monitor butterfly predation to test if it increases
- add paint on the wing (transparent/same brown as the natural colour versus diverse flashy colours) to test if it changes the amount of predation
- monitor predation on paper butterflies with various wing patterns

2) They belong to the same species because they produce fertile hybrids between each other. They were classified as belonging to the same species probably also because they display the same morphological traits (antenna, various body parts besides the wings).

3) - The 5 GWAS give the same result for each comparison:
- a single peak
- on a particular region on chromosome 26
- which contains several genes

4) For the wing pattern phenotype:
The plain (P) allele is recessive over all the other alleles.
The veined (V) allele is dominant over the P allele, recessive over the scrambled (S) allele and co-dominant over the rippled (R) and moldy (M) alleles.
The scrambled allele (S) is dominant over the P allele and V alleles, and co-dominant over the rippled (R) and moldy (M) alleles.
The rippled allele is dominant over the P allele and co-dominant over the V, S and M alleles.
The moldy allele is dominant over the P allele and co-dominant over the V, S and R alleles.

5) injection of Cas9 protein + guide RNAs targetting the cortex gene into butterfly eggs. Raise eggs until adulthood and then screen wings for phenotypic defects and mutations.
The resulting animals are a mosaic of different somatic genomes: they carry mutant clones which harbour either one or two loss-of-function mutations.

6) electrophoresis gel with:
lane 0: molecular markers
lane 1: WT = one band at 301bp
lane 2: individual with deletion 1 = one band at 301 bp + one band at 293 bp (they might show different intensities, depending on the size of the mutant clone)
lane 3: individual with deletion 4 = one band at 301 bp + one band at 297 bp
lane 4: negative control (without butterfly DNA) = no band

7) All are deletions. Deletions 1-3 are not of a multiple of 3 bp, so there are all frameshift mutations: the amino acid sequence after the deletion is completely different from the sequence of the wild-type protein, and there is often a premature stop codon with such mutations. So probably loss-of-function mutations.

Deletion 4 = 9 bp so removal of 3 amino acids and potentially modification of a neighbouring amino acid if the deletion affects four codons. Deletion 4 is likely to have a lower phenotypic impact than deletions 1-3.

8) yes
The gene *cortex* triggers the formation of grey scales around the lateral veins and around the main midrib vein. In absence of *cortex*, different colours are revealed.

9) presence of an inversion at the *cortex* locus

10) Recombinations can occur within inversions but they lead to aneuploidy and thus are usually non viable. See course for the scheme.

11) They probably examined the sequence of the coding region of the *cortex* gene and found no changes or only synonymous mutations between the two morphs.

Gene name	*cortex*
Trait Category	Morphology
Trait	Coloration (wing)
Trait state in Taxon A	Plain morph
Trait state in Taxon B	Vein morph
Taxon A species name	*Kallima inachus*
Taxon B species name	*Kallima inachus*
Taxonomic Status	Intraspecific
Presumptive Null	No
Molecular Type	Cis-regulatory
Aberration Type	Unknown (Note: the inversion may not be causal for the morphological difference between the plain and vein morphs)
Experimental Evidence	Association mapping

Based on:
Wang, S., Teng, D., Li, X., Yang, P., Da, W., Zhang, Y., ... & Zhang, W. (2022). The evolution and diversification of oakleaf butterflies. *Cell*, *185*(17), 3138-3152.