

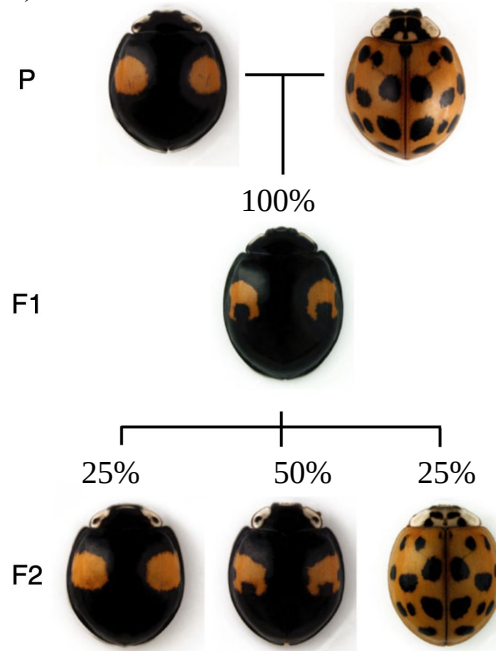
## Exam Genotype-Phenotype week 2 – 2019

(total = 21 points)

### Ladybirds

The harlequin ladybird, *Harmonia axyridis*, is an invasive species originating from Asia, whose individuals display multiple elytral colour patterns. More than 200 colour pattern forms have been described from different localities. This species can be raised easily in the laboratory.

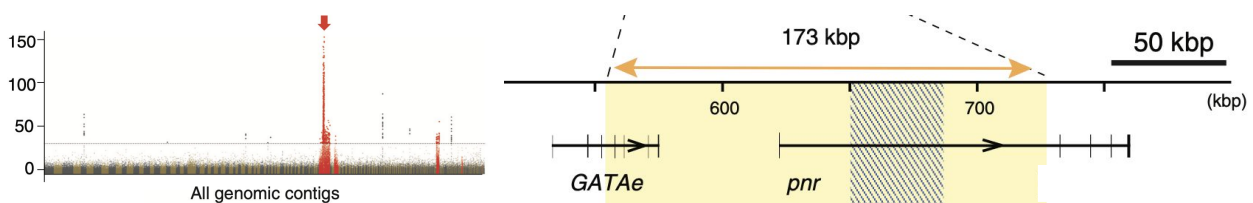
1. Several lines were established. In line 1 (named “Black-2Spots”), all individuals have two large red spots on a black background. In line 2 (named “Red-nSpots”), all individuals have 18-20 small black spots on a red background. Line 1 was crossed with line 2 and the F1 and F2 progenies are indicated below (parent crosses in both directions male/female gave the same results). **Is it possible that a single locus is involved in the colour pattern difference? What can you conclude about the alleles? (2 points)**



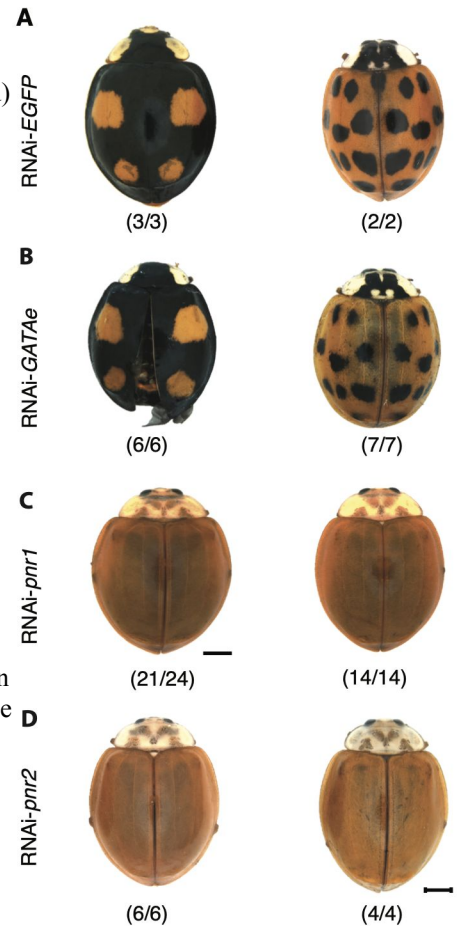
2. To identify genes involved in color polymorphism in *Harmonia axyridis*, a *de novo* genome assembly of the Red-nSpots form was produced using long reads produced by a MinION sequencer (Oxford Nanopore). Then, individuals representative of the world-wide genetic diversity and harbouring the four main colour patterns were pooled into 14 groups, with 7 pools including only Red-nSpots. Each pool was sequenced on a HiSeq 2500 (Illumina).

Genome-wide association study for colour pattern on these 14 pools revealed 56 SNPs with the strongest association signals (Bayes factor > 100 db), delineating a 173-kb region. This region extends from the end of the gene *GATAe* to the first coding exon of the ortholog of the *Drosophila* gene *pannier* (*pnr*).

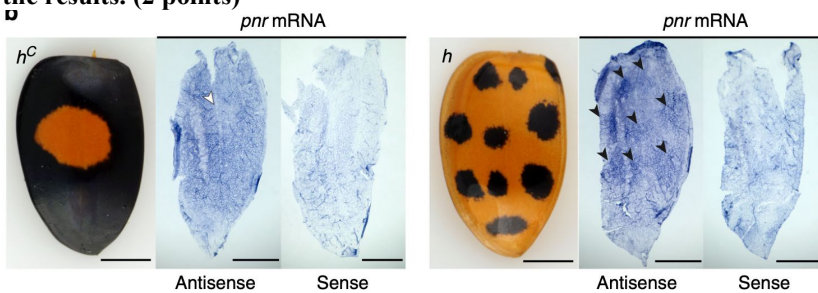
*GATAe* and *pnr* are thus good candidate genes. **Can you imagine an alternative scenario where this QTL does not involve *GATAe* or *pnr* but another gene? (1 point)**



3. Double stranded RNA directed against *EGFP*, *GATAe* and two non-overlapping regions of *pnr* (*pnr1* and *pnr2*) were injected into larvae of line 3 (Black-4Spots, all individuals have four large red spots on a black background) and of line 2 (Red-nSpots, see question 1) and the resulting adults were examined. One representative adult individual is shown for each case. Numbers in parentheses indicate the proportion of adults showing the pigmentation pattern of the representative individual. Scale bar; 1mm. **Why was *EGFP* targeted by RNAi? Why were two non-overlapping regions of *pnr* targeted? What can you conclude from the experiments? (3 points)**



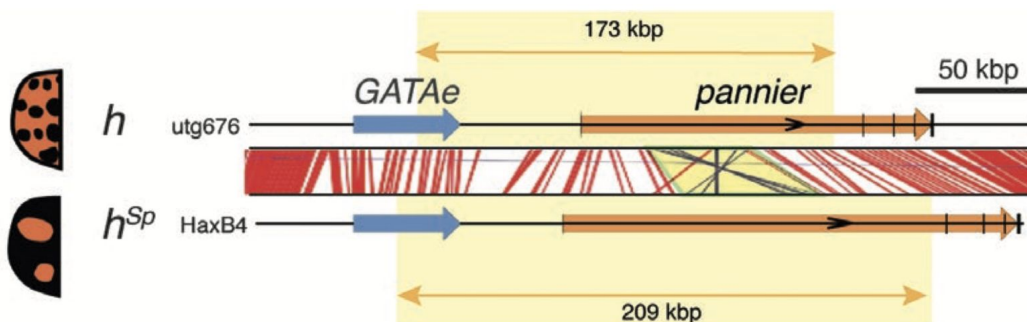
4. In situ hybridization using sense and anti-sense probes for *pnr* coding region was performed on the developing elytral epidermis before pigmentation, in line 1 (Black-2Spots) and line 2 (Red-nSpots). One representative stained elytral epidermis is shown for each case, next to a wild-type adult elytra. Blue coloration indicates in situ hybridization staining. White arrowhead: region with a weak signal. Black arrowheads: regions with intense signals. **Interpret the results. (2 points)**



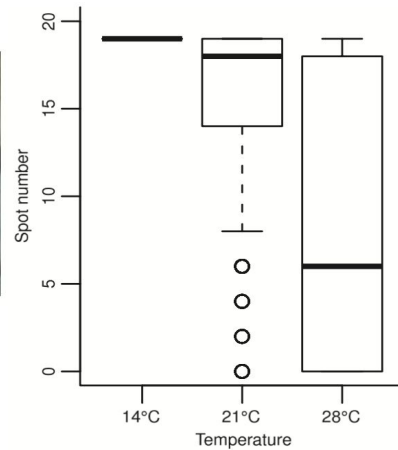
5. Infer the expression pattern of *pnr* in the developing elytra of the F1 progeny of question 1. A drawing can be used. (1 point)

6. *Pannier* encodes a transcription factor. In ladybird elytra, black pigment is melanin and red pigments are carotenoids. **Draw a schematic diagram explaining the role of *pannier* (*pnr*) in ladybird elytra colour patterning. (2 points)**

7. The chromosomal structure around the *pannier* locus was compared between line 2 (Red-nSpots, top) and line 3 (Black-4Spots, bottom). The blue and red diagonal lines between the neighbouring alleles indicate homologous DNA sequence blocks. **What can you infer from the blue diagonal lines? (1 point)**



8. All previous experiments were performed at 21°C. Line 2 (Red-nSpots) was raised at three distinct temperatures. Pictures of representative individuals for the 3 temperature conditions are shown: left: 14°C, middle: 21°C, right: 28°C. In the diagram, the horizontal bar is the median, the box is the interquartile range and open circles are outliers. **What can you conclude? How is this phenomenon called? (2 points)**



9. **Propose one experiment to test whether elytra are used for flight. (1 point)**

10. **Propose one experiment to test whether females choose males based on elytra colour patterns. (2 points)**

11. Another ladybird species, the European 2-spot ladybird, *Adalia bipunctata*, also exhibits a large diversity of elytra colour morphs. Unfortunately, this species cannot hybridize with *Harmonia axyridis* and it cannot be raised in the lab (it is not possible to do crosses nor to collect developing tissues to assess *pnr* expression). **Propose one experiment to test if the *pannier* locus is involved in colour polymorphism in *Adalia bipunctata*. (1 point)**

### Unrelated questions

12. **What is the number of telomeres in a gamete cell right after meiosis if the karyotype of the diploid cell that underwent meiosis was  $2n=32$ ? No need to justify. (1 point)**

13. Transposable elements can insert into the coding region of a gene and disrupt its function, leading to a loss-of-function allele. **Give two other possible scenarios on how transposable elements can affect genes. (2 points)**