Exam - Genomes and Phenotypes – Week 1

I. Gene Drive (10 pts)

A journalist emailed you several questions because he wants to write an article on gene drive. Answer his questions (you can include diagrams if necessary).

1. How would you define CRISPR and gene drive? What is the difference between CRISPR and gene drive? (max 15 lines, 2.5 pts)
2. When was the first paper published on CRISPR technology? (0.5 pt)
3. What are the potential domains of application of gene drive technology?(max 10 lines, 1.5 pt)
4. What are the two main species for which gene drive is most advanced? (1 pt)
5. What is the aim of using gene drives in these two species? (max 10 lines, 2 pts)
6. What are the different risks associated with gene drive? (max 15 lines, 2.5 pts)

II. Red pigmentation in birds (10 pts)

We propose to examine the factors affecting red pigmentation in birds. To produce red pigments, birds ingest yellow carotenoids and endogenously convert them into red ketocarotenoids via an oxidation reaction catalyzed by enzymes.

Red factor canaries

Whereas common canaries (*Serinus canaria*) are all yellow, red factor canaries display red-pigmented plumage. The red factor canaries (also named as red canaries) are hybrids resulting from multiple crosses that were performed starting in the 1920s by bird fanciers between common canaries and red siskins (*Spinus cucullata*), a South American bird with red-pigmented feathers: F1 hybrids between common canaries and red siskins were backcrossed with common canaries over multiple generations to create the red factor canaries (Fig. 1).

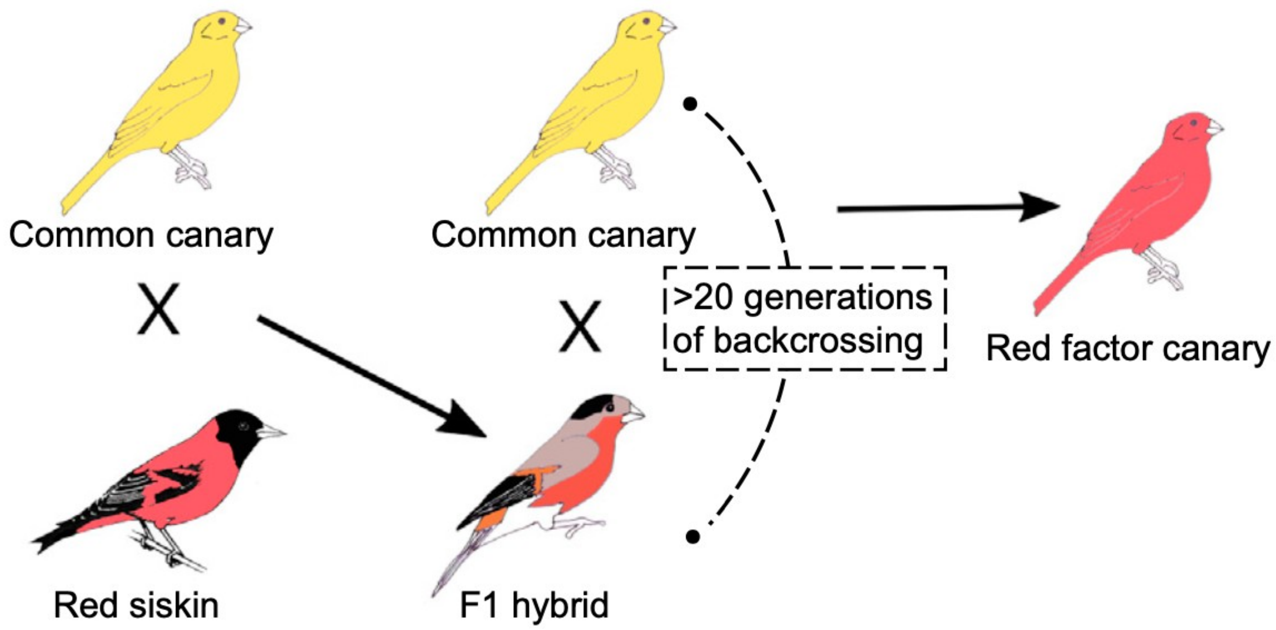

Fig. 1. The Origin of Red Canaries. Red factor canaries were created by crossing common canaries with red siskins. Hybrids were backcrossed with common canaries for many generations to improve the fertility of the line and to remove all siskin characteristics except red coloration. The resulting birds displayed a yellow/orange coloration. They were crossed with each other for several gennerations to obtain breeds of red canaries, which only produce red canaries. The result is a phenotypically normal canary but with the capacity to produce red ketocarotenoids from yellow dietary carotenoids.

1. If you suppose that there is only one gene contributing to the red/yellow plumage color of the belly of the animal, which allele is dominant? (0.5 pt)

2. After 20 generations of backcrossing, at a given locus, which combinations of alleles do you expect in the resulting backcross individual: 2 red siskin alleles, 1 red siskin and 1 common canary allele, or 2 common canary alleles? (0.5 pt)

3. After 20 generations of backcrosses to common canaries, if there was no selection at each generation, what would be the probability for the resulting backcross animal to retain a red siskin allele at a given locus? (1 pt)

4. You have access to blood samples of >100 common canaries, >100 red siskins and >100 red canaries, but you have money to sequence only 100 birds. Your goal is to identify the genetic loci contributing to red pigmentation in red canaries. How many birds of each group will you choose for sequencing? Justify your choice. (1.5 pts)

5. Describe the various experimental steps, going from bird blood samples to obtention of the genome sequences of these 100 birds, using whole genome sequencing of a pool of all 100 individuals and Illumina paired-end sequencing. You don't need to detail the Illumina paired-end sequencing step. (1.5 pts)

6. The researchers found that all chromosomes of red canaries harbour 2 copies of the common canary alleles, except regions on chromosome 8 and 25. What can you conclude about the genetic basis for red coloration based on Fig. 2 (see next page)? (1.5 pts)

7. The researchers identified a bird displaying yellow plumage that was heterozygous for the chr8/NW_007931131 region but homozygous for the common canary allele in the chr25/NW_007931203 region. Second, individuals that are heterozygous for both candidate regions do not express red, but rather a yellow/orange coloration.
What can you conclude about the dominance of the alleles? Is there epistasis between the loci on chromosome 8 and 25? (1 pt)

8. The two QTL contain several candidate genes. To try to identify the causal genes, the adult skin of several red canaries and several yellow canaries was dissected, RNA was extracted and RNAseq was performed. Interpret Fig. 3. (1 pt)

9. The RNAseq experiment presented in Fig. 3 is not sufficient to conclude that mutation(s) in the *CYP2J19* gene contribute(s) to red pigmentation. Propose an explanation which can account for all the observations and where red pigmentation is not caused by mutation(s) in the *CYP2J19* gene. (1 pt)

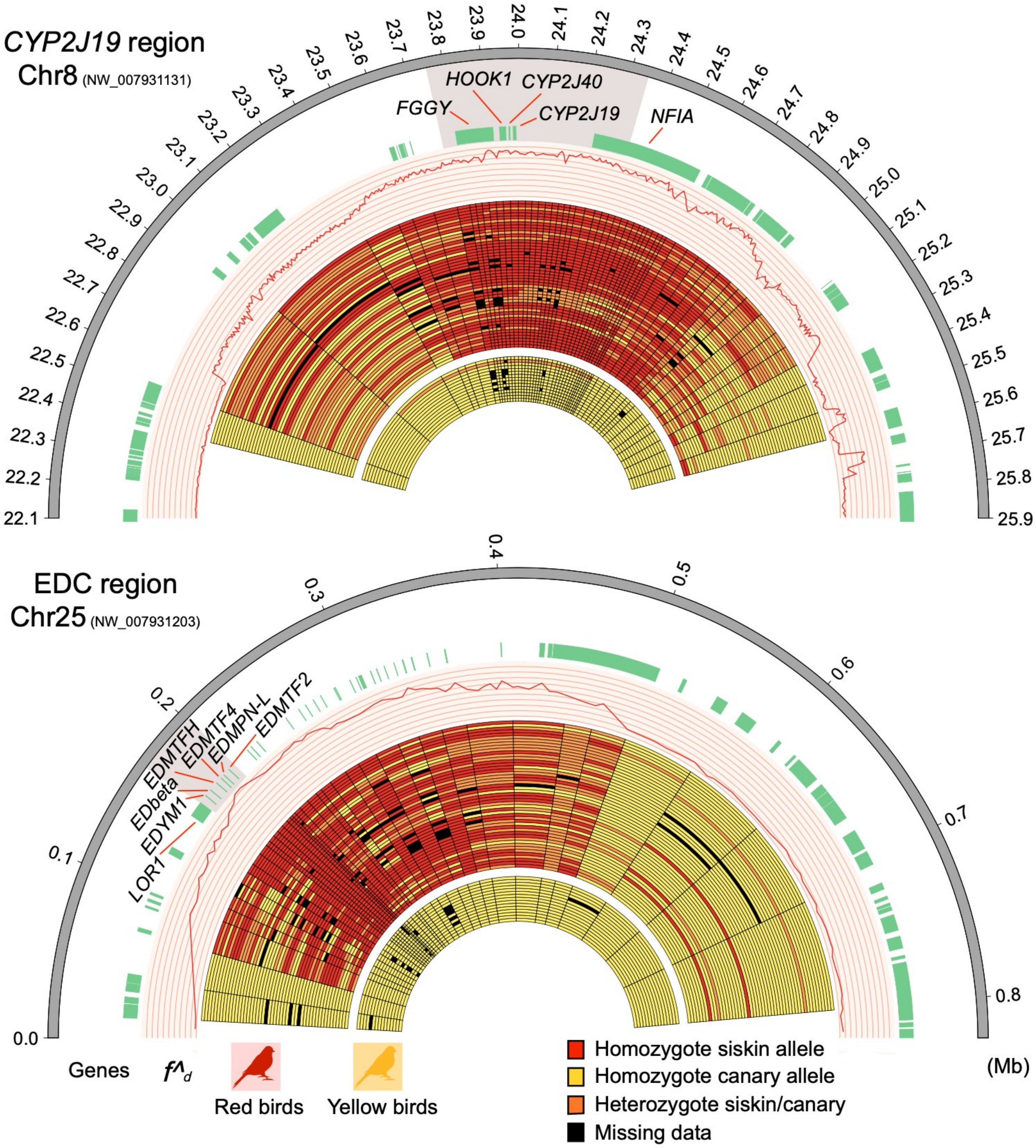

Fig. 2. The outermost semi-circle represents the genomic coordinate in megabases. The next semi-circle (from the outside inward) shows the location of genes from the canary genome annotation (green boxes). In the next semi-circle, f^d values are shown (solid red line). The innermost semi-circles represent the genotyping results for SNPs found to be diagnostic between red siskin and common canaries in 49 red canaries and 15 non-red canaries, as indicated by red and yellow canary silhouettes, respectively. Each row represents one individual, and individuals appear in the same order on both Chr8 and Chr25 graphs. Red, yellow, and orange squares indicate positions homozygous for the red siskin allele, homozygous for the yellow canary allele, and heterozygous for both alleles, respectively. Missing data are represented by black boxes. Light-gray highlighting indicates the longest continuous regions where all red individuals carry at least one copy of the red siskin haplotype.

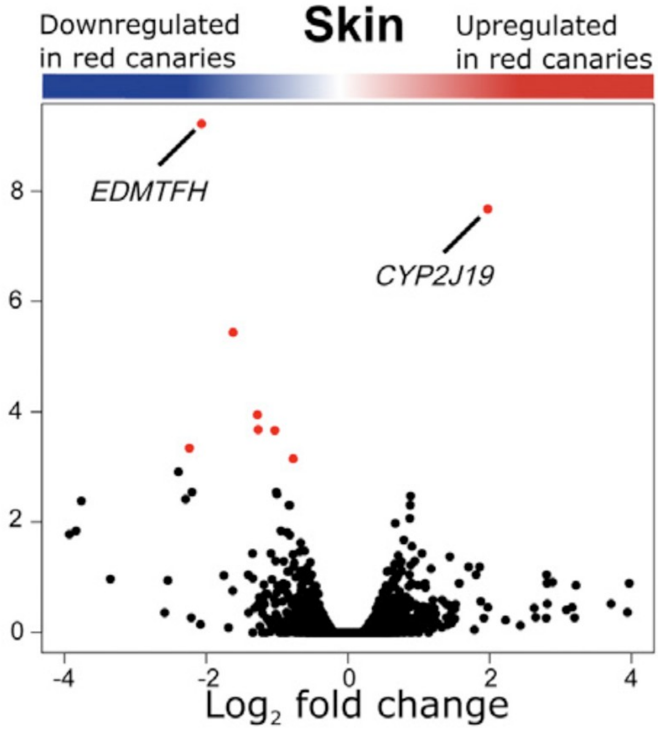

Fig. 3. Gene expression levels. Volcano plot of statistical significance (y axis) against log2 fold change in expression (x axis) for skin using RNA-seq. Significant genes are depicted as red dots, and those that also overlap the candidate regions are labeled.

Male house finches

10. Researchers also investigated carotenoid-based color of feather patches in male house finches *Carpodacus mexicanus*. They recaptured and rescored 63 males and found that the hue of feathers faded significantly over the season. The degree of hue change was a direct function of the amount of time between plumage scores; feathers faded more as the interval between measurements increased. The magnitude of hue change was not, however, related to an individual's age or initial plumage redness, which suggests that certain birds are not more or less prone to fading.
What is the name of the general phenomenon underlying the observed variation in hue? (0.5 pt)

Correction

I.

1. CRISPR: (clustered regularly interspaced short palindromic repeats, originally: a family of DNA sequences present in bacteria and used to detect and destroy virus DNA)

- technology that allows to cut a specific DNA sequence (recognized by a guide RNA) → can create targeted mutations and even replace a DNA sequence by a new DNA sequence of interest

More versatile than restriction enzymes, Zn finger nucleases and transcription activator-like effector nucleases (TALENs). So far works in all tested species.

gene drive: A novel biotechnology under development which aims to bias inheritance and control disease vectors, invasive species and other pests. Reduction or suppression of natural population. Invasive GMO.

CRISPR= many applications, gene drive: under development in laboratories

Most gene drive technologies under development are based on CRISPR.

2. 2012
3. Applications in: Public health, agriculture, conservation biology
4. Drosophila suzukii & Anopheles mosquitoes vectors of malaria
5. Drosophila suzukii: pest species, agricultural damage, goal = eliminate the populations

Anopheles: eliminate malaria, either by eliminating mosquito populations, or by making them resistant to malaria parasite development

6. Molecular off-targets

Propagation to non-target populations and species

Consequences for ecosystems

Failure of counter-measures

Potentially less efficient than expected (resistance via mutations in the target site, cryptic species)

II.

1. the red/red siskin allele is dominant according to figure 1. (semi-dominant according to the description of the figure 1.)
2. two possibilities: 1 red siskin and 1 common canary allele, or 2 common canary alleles
3. After the first backcross: 50% chance to retain a red siskin allele, after the second: 0.5x0.5= 25% chance, after 20 backcrosses: $0.5^{20}=9.10^{-7}$

very little probability

4. best choice: a few red siskins, a few more common canaries, and a lot of red canaries coming from different series of crosses if possible. The goal is to identify the genomic regions in red canaries which harbour 1 red siskin and 1 common canary allele. It is better to sequence a few red siskins and a few common canaries (rather than just one individual for each) because we want to have an estimation of the genetic diversity in each species. Furthermore, it is good to sequence as many red canaries as possible: the more are sequenced, the better the resolution of the QTLs.

Note: we don't know which common canary strains were used for the generation of red canaries, so it might be good also to sequence many red canaries and many common canaries

good to do more than one red siskin individual, just in case there is an issue with one sample (just in case this is not a "real" siskin, the sample quality is bad, the sample library preparation failed, etc.)

5. extraction of DNA,

fragmentation of DNA

ligation of a specific tag to each DNA sample
pool of the 100 DNA in equimolar concentrations
preparation of the Illumina library for paired-end sequencing
paired-end Illumina sequencing
attributing reads to each bird based on the tag sequences
mapping the reads to the canary genome or assembling the reads to obtain full genome sequences for each bird
0.25 pts per step
8x0.25=2 pts but this question is 1.5 pts because no student proposed all the steps

6. red canaries are all homozygous for the siskin allele for two regions: one on chromosome 8, one on chromosome 25. Remaining parts of the genome are siskin/common canary or common canary/common canary.
So two loci involved in red pigmentation, one on chromosome 8, one on chromosome 25, several candidate genes in each QTL.

7. Semi-dominance of both alleles, both regions are necessary to produce red coloration. We don't know if there is epistasis. Maybe the effects are fully additive and once it reaches a threshold (obtained in individuals homozygous for the chr25 locus and heterozygous for the chr8 locus), the red pigmentation is obtained.

8. one gene *CYP2J19* shows increased expression in red canaries → good candidate gene, one gene from the other QTL, *EDMTFH*, hows decreased expression in red canaries → good candidate gene also.
9. It could be a mutation in a gene acting upstream of *CYP2J19*. This gene has to be in the QTL.
10. plasticity

Based on:

McGraw, K. J., & Hill, G. E. (2004). Plumage color as a dynamic trait: carotenoid pigmentation of male house finches (Carpodacus mexicanus) fades during the breeding season. Canadian Journal of Zoology, 82(5), 734-738.

Lopes, R. J., Johnson, J. D., Toomey, M. B., Ferreira, M. S., Araujo, P. M., Melo-Ferreira, J., ... & Carneiro, M. (2016). Genetic basis for red coloration in birds. Current Biology, 26(11), 1427-1434.