

Investigating the Heterogeneity of Altitudinal Pigmentation in African *Drosophila melanogaster* Populations

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How Do Polygenic Traits Evolve in Populations?

- When polygenic traits are under selective pressure, causative alleles may not entirely sweep through a population
- This results in heterogenous patchworks of different genes that, when combined, produce similar phenotypes
- Fitness-affecting alleles may also experience epistatic effects, resulting in cryptic genetic variation¹
- How does cryptic genetic variation complicate our picture of evolution in populations?

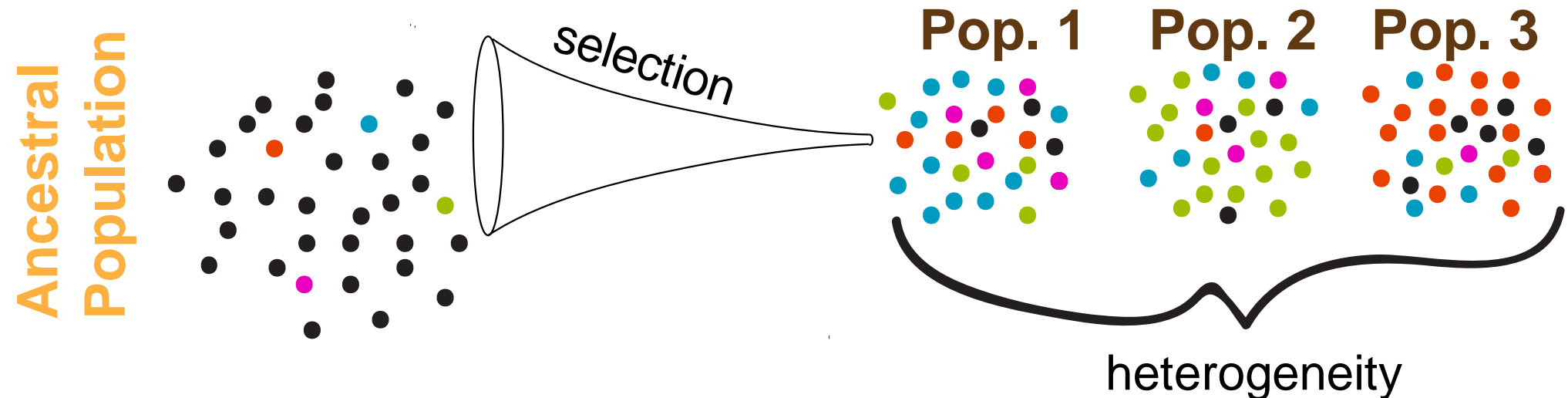


Fig. 1. Different combinations of genes may produce similar phenotypes.

African Fruit Flies Exhibit Pigmentation Differences Correlated to Altitude

- Flies from a high-altitude environment exhibit increased melanization in comparison to flies from a low-altitude environment
- Increased melanization is broadly correlated to increasing altitude²
- Bulk-segregant analysis (BSA) implicates multiple loci that underlie dark pigmentation phenotype, and the identities of these loci differ between distinct isofemale lines³

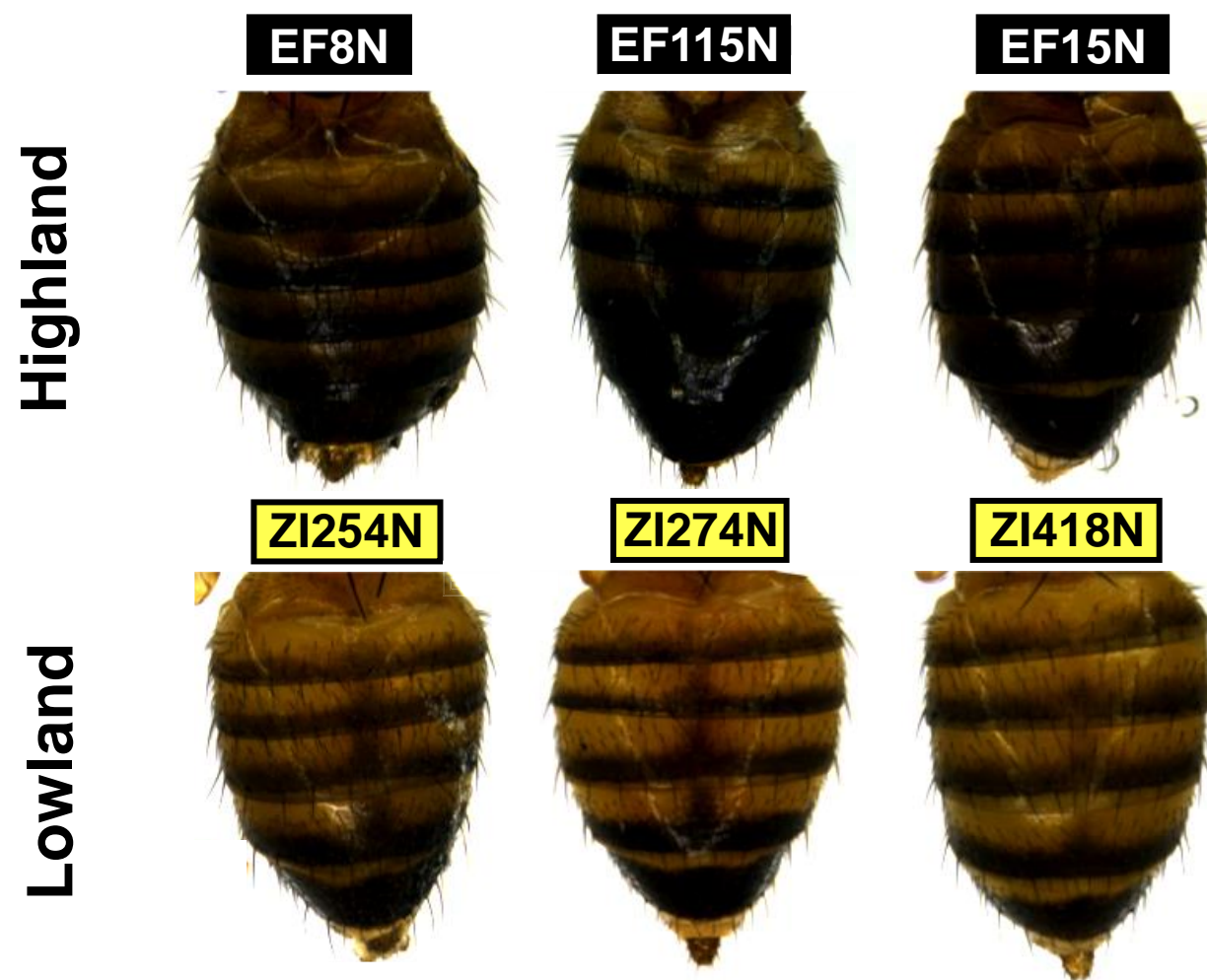


Fig. 2. Highland fruit flies from Piche, Ethiopia exhibit significantly darker pigmentation than lowland flies from Siavonga, Zambia, which display ancestral coloration.

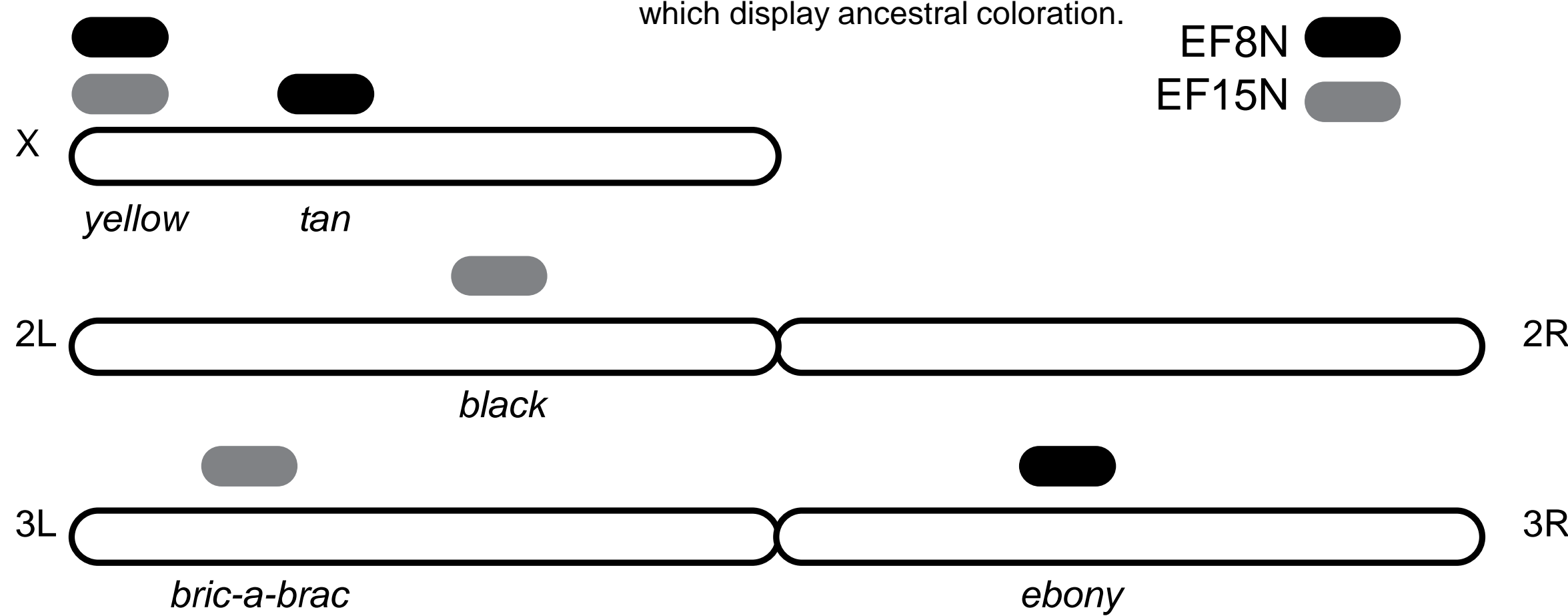
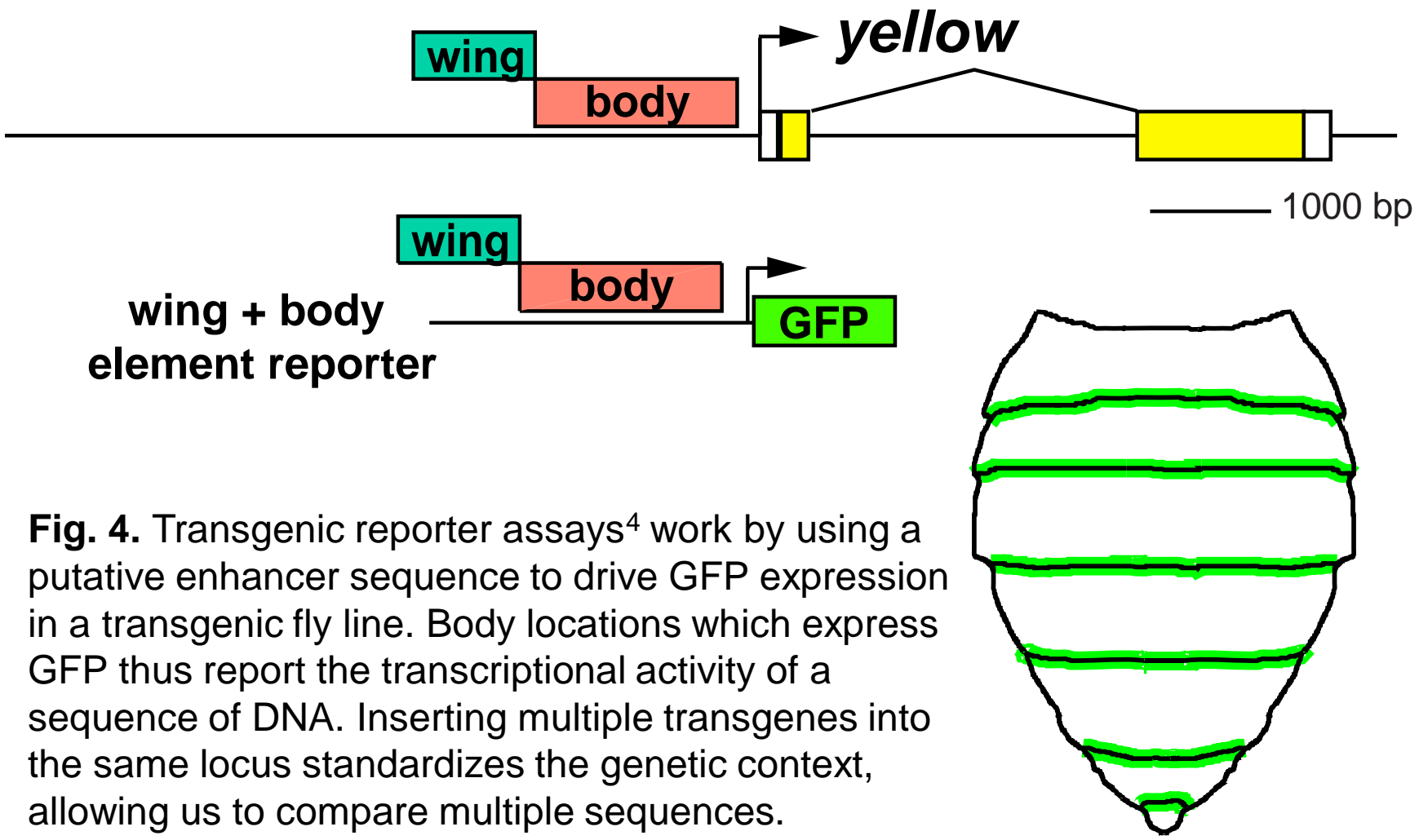


Fig. 3. Bulk-segregant analysis implicated three loci underlying increased melanization for both EF8N and EF15N. Strikingly, the only locus implicated in both lines was *yellow*, with *ebony* and *tan* predicted for EF8N and *black* and *bric-a-brac* predicted for EF15N. Adapted from Bastide et al. 2016³.

Methods



- in situ* hybridization was used to look at mRNA accumulation of known pigmentation genes *ebony*, a light pigment promoter, and *yellow*, a dark pigment promoter
- The expression patterns of the canonical *yellow* wing + body regulatory element were examined using transgenic reporter constructs in the 51D attP2 landing site⁴

Fig. 4. Transgenic reporter assays⁴ work by using a putative enhancer sequence to drive GFP expression in a transgenic fly line. Body locations which express GFP thus report the transcriptional activity of a sequence of DNA. Inserting multiple transgenes into the same locus standardizes the genetic context, allowing us to compare multiple sequences.

ebony *in situ* hybridization

- In ZI254N and ZI274N, high *ebony* expression correlates to lightly-pigmented regions, as predicted for lightly-colored flies
- In EF115N, *ebony* expression resembles ZI254N and ZI274N expression patterns
- In EF8N and EF15N, *ebony* expression is low, as expected for darkly-colored flies
- In ZI418N, *ebony* expression resembles EF8N and EF15N expression patterns

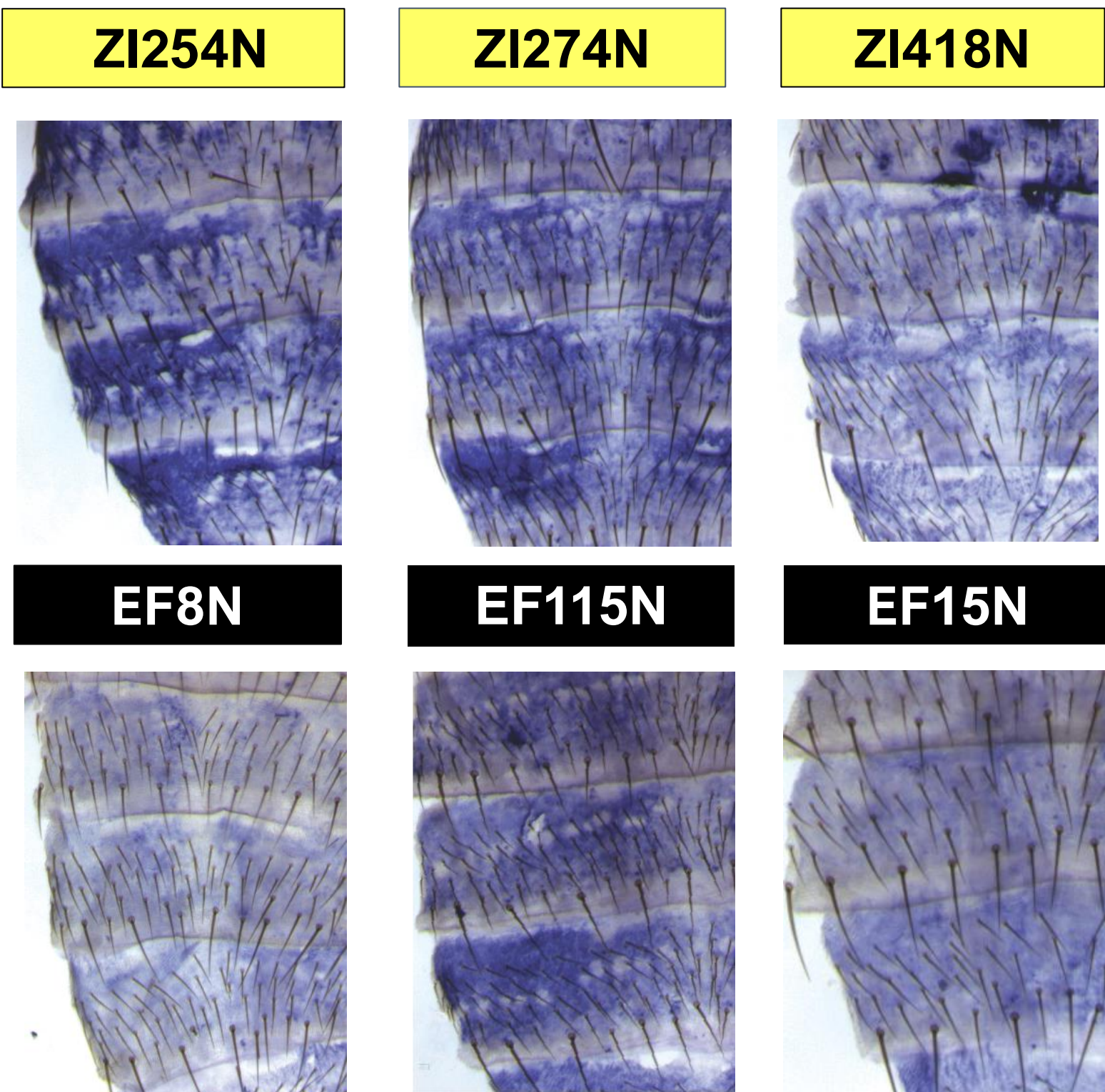


Fig. 5. *ebony* *in situ* hybridization on abdomens dissected at eclosion. Samples represent n>15 for 20 samples across 2 replicates.

yellow *in situ* hybridization

- In all ZI fly lines, *yellow* expression correlates to the darkly-pigmented stripes seen in adults
- In EF8N and EF115N, *yellow* expression is still localized to the stripes, although the stripes appear thicker than in ZI lines
- In EF15N, *yellow* is expressed variably throughout the abdomen, with some samples exhibiting expression patterns similar to EF8N and EF115N, and others exhibiting broad expansion of *yellow* expression. This variability requires further investigation into EF15N's *yellow* expression.

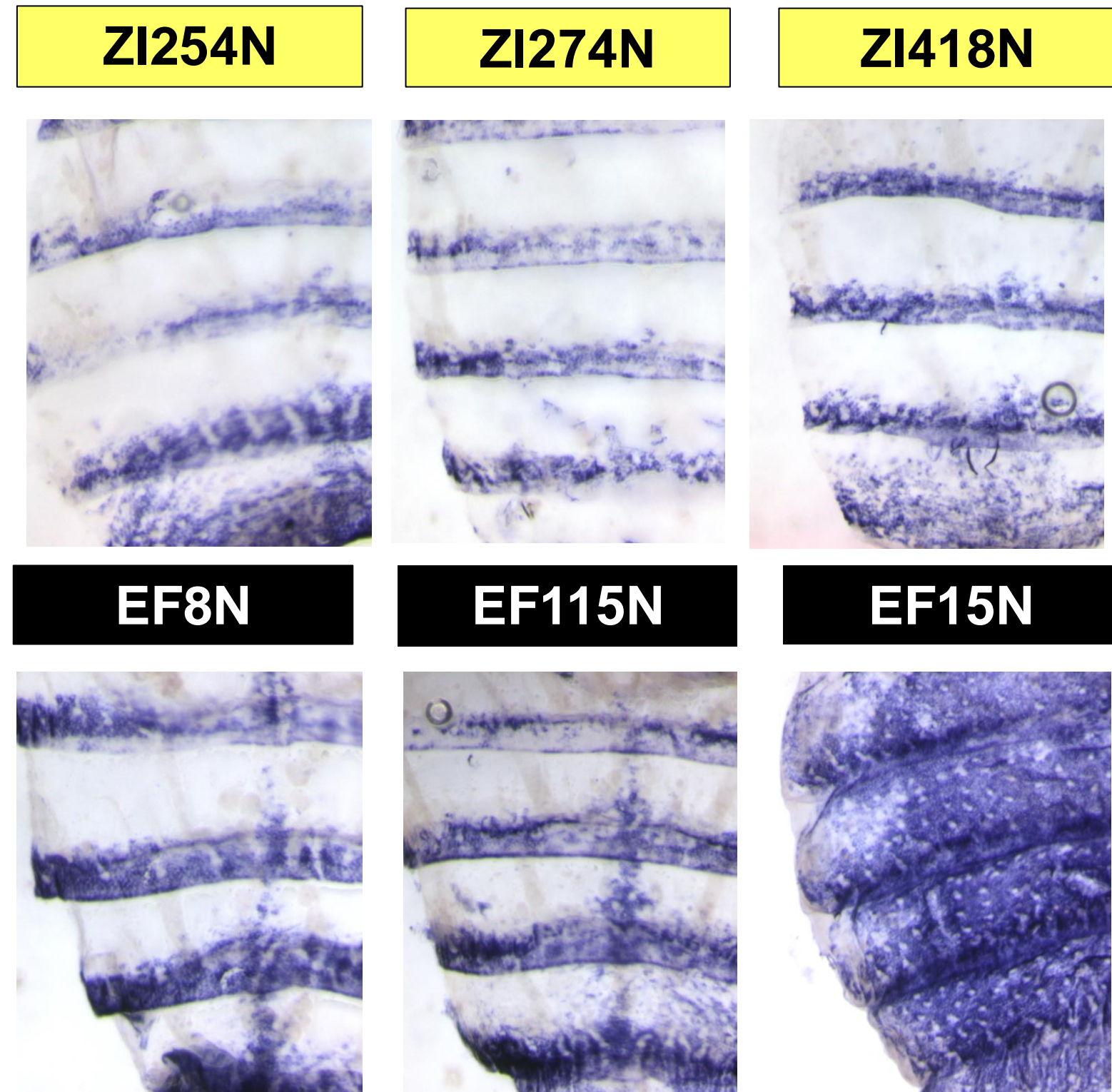


Fig. 5. *yellow* *in situ* hybridization on abdomens dissected at 72 hours after pupal formation, n>7 for 10 samples for all lines except EF15N.

yellow wing + body element reporter

- GFP expression correlates to *yellow* expression as seen in the *in situ* hybridization samples.
- In ZI254N, GFP is expressed at the stripes.
- In EF115N, GFP is expressed at the stripes.
- In EF15N, GFP is expressed throughout the abdomen. Notably, although the *in situ* expression patterns are highly variable, the GFP patterns in the reporters are highly consistent between samples

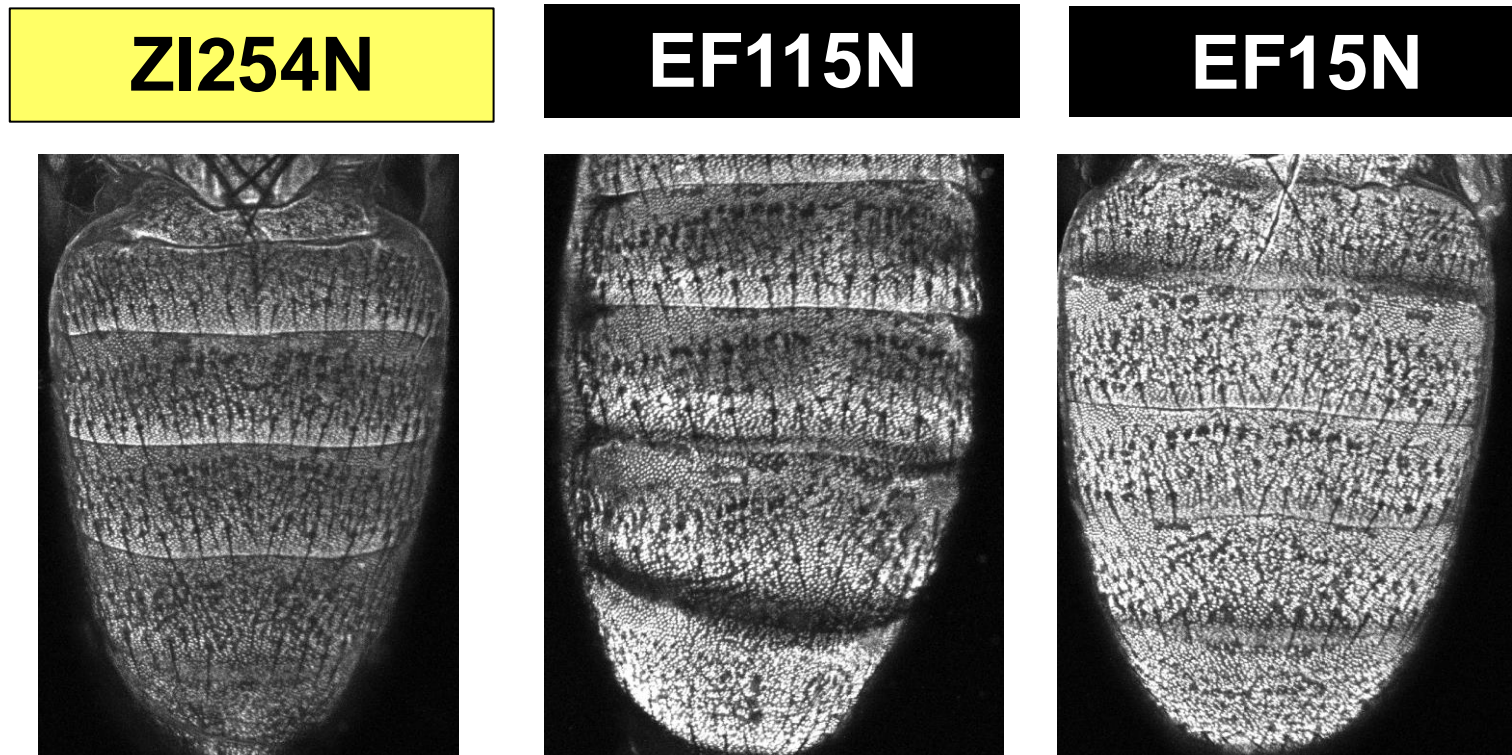


Fig. 6. GFP-reporters for the *yellow* wing + body element, imaged at 96hrs after pupal formation, n>20.

Future Directions

- Examine expression patterns for *tan*, *black*, and *bric-a-brac*
- Investigate known enhancer regions for *ebony*, *tan*, and *bric-a-brac*
- Generate transgenic chimeras to further narrow down the sequence responsible for expression differences in *yellow*
- Confirm an allele's contribution to the dark phenotype via CRISPR/Cas9-mediated reciprocal hemizygosity assays

Conclusions

- Some of the BSA predictions were supported, with low *ebony* expression in EF8N and high *yellow* expression in EF15N. For example, the BSA did not predict low *ebony* in EF15N, but low *ebony* expression is observed.
- Such discrepancies in BSA results have a few explanations:
 - 1) a trans-regulator that was not detected in the BSA or that is unknown may underlie differences in expression;
 - 2) the light line used to cross to a particular dark line may have a similar allele persisting as a cryptic variant, preventing the BSA from detecting the variant
 - 3) a particular variant was a false negative in the BSA.
- Surprisingly, one light line (ZI418N) expresses low *ebony*, suggesting that ZI418N's light coloration comes from additional changes that mask a low-*ebony* phenotype.
- Cis-regulatory changes in the wing + body element are sufficient to drive the *yellow* expression patterns seen in light line ZI254N and dark lines EF115N and EF15N.

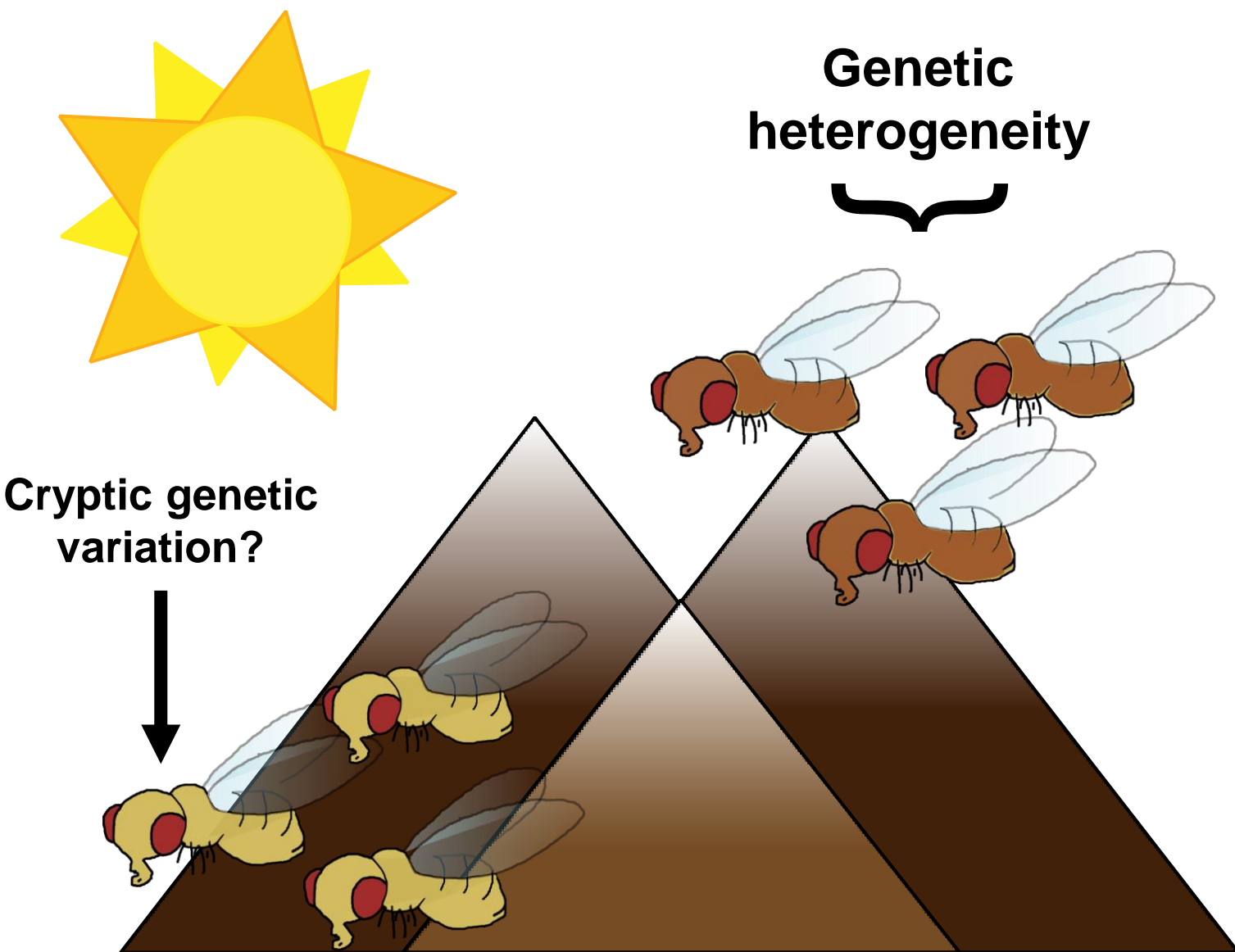


Fig. 7. African fruit flies exhibit genetic heterogeneity at the expression level and possibly contain cryptic genetic variants.

Funding & Acknowledgements

This project was funded by the NIH.

We would like to acknowledge the efforts of Tiago Ribeira (UW Madison), who has been essential in forwarding this project.

References

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