

# Small Populations, Lasting Signatures: Demography and Genetic Load in the Endemic Butterfly

## *Hipparchia sbordonii*

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### Background

*Hipparchia sbordonii* is an endangered butterfly endemic to the Pontine Islands (Italy) (Figure 1), surviving in small, isolated populations.

Its relative *Hipparchia semele* is widespread and abundant across mainland.

Small populations:

- ↓ Genetic diversity
- ↑ Genetic drift & inbreeding
- ↑ Harmful mutations can accumulate
- ↑ But purging of strongly deleterious mutations may occur (via homozygosity)

Large populations:

- ↑ Diversity
- ↑ Harmful mutations in heterozygosity (masked load)

Despite concerns over the "insect apocalypse", we lack genomic data on how demography affects genome health in insects.

Understanding these effects in *H. sbordonii* helps assess extinction risk and adaptive potential in threatened species.

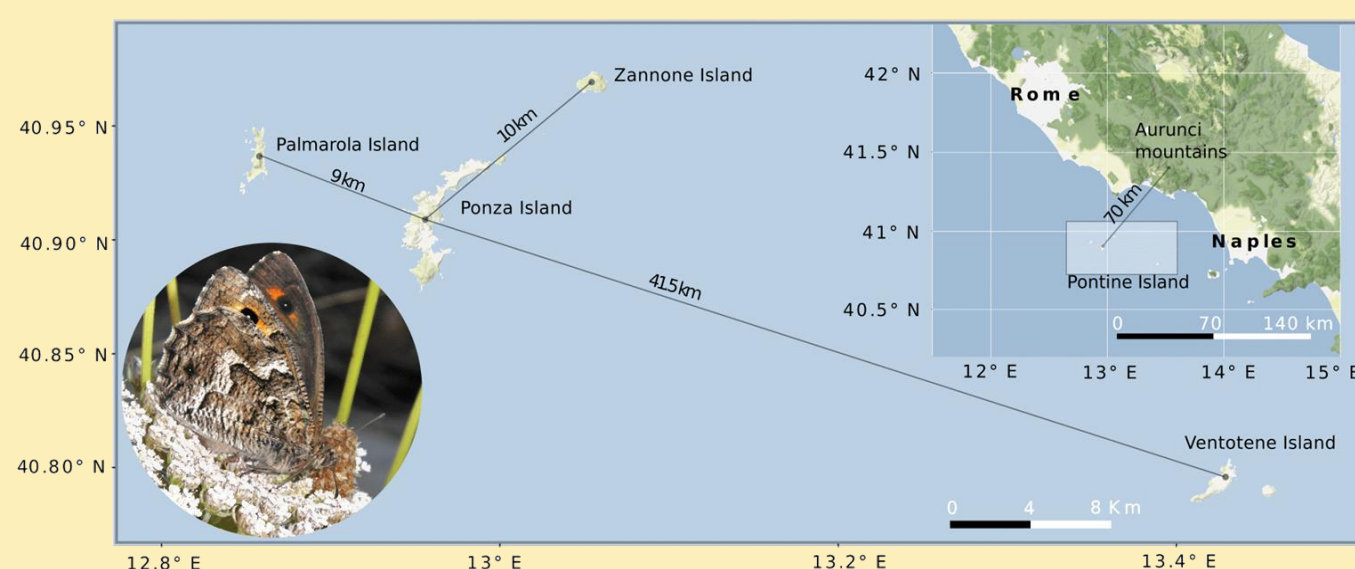


Figure 1: *H. sbordonii* distribution on Pontine Islands. Now restricted to Ponza; islands are isolated from each other and the mainland.

### Next steps

### Aims

- 1) Compare genetic diversity and inbreeding between *H. sbordonii* and *H. semele*, including  $\pi$  and Tajima's D.
- 2) Reconstruct demographic history of *H. sbordonii* to detect long-term small population size and recent declines.
- 3) Assess deleterious mutation load in both species and test for purging in *H. sbordonii*
- 4) Explore selection on variants in *H. sbordonii*, as potential island-specific adaptations

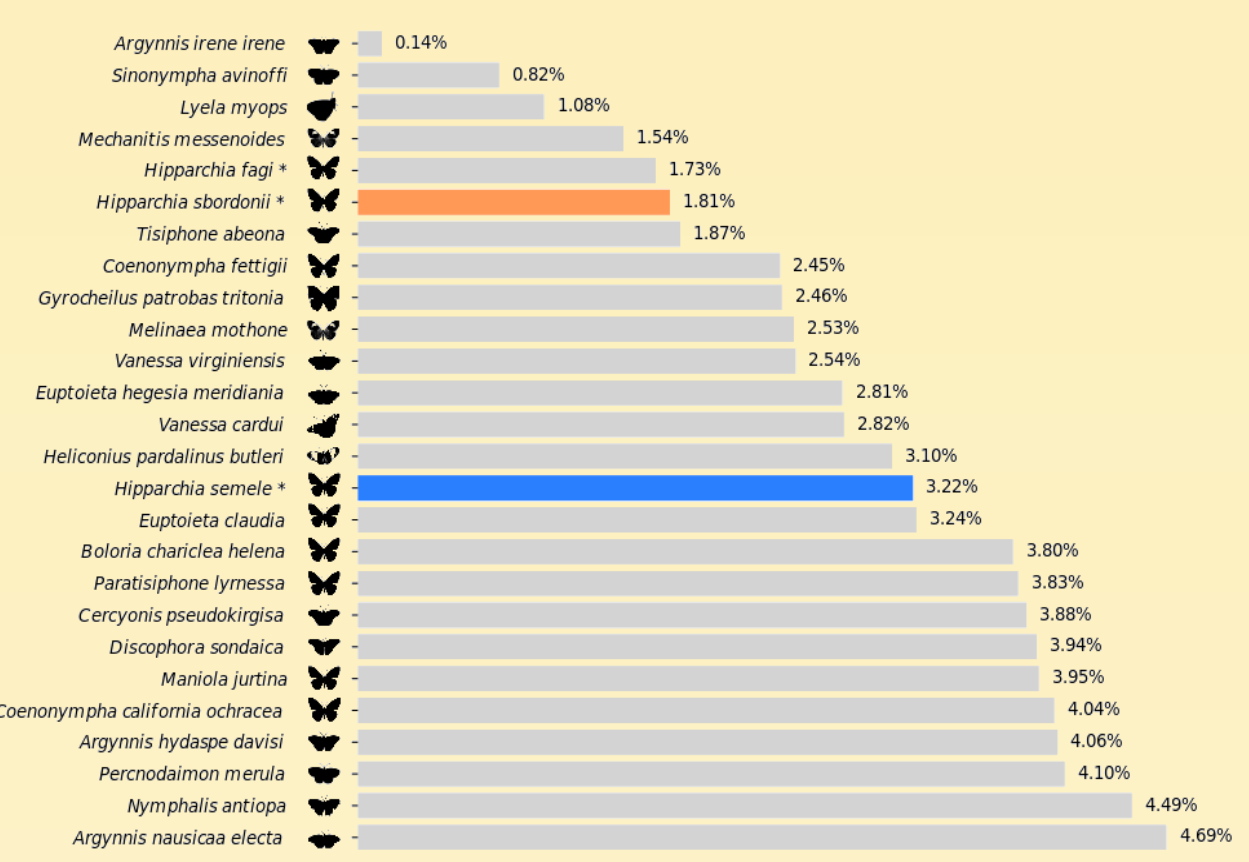
### M&M

22 individuals resequenced: ten *H. sbordonii*, ten *H. semele*, two outgroups (*H. fagi*, *H. mersina*). Reads mapped to the *H. sbordonii* reference genome; variants called with GATK.

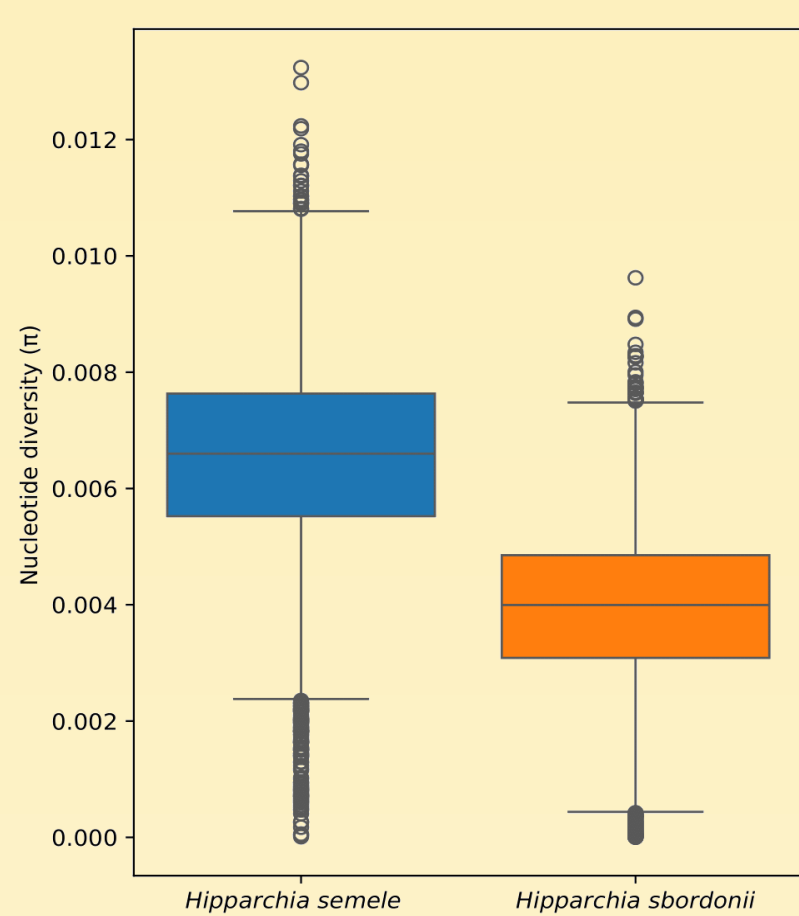
- Aim 1)** Heterozygosity: estimated via k-mer analysis (GenomeScope, Jellyfish).  $\pi$  and Tajima's D: calculated with VCFtools. ROH: estimated with ROHan, categorized by length.
- Aim 2)** Demographic history: inferred with SMC++ (past) and GONE (recent).
- Aim 3)** SNP annotation: done with SnpEff; variants polarized with outgroups. Genetic load difference: assessed via difference in derived allele frequencies ratio using R'xy across mutational categories.
- Aim 4) Next steps** Fixed SNPs in *H. sbordonii*: identified if rare/absent in *H. semele*; mapped to genes. Network analyses: used to infer functional and adaptive relevance.

### Results and Discussions

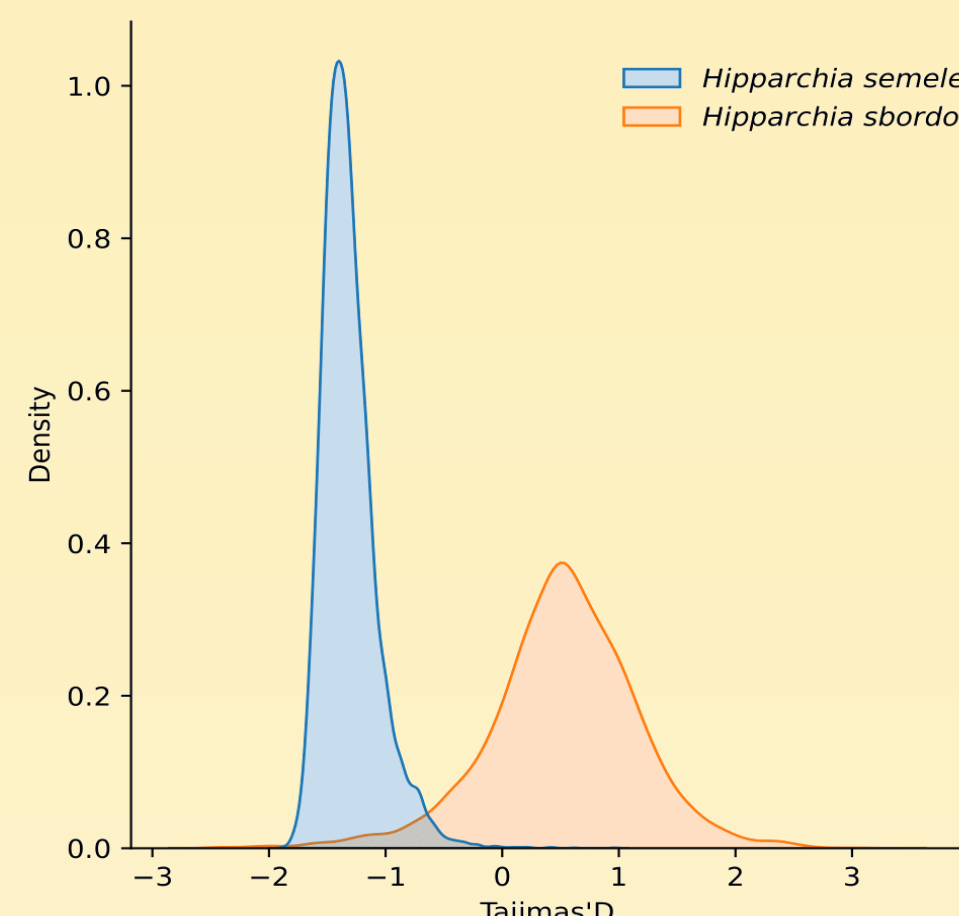
#### Heterozygosity comparison, Nucleotide diversity, Tajima's D, ROH



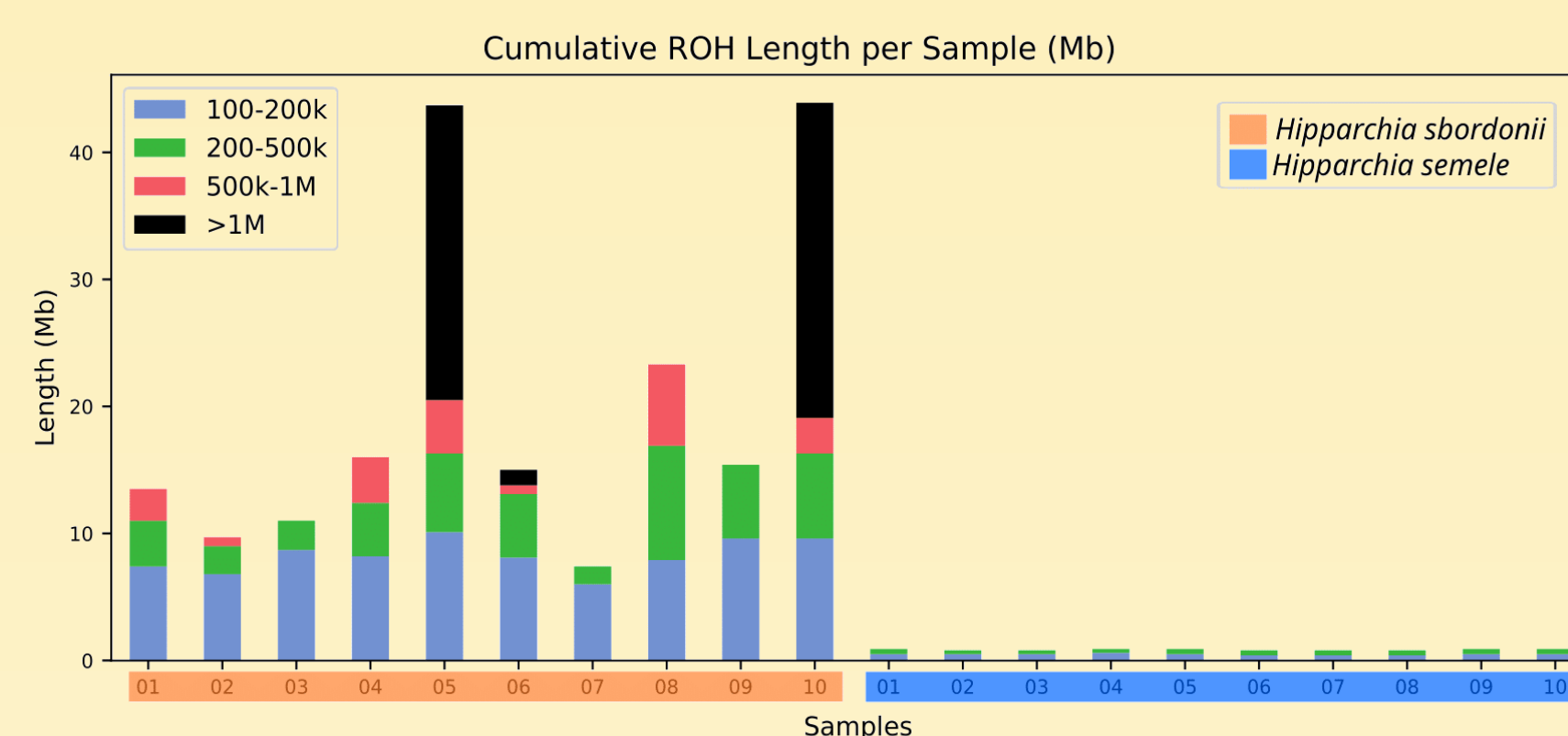
Genome-wide heterozygosity %: *H. sbordonii* shows reduced heterozygosity vs. other Nymphalidae, consistent with small, isolated populations



Nucleotide diversity ( $\pi$ ): Much lower in *H. sbordonii* than *H. semele*, reflecting its insular demography.

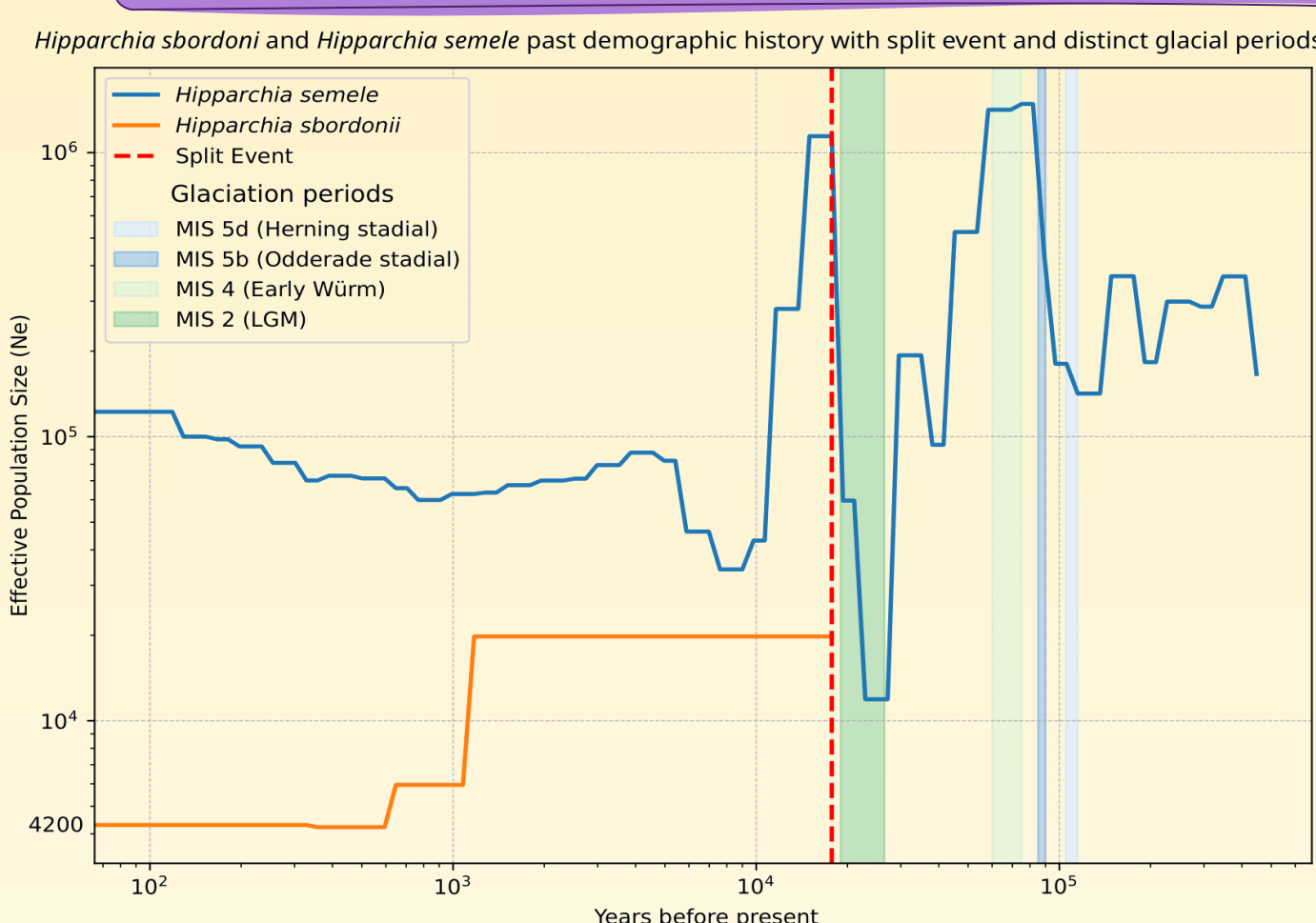


Tajima's D: Negative in *H. semele* (expansion); slightly positive in *H. sbordonii* (recent bottleneck).



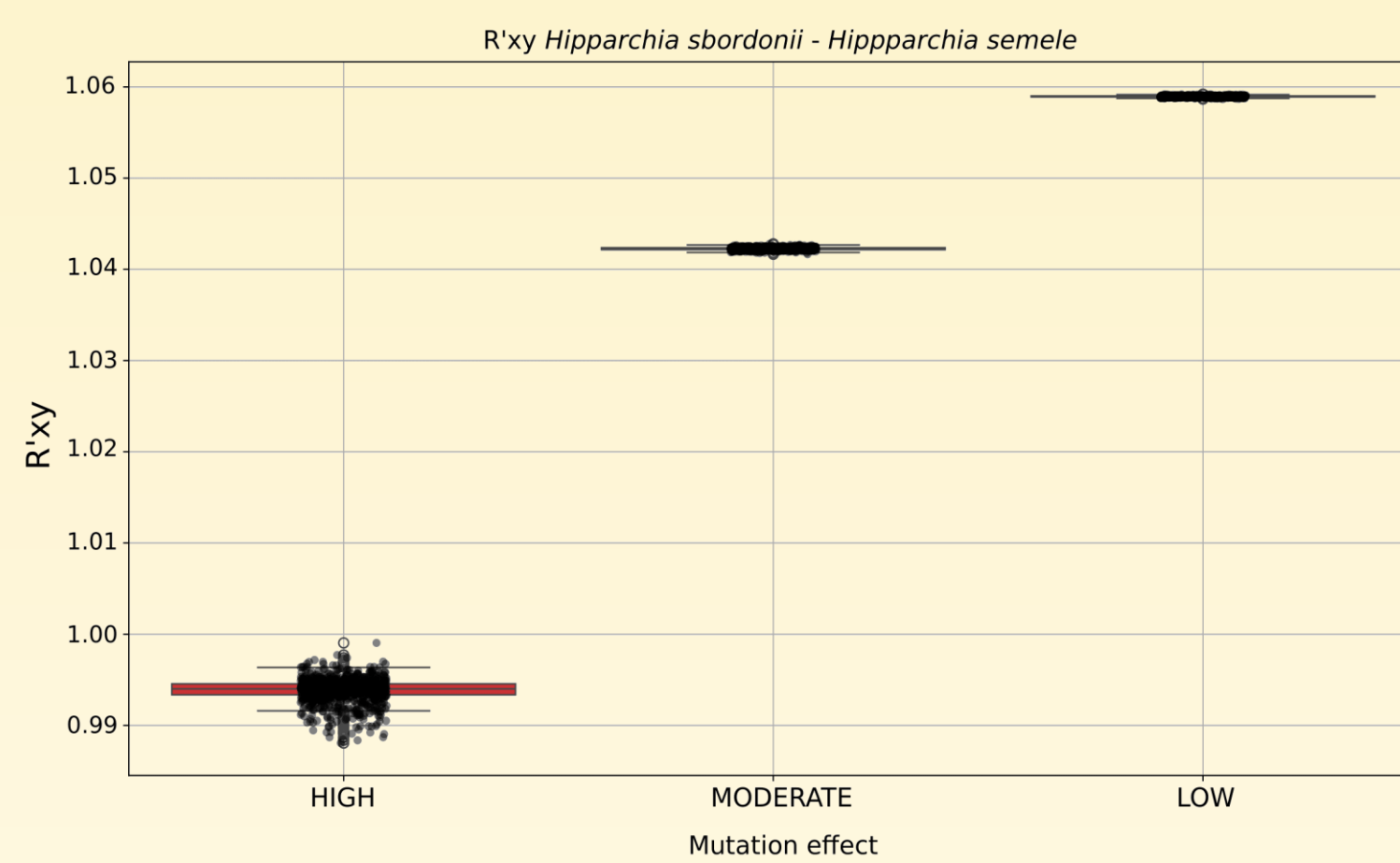
ROH length per individual: *H. sbordonii* has more/longer ROHs, indicating inbreeding and recent small  $N_e$ .

#### Past and recent demographic history



SMC++ demography: *H. sbordonii* shows persistently low  $N_e$ ; species split ~17 kya.

#### Difference in derived allele frequencies: R'xy



Derived allele frequencies ratio (R'xy): *H. sbordonii* depleted in high-effect mutations (purging), enriched in moderate/low-effect ones (possible directional selection).

### Conclusions

*H. sbordonii* shows signs of long-term isolation:

- ↓ Diversity, ↑ ROH
- ↑ Possible purging of highly deleterious mutations

Some fixed variants may reflect island-specific adaptation

#### Next steps workflow

Identify fixed SNPs in *H. sbordonii* (rare/absent in *H. semele*)

Map to genes and perform gene network analysis

Highlight candidates for adaptive island traits

GONE demography: Recent sharp  $N_e$  drop in *H. sbordonii*, starting ~30 years ago.

References  
- Bertorelle, G., Raffini, F., Bosse, M., Bortoluzzi, C., Iannucci, A., Trucchi, E., ... & Van Oosterhout, C. (2022). Genetic load: genomic estimates and applications in non-model animals. *Nature Reviews Genetics*.  
- Webster, M. T., Beaurepaire, A., Neumann, P., & Stolle, E. (2023). Population genomics for insect conservation. *Annual Review of Animal Biosciences*.  
- Fava, S., Solitto, M., Racaku, M., Iannucci, A., Benazzo, A., Ancona, L., ... & Trucchi, E. (2024). Chromosome-Level Reference Genome of the Ponza Grayling (*Hipparchia sbordonii*), an Italian Endemic and Endangered Butterfly. *Genome Biology and Evolution*, 16(7), eaa136.  
- Do, R., Balick, D., Li, H., Adzhubei, I., Sunyaev, S., & Reich, D. (2015). No evidence that selection has been less effective at removing deleterious mutations in Europeans than in Africans. *Nature genetics*.



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