Experimental Speciation: Sexual and Gametic Reproductive Isolation

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1. Background

A species can be defined as a group of interbreeding natural populations that is reproductively isolated from other such groups. The study of speciation attempts to understand the way in which reproductive isolating barriers are formed and maintained. The maintenance of reproductive isolation is a particular challenge in speciation because the flow of genetic information between populations normally acts to homogenize incipient species.[1]

Reinforcement is one way in which reproductive isolation might be strengthened even in the face of gene flow. Reinforcement occurs when natural selection favors avoidance of hybridization, because hybrid offspring have reduced fitness, and results in increased pre-mating (sexual) or post-mating pre-zygotic (gametic) isolation. In fruit fly species of the genus Drosophila, sexual isolation is often mediated by differences between populations or species in courtship behavior, pheromones, or other signals.[2,3] Gametic isolation, on the other hand, can take the form of incompatibilities between sperm and egg that prevent fertilization or sperm precedence, where conspecific sperm are favored over heterospecific sperm in the fertilization of eggs by females.[4]

In order to test whether reinforcement can strengthen pre-zygotic reproductive isolation, we utilized two populations of the fruit fly *Drosophila melanogaster* that are highly genetically diverged (Fig. 1).[5] Using experimental evolution, we mimicked the natural conditions needed for reinforcement (maladaptive hybridization with gene flow) in a laboratory setting with many replicate experimental populations. After a period of evolution in the lab, we tested for evolved changes in reproductive isolation.

If maladaptive hybridization can strengthen prezygotic isolation even when gene flow is present, we predicted an increase in conpopulation mating when females are given a choice between within-population and migrant males (i.e. an increase in sexual isolation). We also predicted an increase in conpopulation sperm precedence (i.e. an increase in gametic isolation) when females are mated to both within-population and migrant males.

IV and LHm *Drosophila melanogaster* Lab Populations

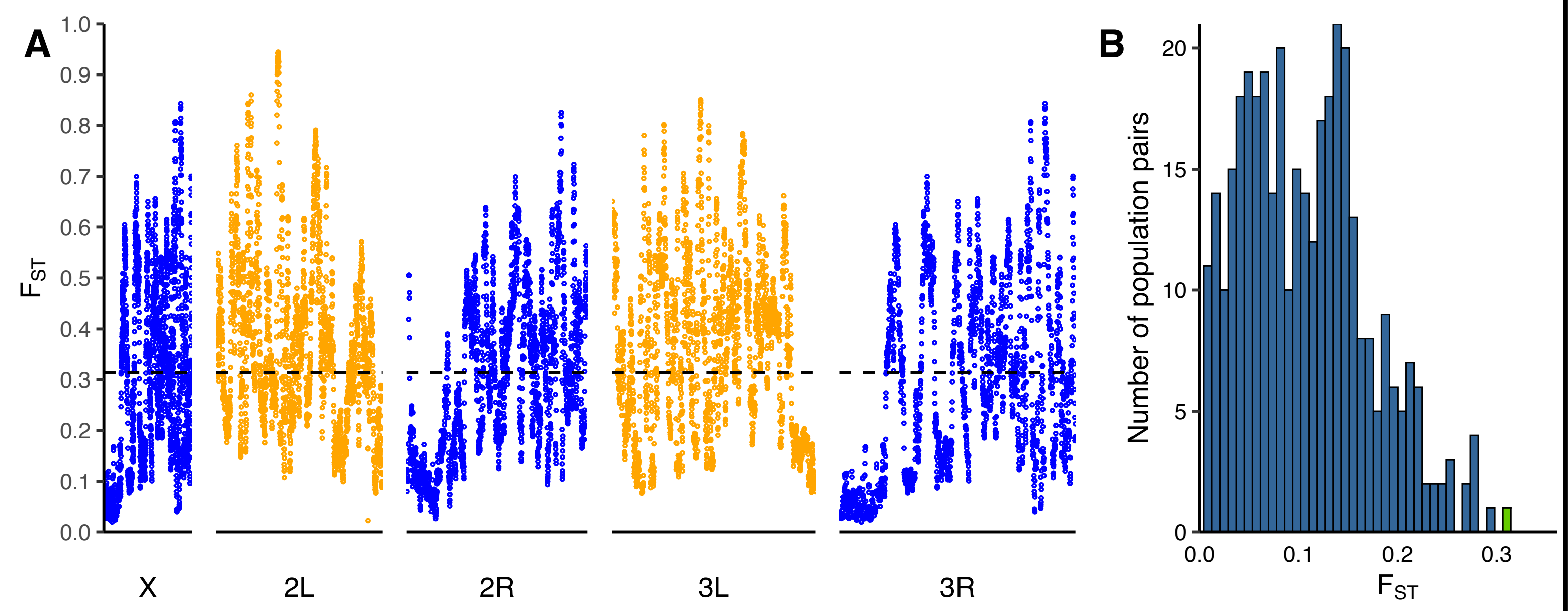


Figure 1. A measure of genetic differentiation (F_{ST}) between IV and LHm *D. melanogaster* lab populations across the three major chromosomes, demonstrating the genome-wide differences that have accumulated over the last 45+ years (> 1000 generations) in allopatry. The dashed line indicates the genome-wide mean F_{ST} of 0.31. **(B)** F_{ST} estimates for pairs of *D. melanogaster* populations collected from around the world (Lack et al. 2016), with the IV-LHm F_{ST} estimate included in green.

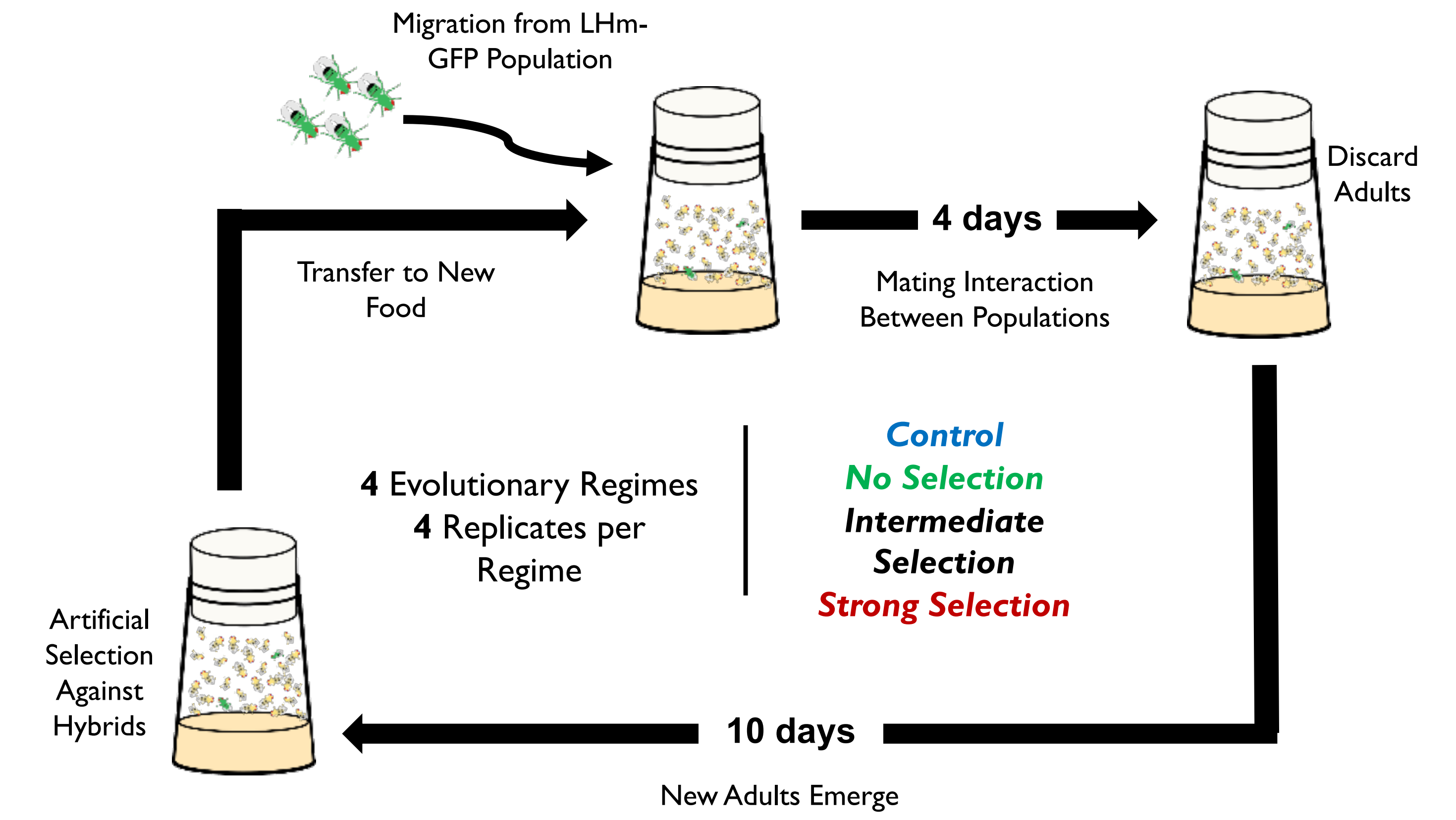
References

(1) Coyne, J. A., & Orr, H. A. (2004). Species: Reality and Concepts. In *Speciation* (p. 27). essay, Sinauer Associates.
(2) Matute, D. R. (2010). Reinforcement Can Overcome Gene Flow during Speciation in Drosophila. *Current Biology*,
(3) Coyne, J. A., & Orr, H. A. (2004). Reinforcement. In *Speciation* (pp. 353–354). essay, Sinauer Associates.
(4) Price, C. (1997). Conspecific sperm precedence in Drosophila; Nature
(5) Lack, J. B. et al. (2016). A Thousand Fly Genomes: An Expanded Drosophila Genome Nexus; Molecular Biology and Evolution.

2. Methods

Evolutionary Regime Maintenance

We used experimental evolution to track the course of reinforcement in real time utilizing the IV and LHm lab populations of *Drosophila melanogaster*. By mimicking the natural conditions of reproductive isolation via reinforcement in the lab we can observe real world changes in a controlled environment.



Migration from LHm-GFP Population

Transfer to New Food

4 days

Mating Interaction Between Populations

Discard Adults

4 Evolutionary Regimes
4 Replicates per Regime

Control
No Selection
Intermediate Selection
Strong Selection

Artificial Selection Against Hybrids

10 days

New Adults Emerge

Figure 2. Experimental evolution scheme depicting migration and selection of the four experimental regimes. The "control" regime receives no migration from LHm males.

Testing Sexual Reproductive Isolation

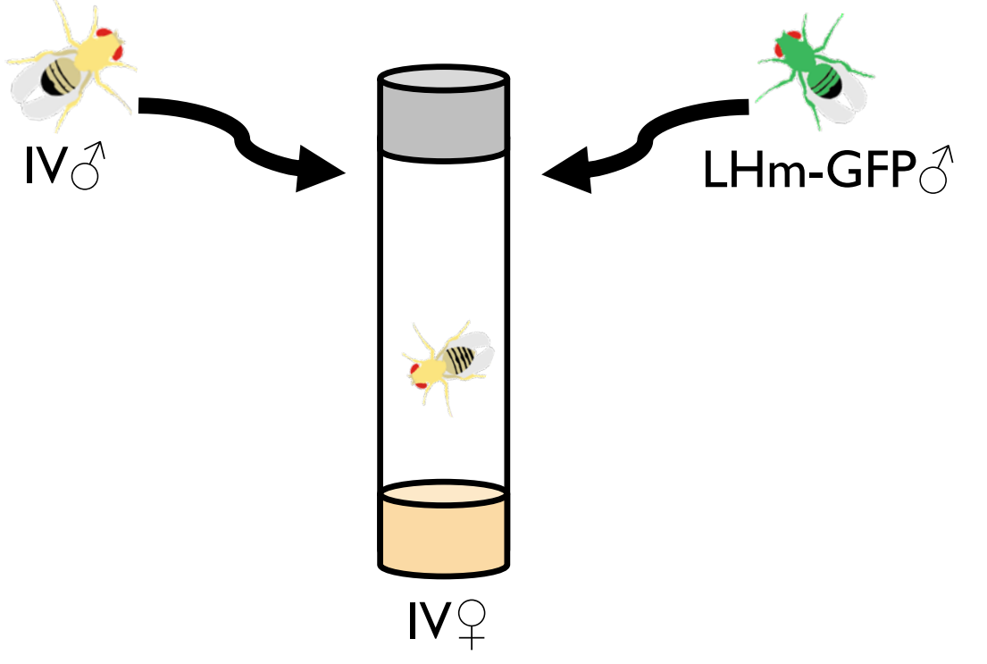


Figure 3. Experimental scheme of competition assay.

To test IV female willingness to mate conpopulation male versus heteropopulation male we set up a simple competition assay (Fig. 3). We assessed female mating preference by directly competing LHm and IV males for an IV female and observing the outcome (n = 75 matings per replicate population for each regime). Sexual reproductive isolation was tested at generations 22 and 23.

Testing Gametic Reproductive Isolation

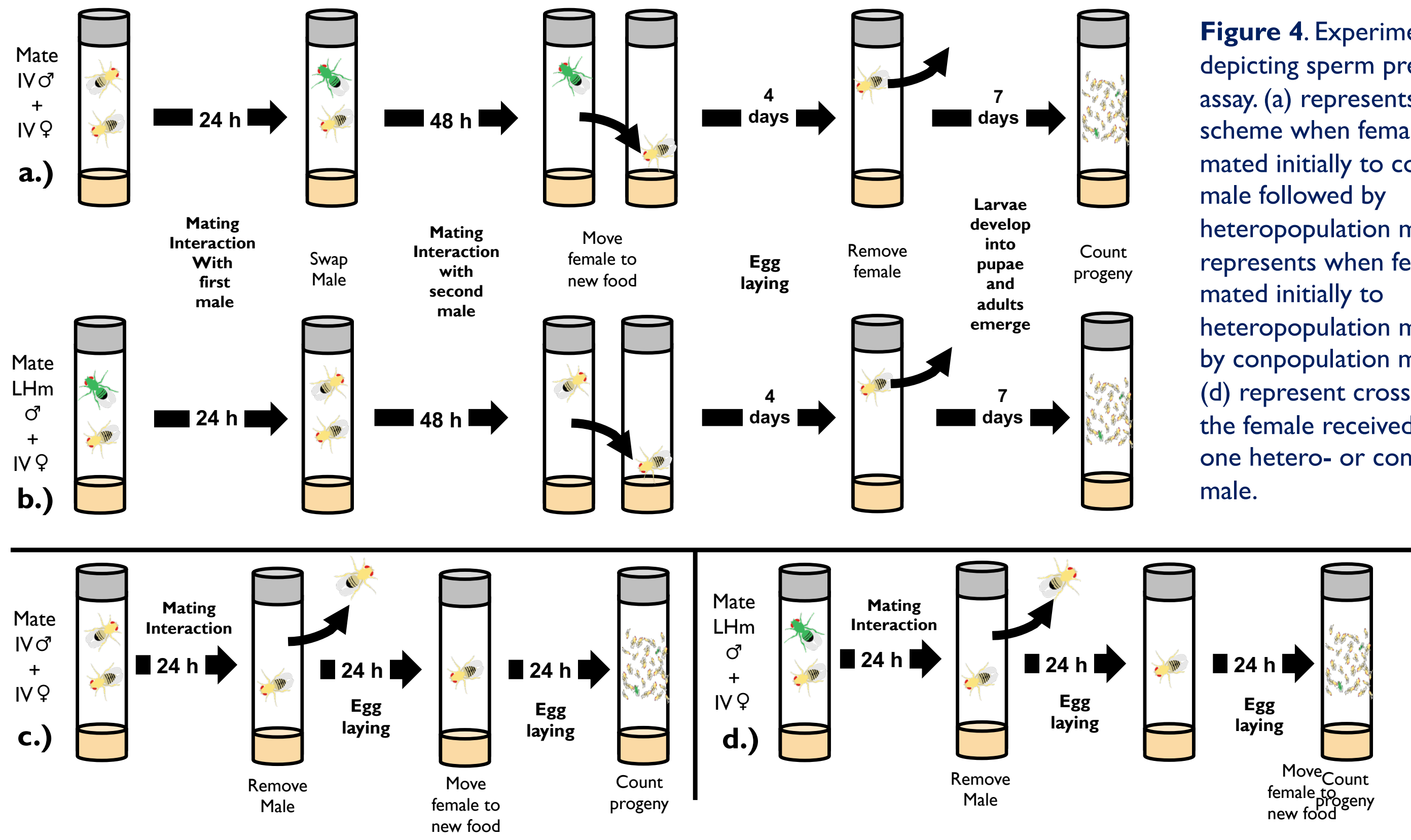


Figure 4. Experimental scheme depicting sperm precedence assay. (a) represents mating scheme when female was mated initially to conpopulation male followed by heteropopulation male. (b) represents when female was mated initially to heteropopulation male followed by conpopulation male. (c) and (d) represent crosses where the female received exclusively one hetero- or con-population male.

To test gametic reproductive isolation we tested female sperm precedence between hetero- and con-population males. Females were doubly inseminated where some females were mated to conpopulation males first (n = 20) (Fig. 4a) and other females were mated to heteropopulation males first (n = 20) (Fig. 4b). In addition to the double mated females we also mated females exclusively to either a hetero- or con-population male (Fig. 4c & d) (n = 10) and counted the number of progeny. Gametic reproductive isolation was tested at generations 24 and 25.

3. Results

Sexual Isolation

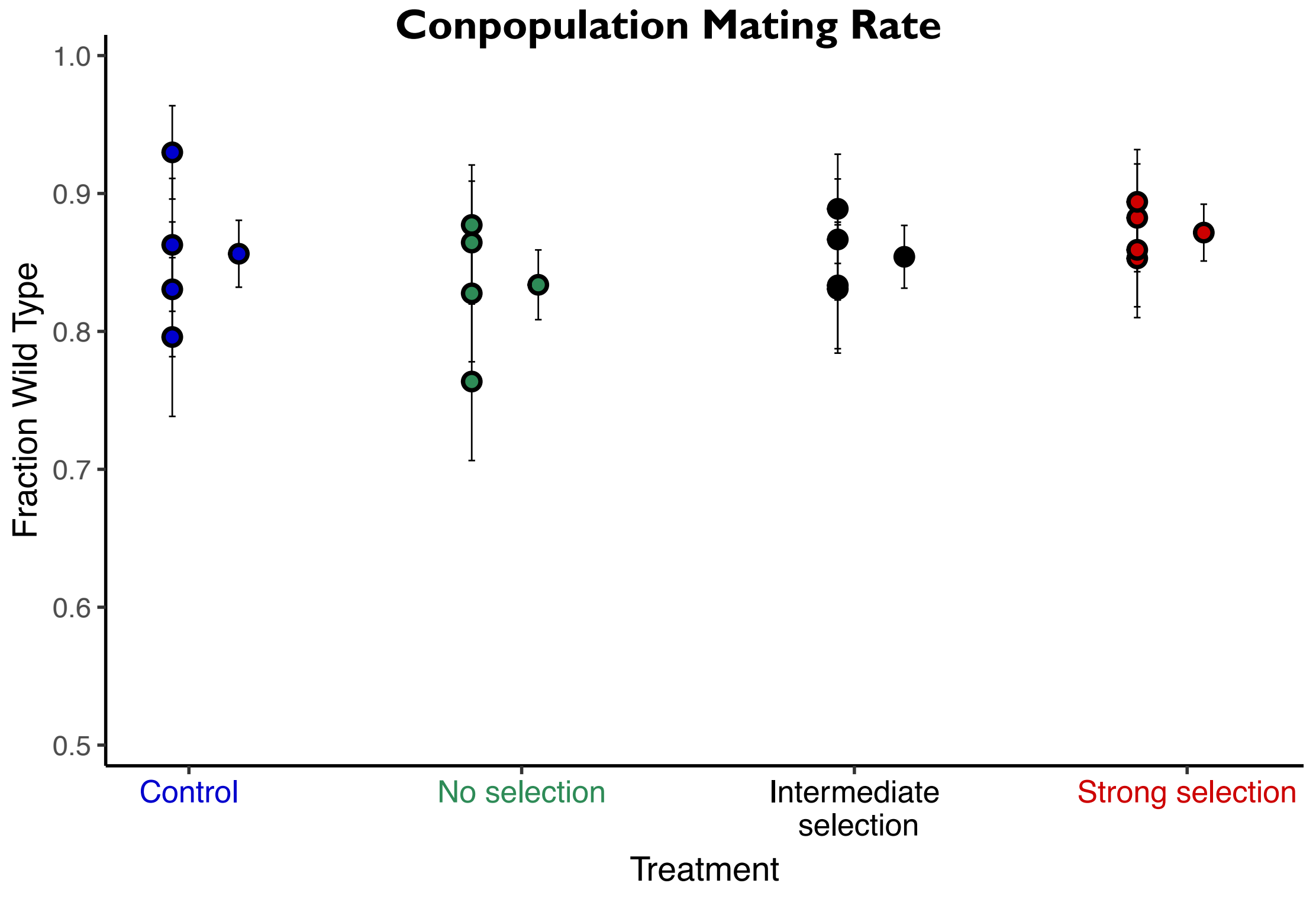


Figure 5. Proportion of IV females who mated IV males across the four evolutionary regimes. Stacked points represent individual population mating rates. Lone point represents treatment-wide mating rates. There was no significant effect of evolutionary regime on the probability conpopulation mating (X^2 = 1.43, p = .70).

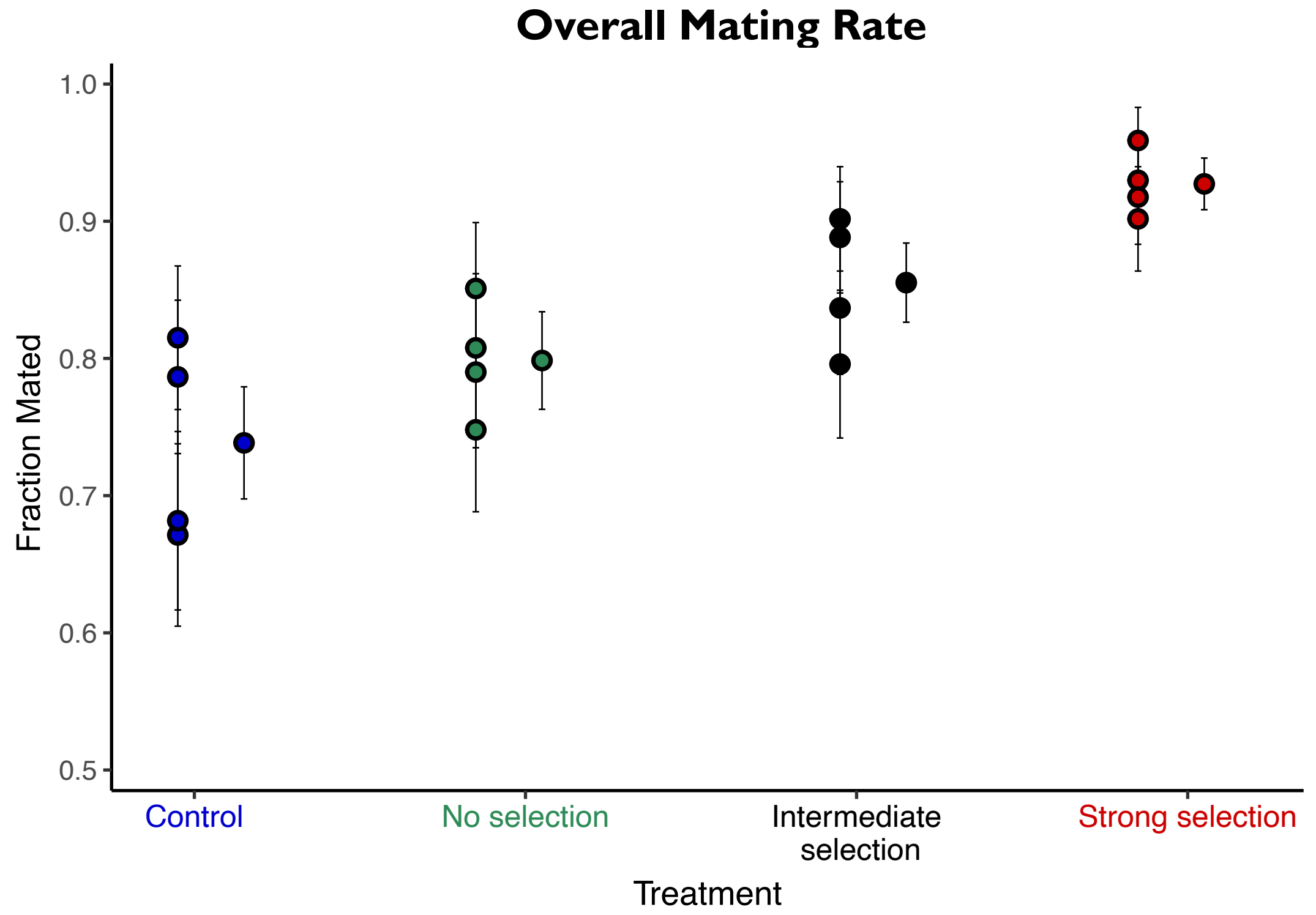


Figure 6. Proportion of IV females who mated either an IV male or a GFP male across evolutionary regimes. Stacked points represent individual population mating rates. Lone point represents treatment-wide mating rates. There was a significant regime effect on the willingness to mate (X^2 = 22.288, p < .001)

Gametic Isolation

Singly mated crosses between IV females and hetero- or con-population males showed no clear difference in the number of progeny sired by the female.

For sperm competition outcomes (i.e. doubly mated females), we do not yet have the data available.

4. Conclusions

- No significant difference was detected in mating preference between conpopulation and heteropopulation individuals across experimental regimes.
- Both the Strong and Intermediate regimes (regimes experiencing strongest reinforcing selection) differed from Control regime in their overall willingness to mate.

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