

Additional File 1: Supplementary information

Supplementary Tables

Table S1. Estimated effective population size from generation 0 to generation 60.

Population	Cons		Fluc	
	autosome	X chromosome	autosome	X chromosome
Replicate 1	216	197	244	179
Replicate 2	218	219	253	146
Replicate 3	279	191	265	213
Replicate 4	185	184	293	220
Replicate 5	225	173	233	173

#p-value=0.09524 for autosome (Wilcoxon test)

#p-value=0.7533 for X chromosome (Wilcoxon test)

Table S2. Number of SNPs that showed replicate-specific signal detected from adapted Chi-square test between generation 0 and generation 60.

Population	Cons	Fluc
Replicate 1	14,271	14,528
Replicate 2	19,454	14,091
Replicate 3	8,593	15,205
Replicate 4	21,762	12,771
Replicate 5	14,971	16,373
Total	76,491	71,004

Supplementary Figures

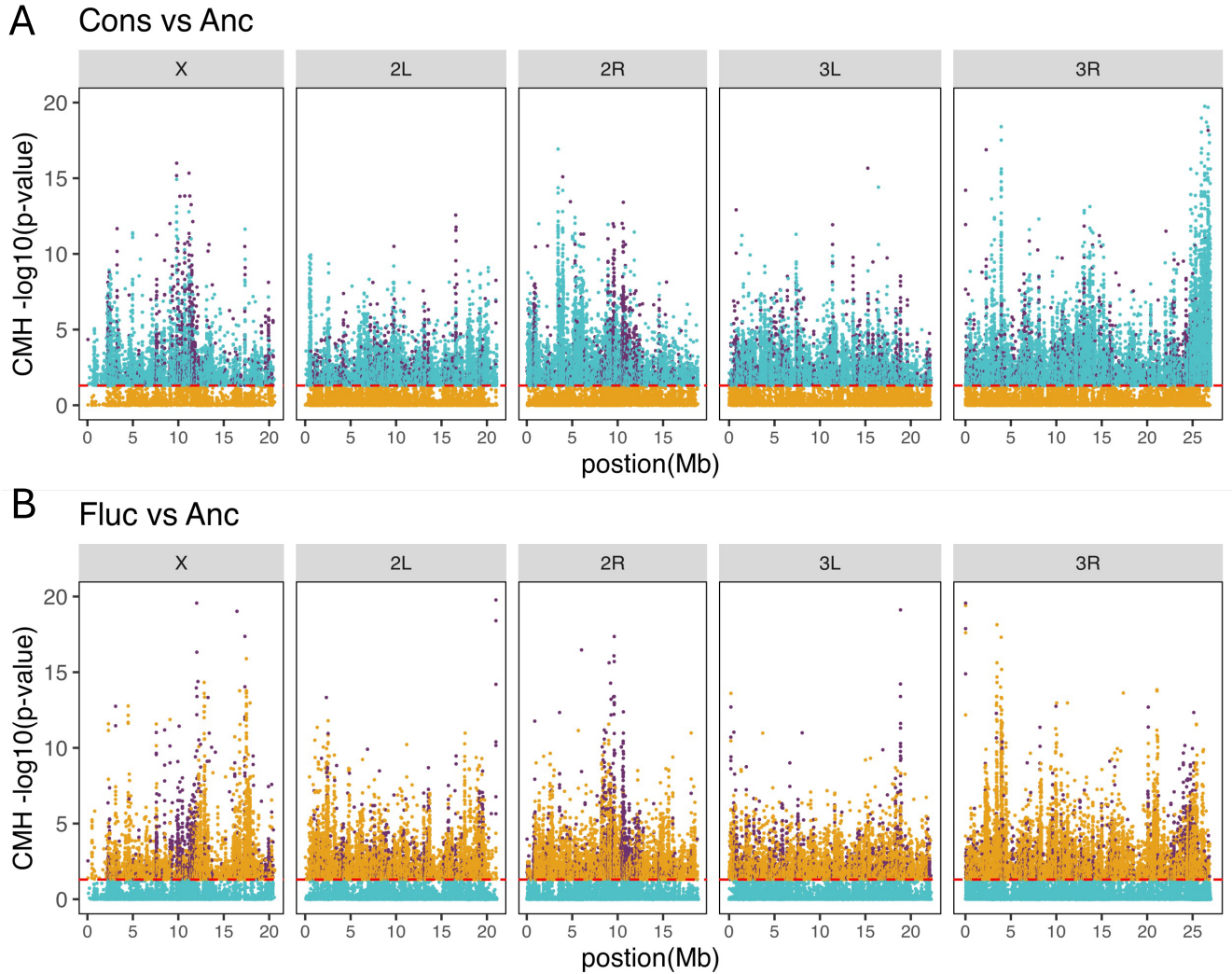
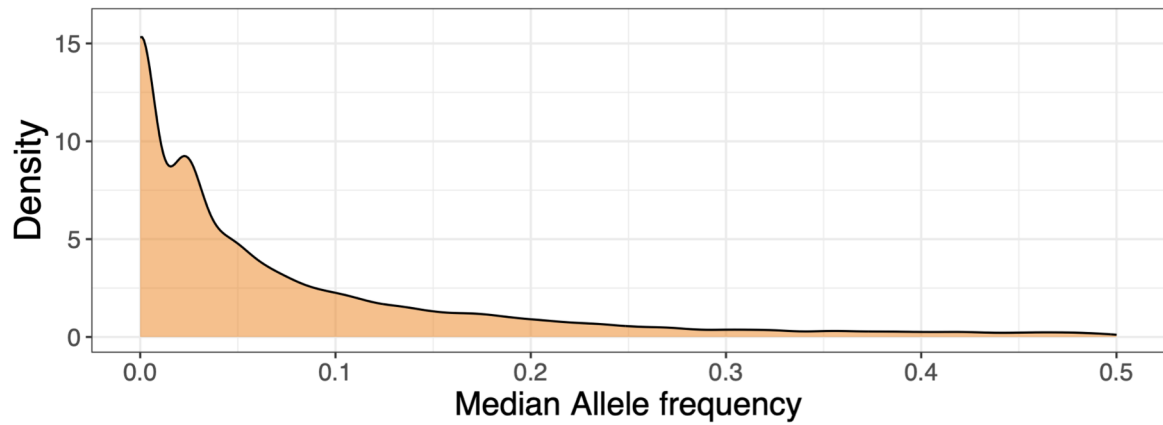
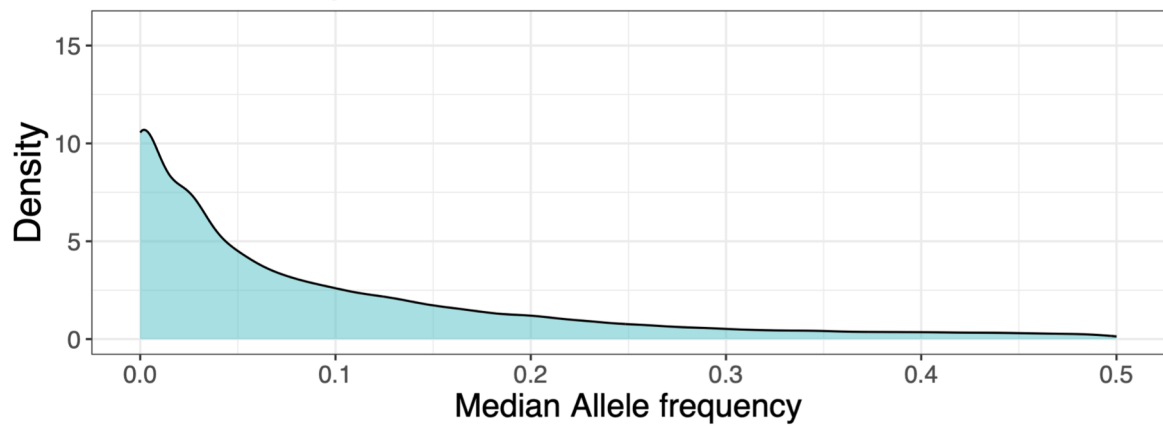


Fig S1. Manhattan plot based on the allele frequency differences between the founder population and evolved population at generation 60 using the adapted CMH test. (A) Adaptive CMH test between five replicates of the Cons population (F60) and the Anc population. (B) Adaptive CMH test between five replicates of the Fluc population (F60) and the Anc population. The color indicates the treatment in which a SNP exhibits a more pronounced allele frequency change across replicates than expected under neutrality. Blue: SNPs significant only in the constant treatment; orange: SNPs significant only in the fluctuating treatment; purple: SNPs significant in both treatments; grey: non-significant SNPs.

A Fluctuating Significant SNPs



B Constant Signifiacnt SNPs



C Genomewide SNPs

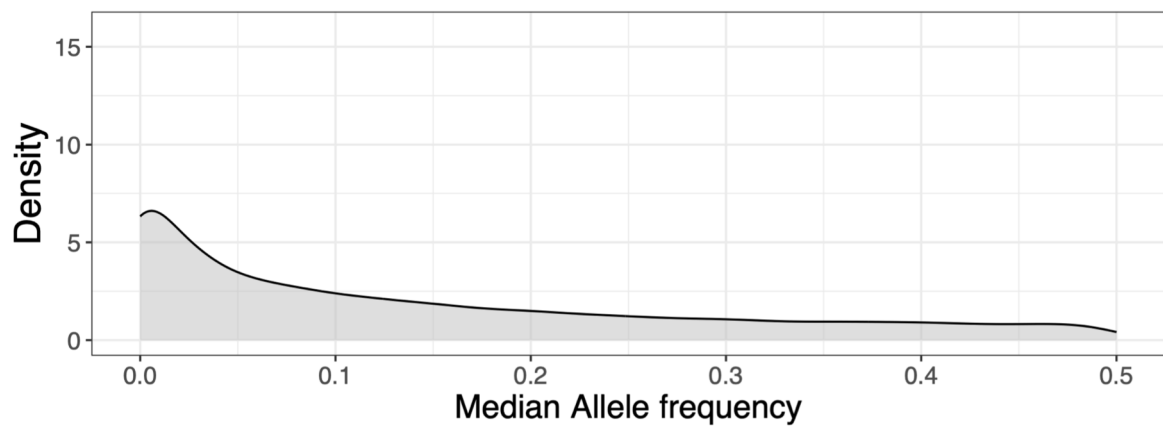


Fig S2 Frequency distribution in the founder population of SNPs that respond to selection. The median frequency across five replicates is shown. (A) SNPs with a significant adapted CMH test in the constant treatment (F60). (B) SNPs with a significant adapted CMH test in the fluctuating treatment (F60). (C) Randomly sampled SNPs from the genome-wide; the number of sampled SNPs is the average of significant SNPs identified in the fluctuating and constant populations.

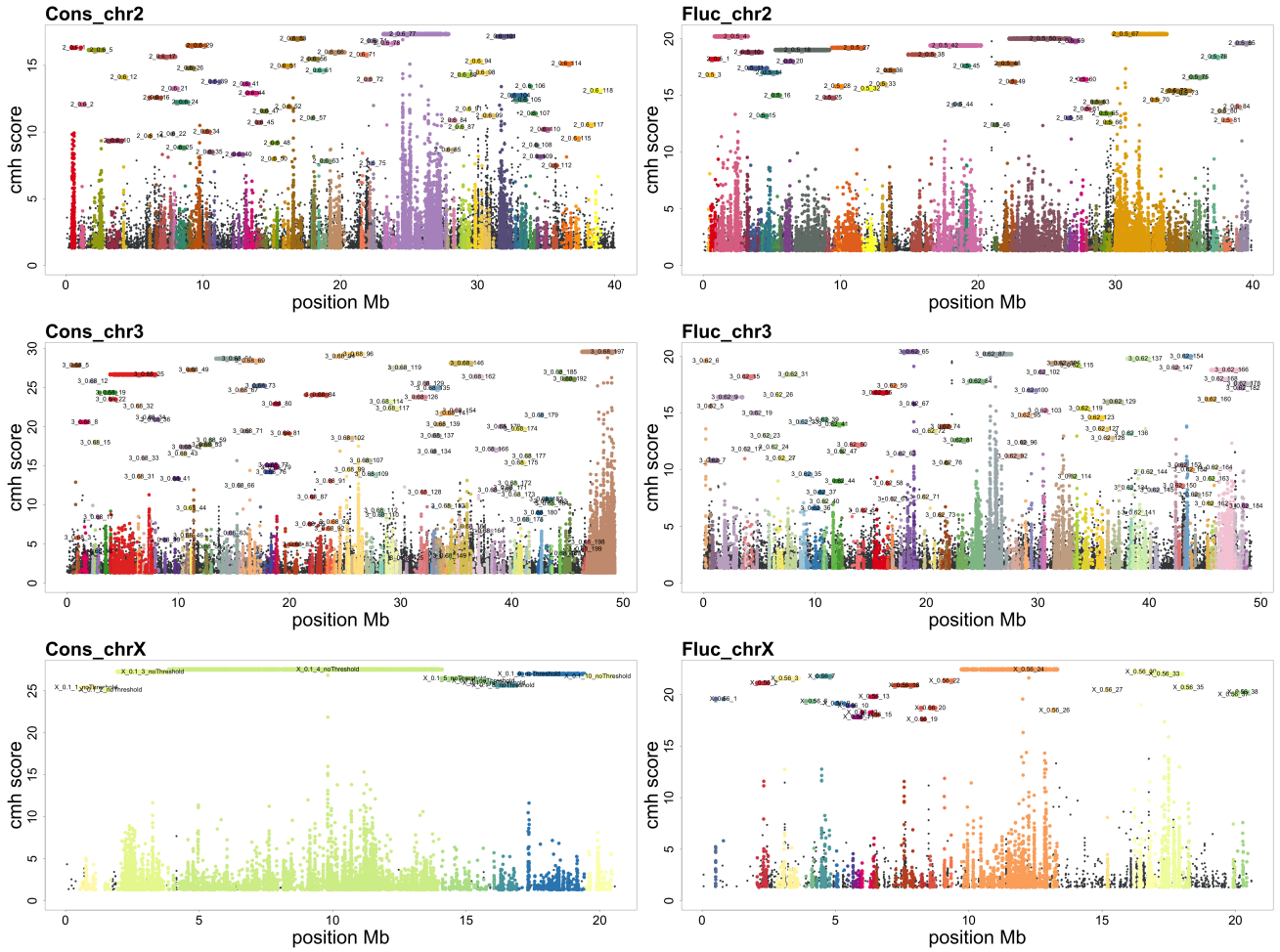


Fig S3. Overview of reconstructed Haplotypes. SNPs belonging to haplotype blocks identified by Haplovalidate are shown in different colors. The horizontal lines show the genomic regions spanned by haplotype blocks. Significant SNPs identified from the adapted CMH test in the fluctuating and constant populations were used for haplotype reconstruction separately.

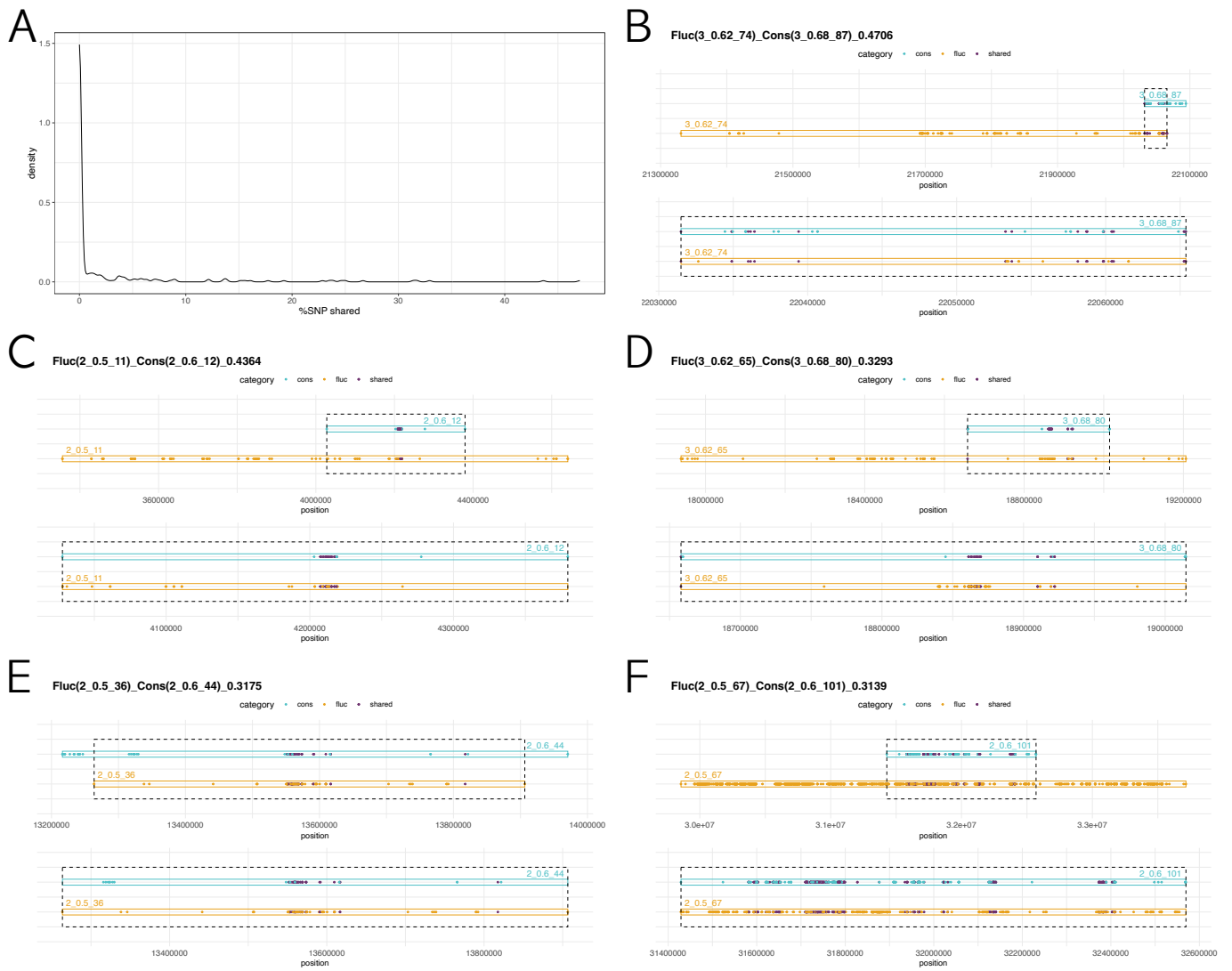


Fig S4. No shared haplotype between fluctuating and constant populations. (A) Distribution of proportion of SNPs being shared between haplotypes from fluctuating and constant populations. (B)-(F) Visualization of overlapping haplotype blocks detected in fluctuating and constant environments. Haplotype blocks identified in the fluctuating environment (orange) and the constant environment (blue) are shown as horizontal rectangles spanning the genomic positions of the SNPs assigned to each haplotype. Points indicate SNPs used for haplotype clustering. SNP colors represent their significance in environment-specific CMH tests: orange, significant only in the fluctuating environment; blue, significant only in the constant environment; purple, significant in both environments. The dashed rectangle highlights the genomic region where the two haplotype blocks overlap. The lower panel shows a magnified view of the overlapping region, illustrating the distribution of shared and environment-specific candidate SNPs within the overlap.

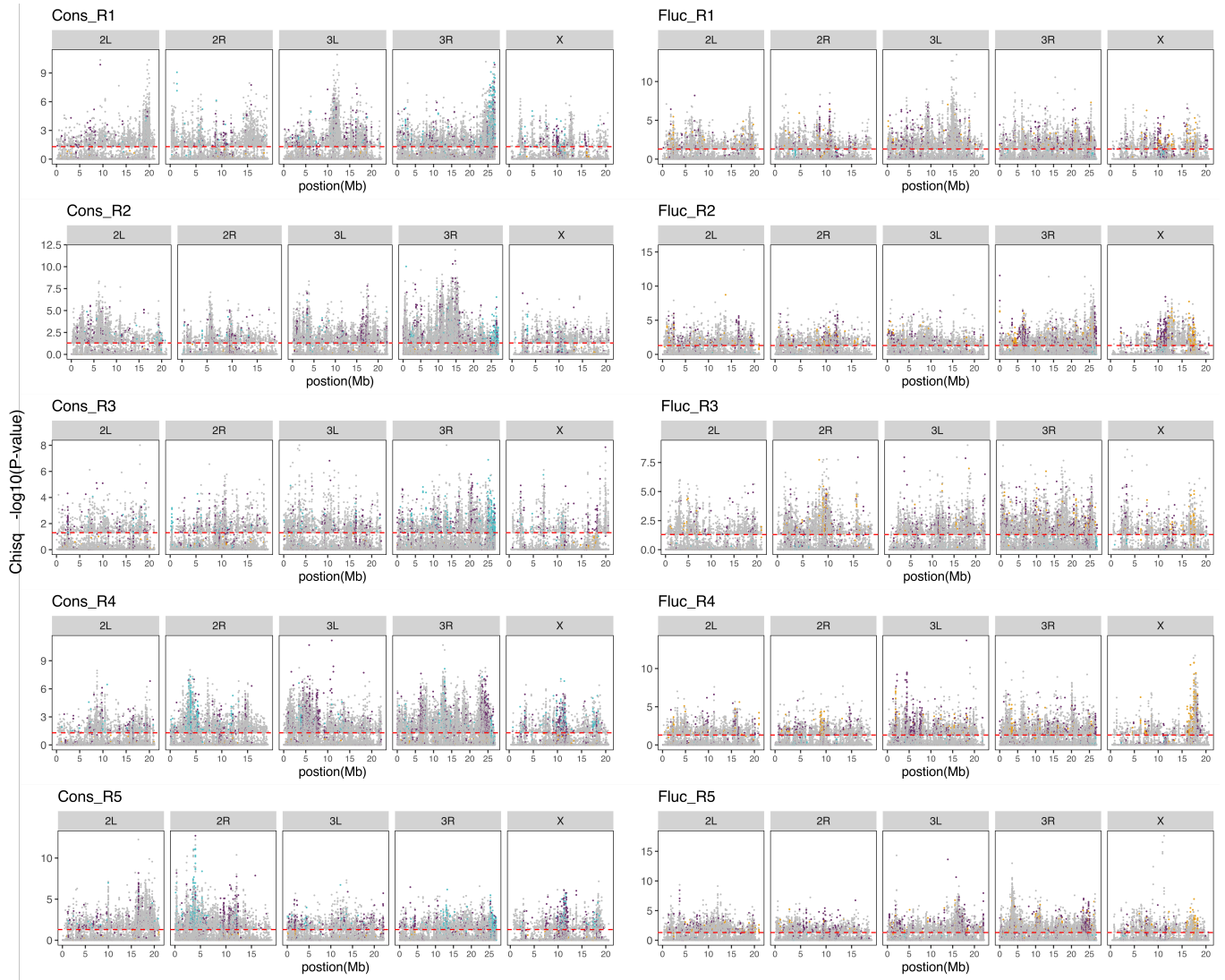


Fig S5. Manhattan plot based on the allele frequency differences between the founder population and evolved population at generation 60. P-values were obtained for each replicates using the adapted Chisq-test. The left panel showed the p-value of five replicates from the Cons populations. The right panel showed the p-value of five replicates from the Fluc populations. The color indicates the treatment in which a SNP exhibits a more pronounced allele frequency change across replicates than expected under neutrality ($p < 0.05$). **Grey**: SNPs only significant in the focal replicate. **Blue**: SNPs significant in more than one replicate adapting to the constant environment, but not significant in the fluctuating environment. **Orange**: SNPs significant in more than one replicate adapting to the fluctuating environment, but not significant in the constant environment. **Purple**: SNPs significant in at least one replicate in both environments. SNPs that were not significant in any replicate were not visualized.

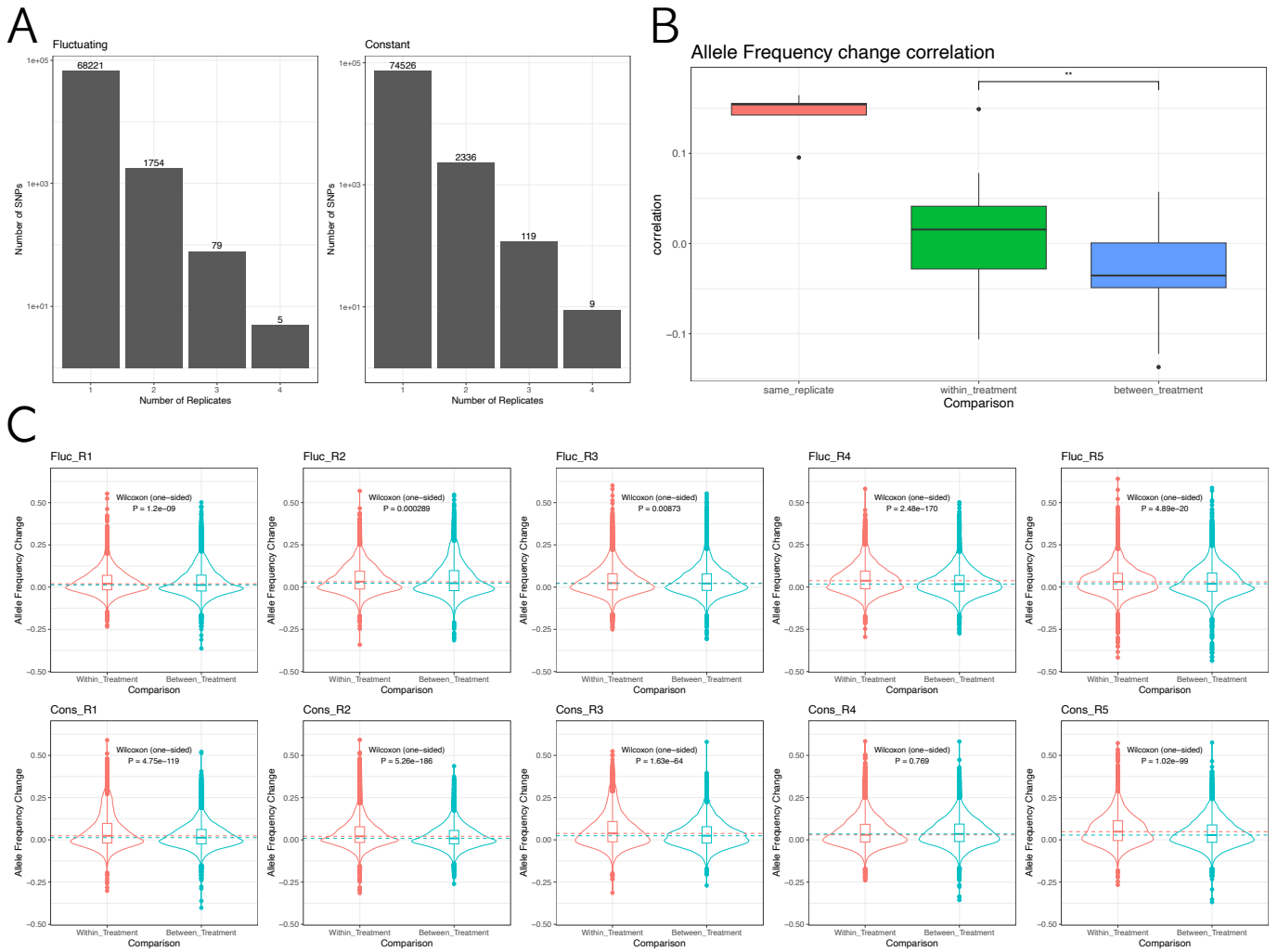


Fig S6. Higher similarity in genomic response of replicates within the treatment than between treatments. (A) Replicate frequency spectrum of SNPs identified as significant from the adapted chisq-test between generation 0 and generation 60. The x-axis shows the number of replicates in which SNPs show significance, and the y-axis indicates the number of SNPs. (B) Pearson correlation of allele frequency change between F0 and F60. (C) Mean allele frequency change distributions between generation 0 and generation 60. For each focal replicate, significant SNPs from the adapted chisq-test were extracted and polarized to increase in frequency in the focal replicate. Then the mean allele frequency changes of the remaining replicates within the same treatment (red) and the matching four replicates in the other treatment (blue) were plotted separately. We compared the distributions with one-sided Wilcoxon tests. Replicates derived from the same ancestral population but evolved in the alternative environment were excluded from the analysis to avoid non-independence arising from shared ancestry.

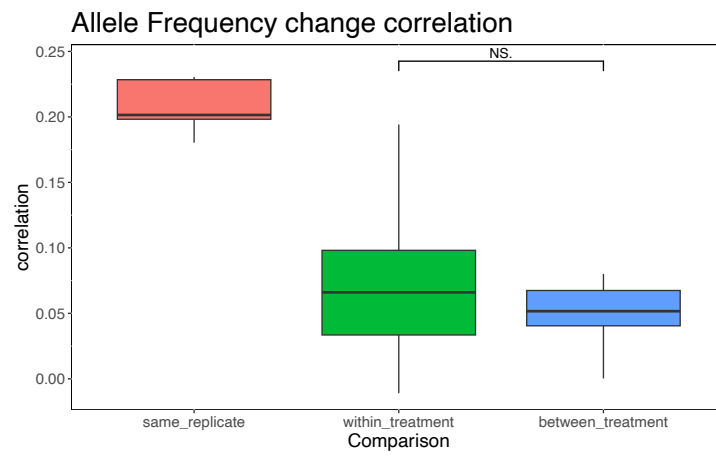


Fig S7. Pearson correlation of allele frequency change between F0 and F60 calculated from genome-wide SNPs.

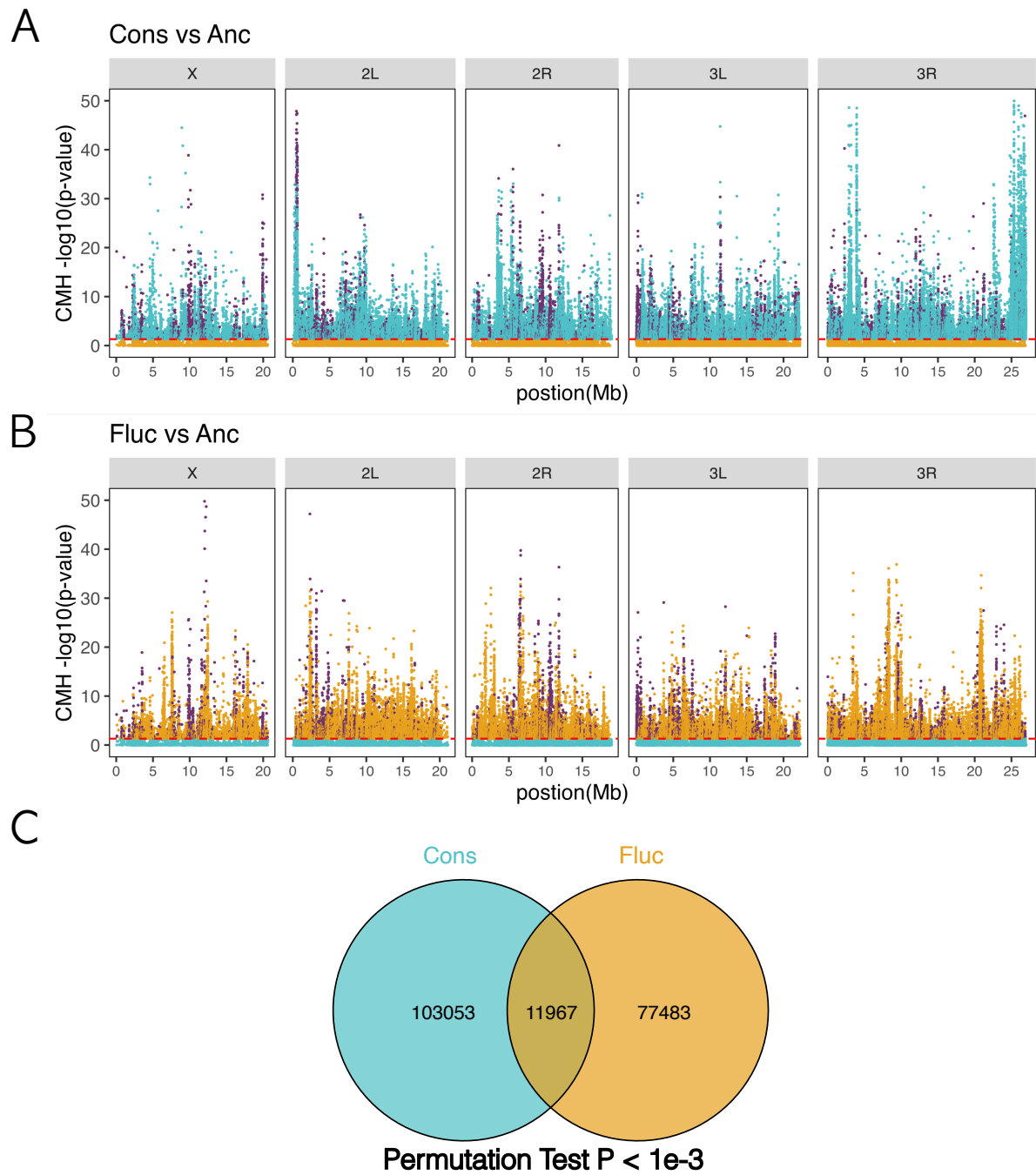


Fig S8. Only a few selected SNPs are shared between fluctuating and constant treatments at generation 160. (A) Manhattan plot showing the adaptive CMH test results between five replicates of the Cons populations and the Anc populations. (B) Manhattan plot showing the adapted CMH test results between five replicates of the Fluc populations and the Anc populations. The color indicates the treatment in which a SNP exhibits a more pronounced allele frequency change across replicates than expected under neutrality. Blue: SNPs significant only in the constant treatment; orange: SNPs significant only in the fluctuating treatment; purple: SNPs significant in both treatments. SNPs that were not significant in any treatment were not visualized. (C) Venn diagram based on the significant SNPs detected in the fluctuating/constant treatments.

Model1

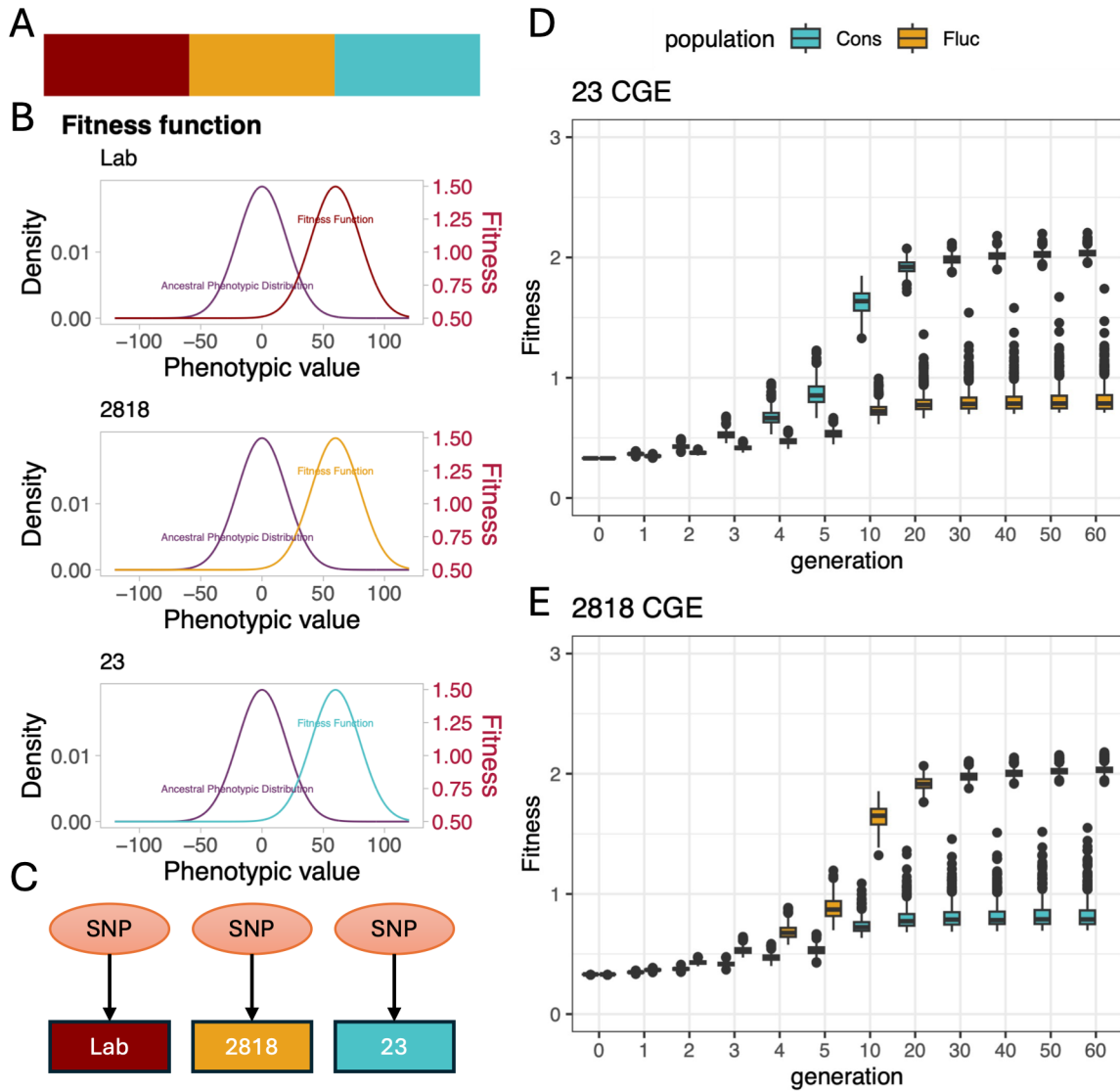


Fig S9. Forward simulation results of model 1. Panels (A)-(C) describe the simulation scenario: (A) Color areas schematically represent the number of loci contributing to each trait. The same number of loci contribute to each trait. (B) Fitness function for the three traits in the simulation. Lab: traits are shared between two environments. 2818: traits that are specific to the fluctuating environment. 23: traits that are specific to the constant environment. Equal selection strength of three simulated traits. (C) Non-pleiotropy: each SNP only contributes to one trait. (D, E) Fitness estimates in the 23 °C CGE (upper panel) and 2818 °C CGE (lower panel) of populations adapting to the fluctuating and constant environments.

Model2

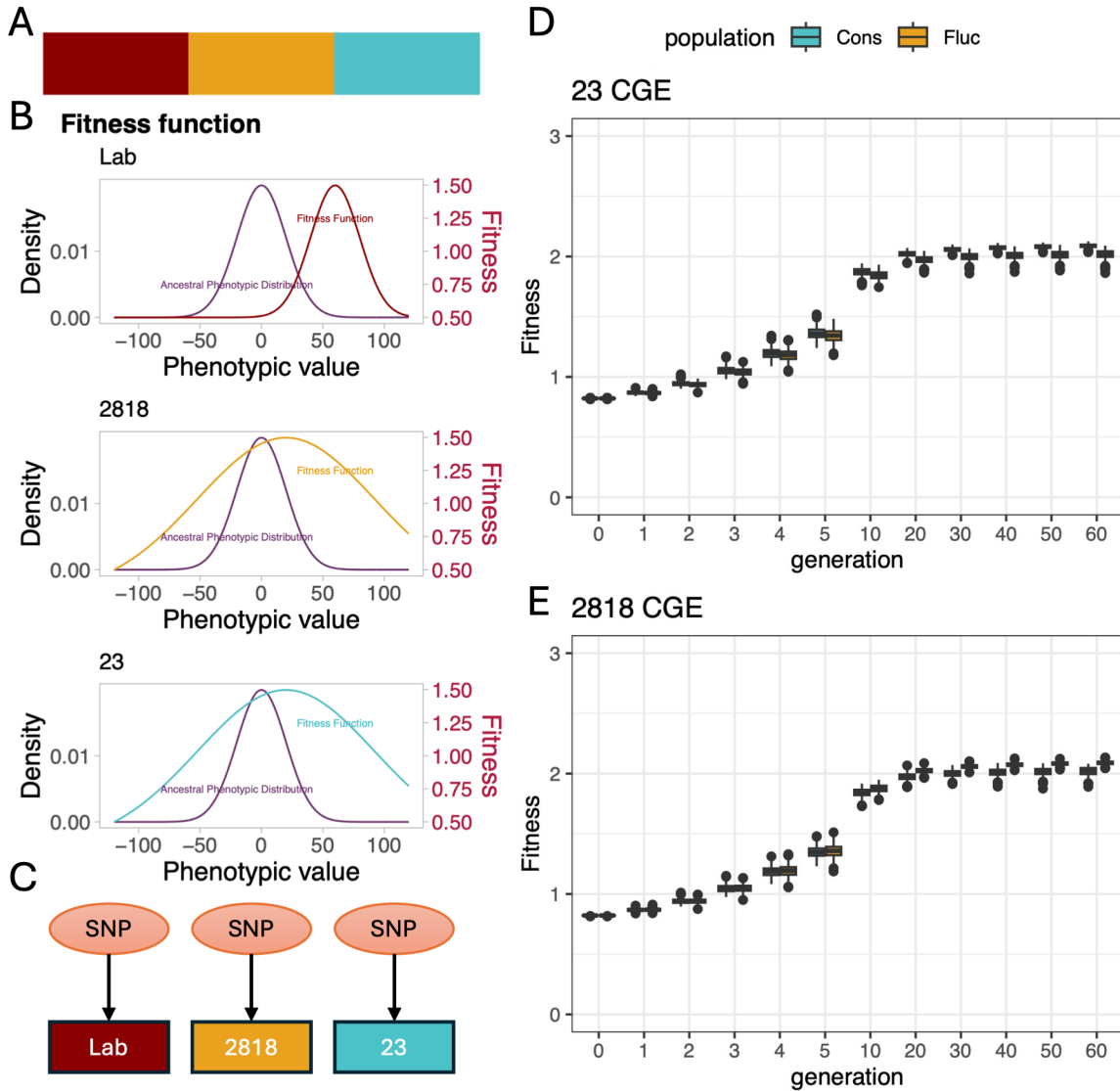


Fig S10. Forward simulation results of model 2. Panels (A)-(C) describe the simulation scenario: (A) Color areas schematically represent the number of loci contributing to each trait. The same number of loci contributes to each trait. (B) Fitness function for the three traits in the simulation. Lab: traits are shared between two environments. 2818: traits that are specific to the 28/18°C environment. 23: traits that are specific to the 23°C environment. Stronger selection strength for lab traits than temperature-specific traits. (C) Non-pleiotropy: each SNP only contributes to one trait. (D, E) Fitness estimates in the 23 °C CGE (upper panel) and 2818 °C CGE (lower panel) of populations adapting to the fluctuating and constant environments.

Model3

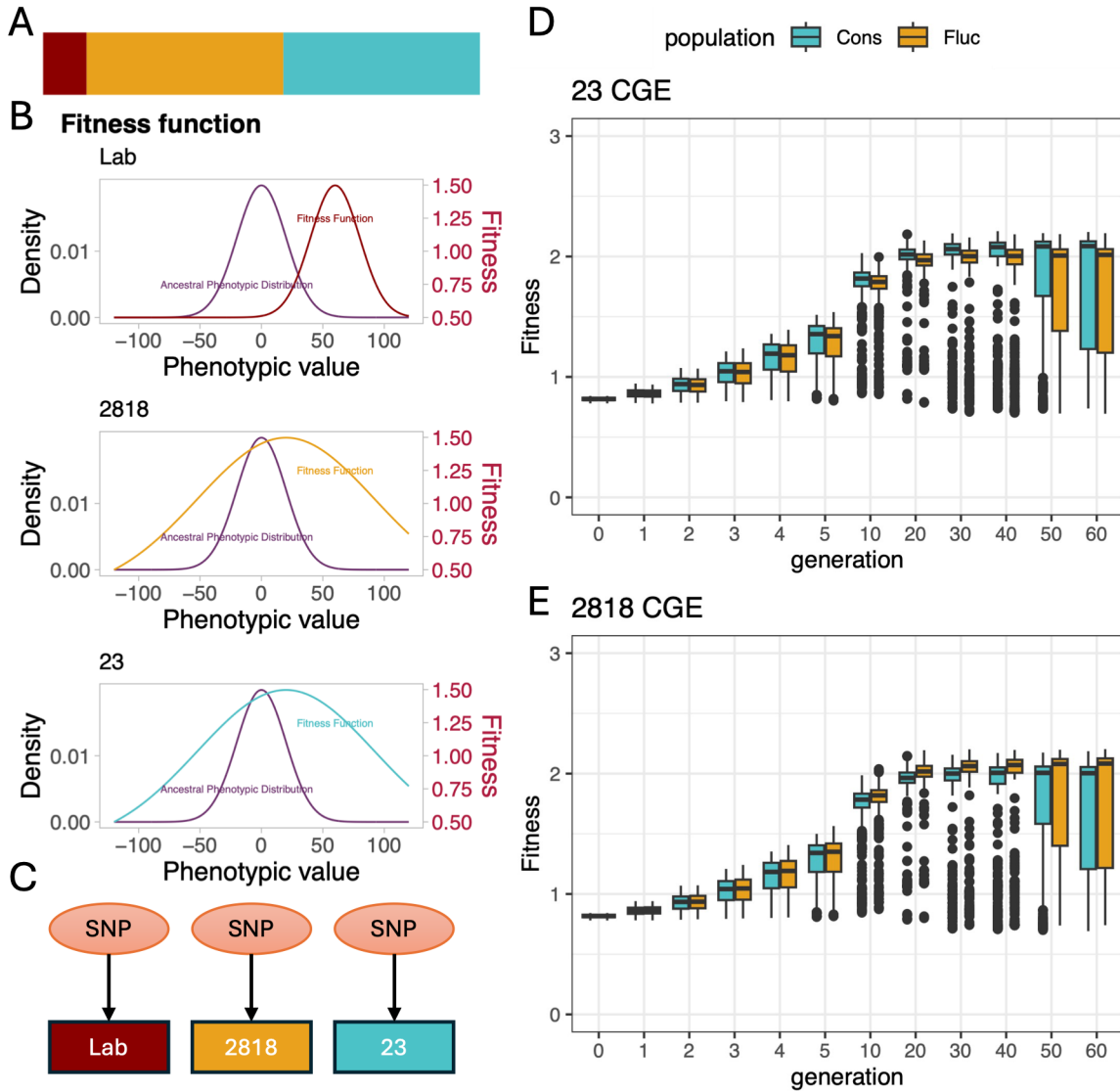


Fig S11. Forward simulation results of model 3. Panels (A)-(C) describe the simulation scenario: (A) Color areas schematically represent the number of loci contributing to each trait. A few SNPs contribute to lab traits (10 SNPs, dark red), while more SNPs contribute to temperature-specific traits (4,995 SNPs each, orange and blue). (B) Fitness function for the three traits in the simulation. Lab: traits are shared between two environments. 2818: traits that are specific to the 28/18°C environment. 23: traits that are specific to the 23°C environment. Stronger selection strength for lab traits than temperature-specific traits. (C) Non-pleiotropy: each SNP only contributes to one trait. (D, E) Fitness estimates in the 23 °C CGE (upper panel) and 2818 °C CGE (lower panel) of populations adapting to the fluctuating and constant environments.

Model4

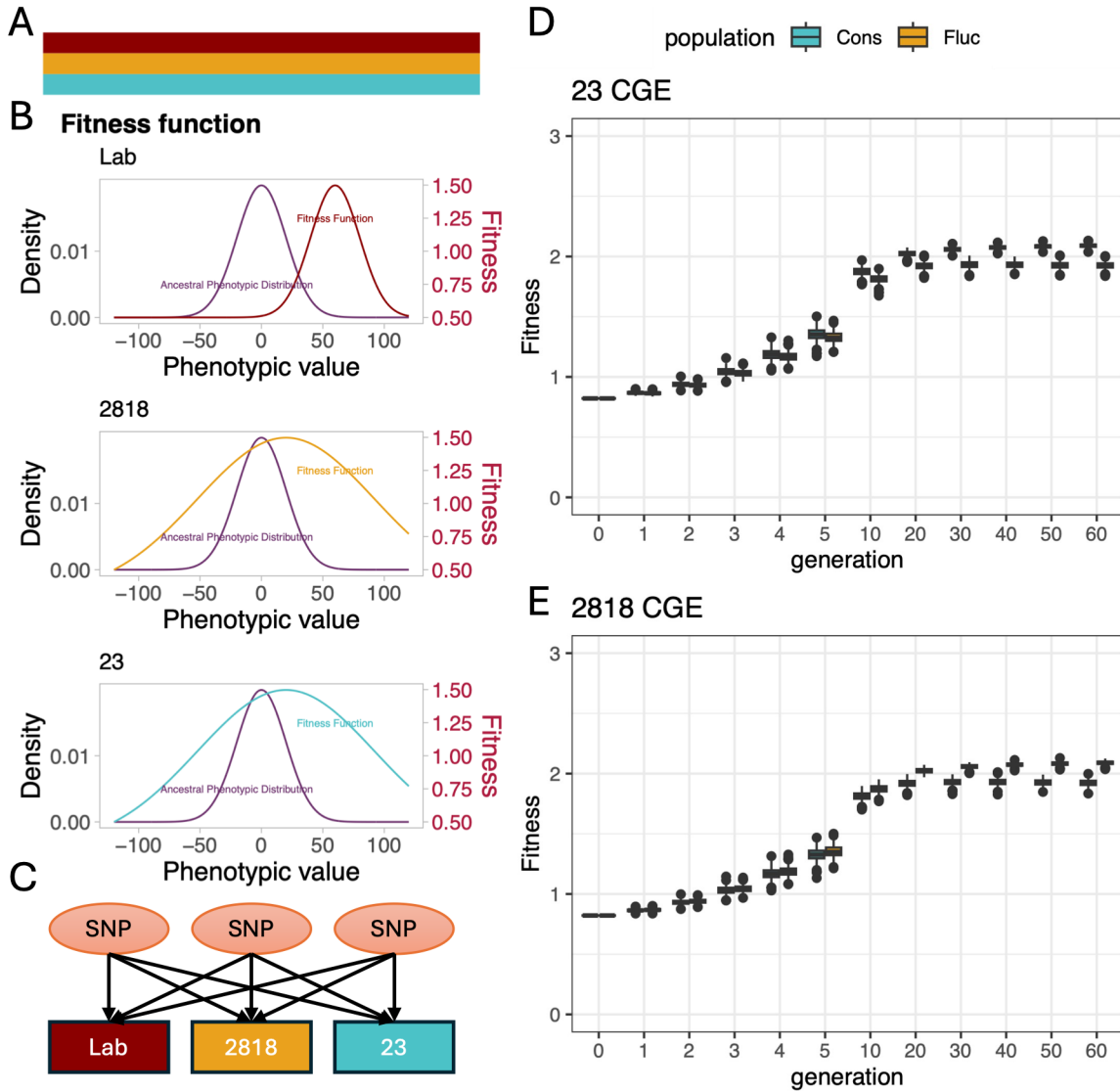


Fig S12. Forward simulation results of model 4. Panels (A)-(C) describe the simulation scenario: (A) Color areas schematically represent the number of loci contributing to each trait. Each SNP contributes to all three simulated traits. (B) Fitness function for the three traits in the simulation. Lab: traits are shared between two environments. 2818: traits that are specific to the 28/18°C environment. 23: traits that are specific to the 23°C environment. Stronger selection strength for lab traits than temperature-specific traits. (C) Pleiotropy: each SNP contributes to all three simulated traits. (D, E) Fitness estimates in the 23 °C CGE (upper panel) and 2818 °C CGE (lower panel) of populations adapting to the fluctuating and constant environments.

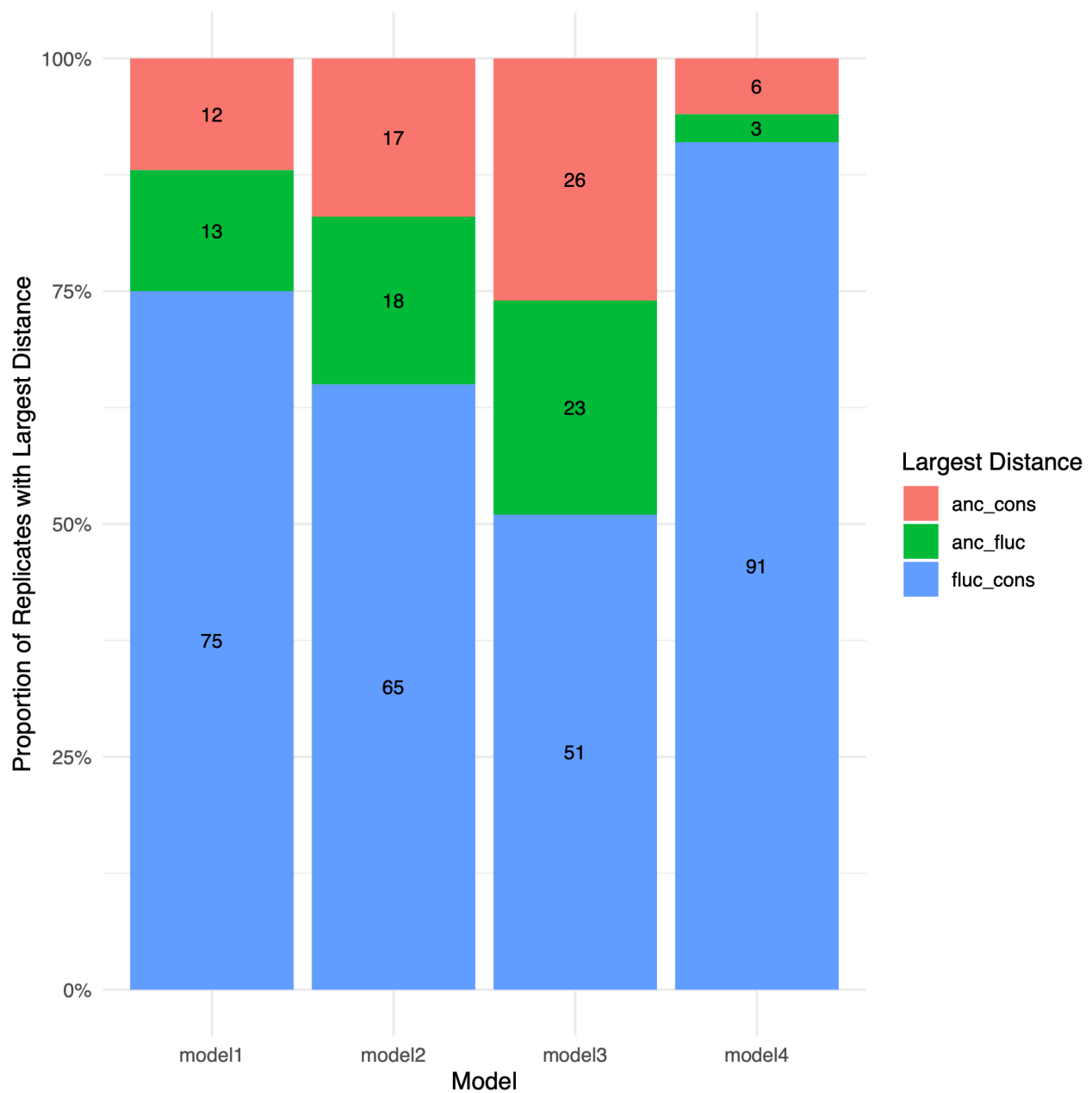


Fig S13. Number of replicate counts for maximum distance across simulated models. The Euclidean distances were calculated between the Anc (F0), Fluc (F60) and Cons (F60) populations using the PC1 scores. The color indicates the comparison yielding the largest distance.

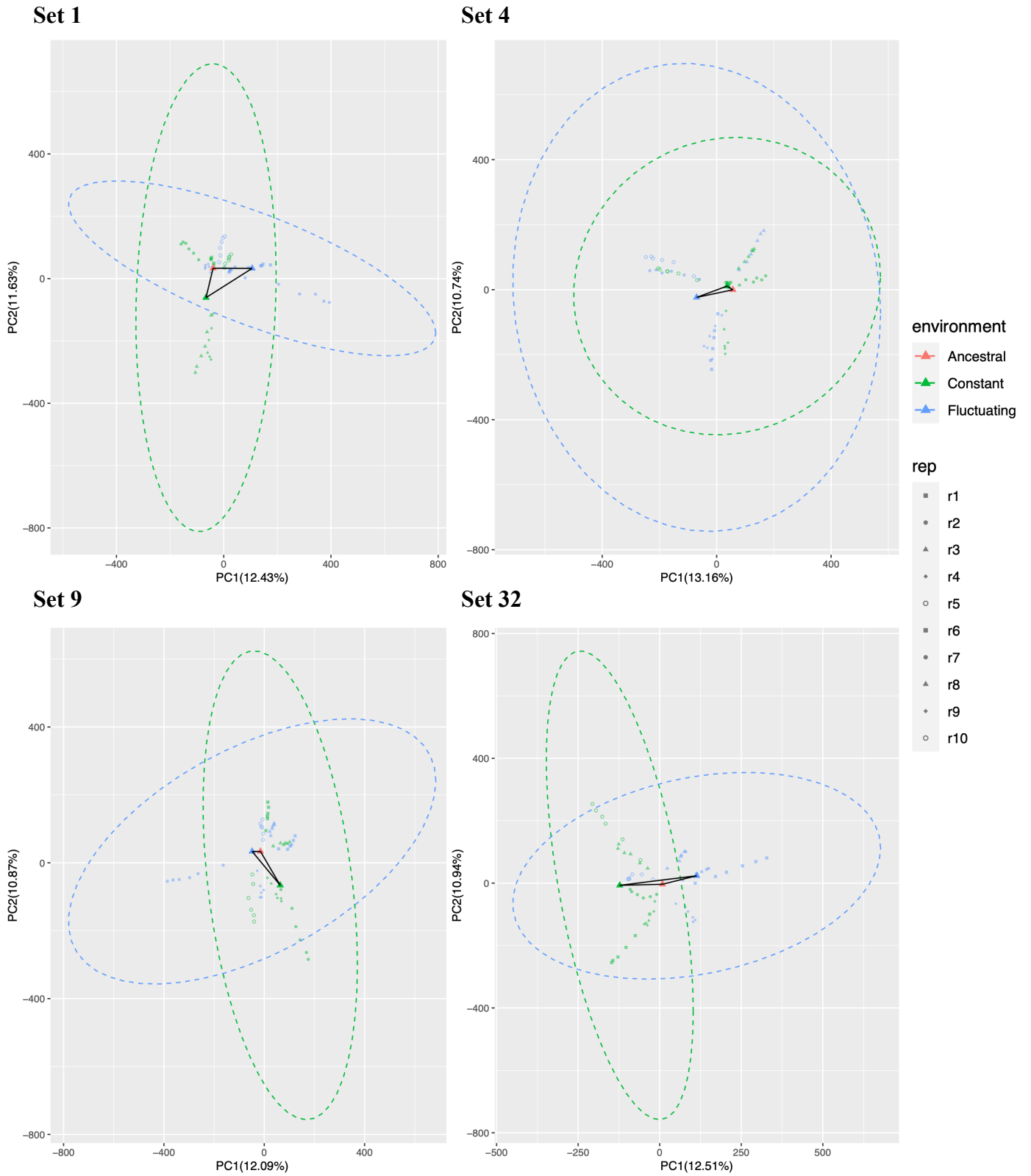


Fig S14. example of PCA results from the simulation under model 2.

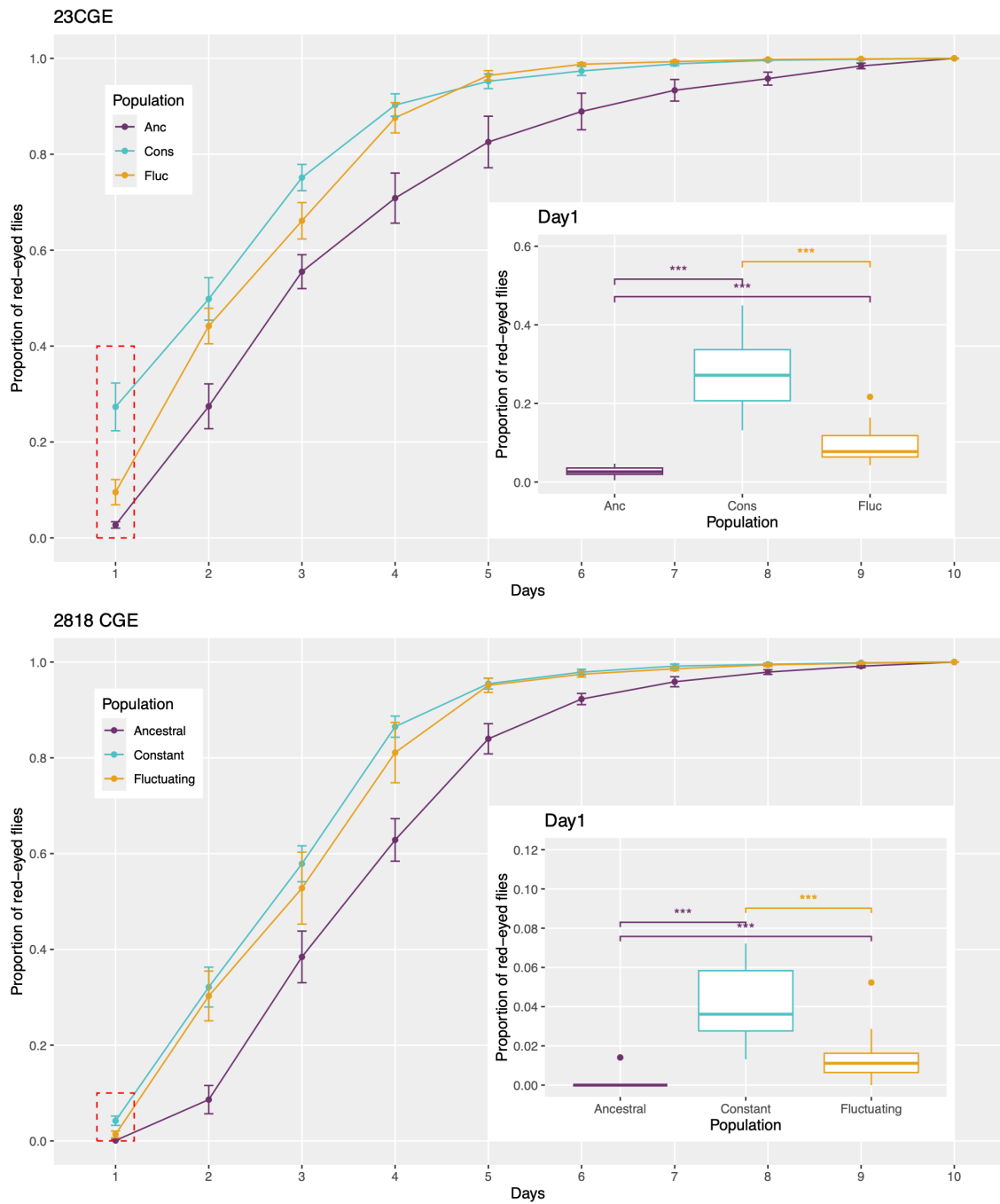


Fig S15. Eclosion trajectory of red-eyed flies under competitive fitness assay. (A) The proportion of red-eyed flies eclosed during the competitive assay under constant 23 °C CGE. **(B)** The proportion of red-eyed flies eclosed during the competitive assay under fluctuating 2818 °C CGE. The proportion of red-eyed flies eclosed on the first day was compared using the Wilcoxon test.