SUPPLEMENTARY MATERIALS FOR

Antagonistic pleiotropy conceals molecular adaptations in changing environments

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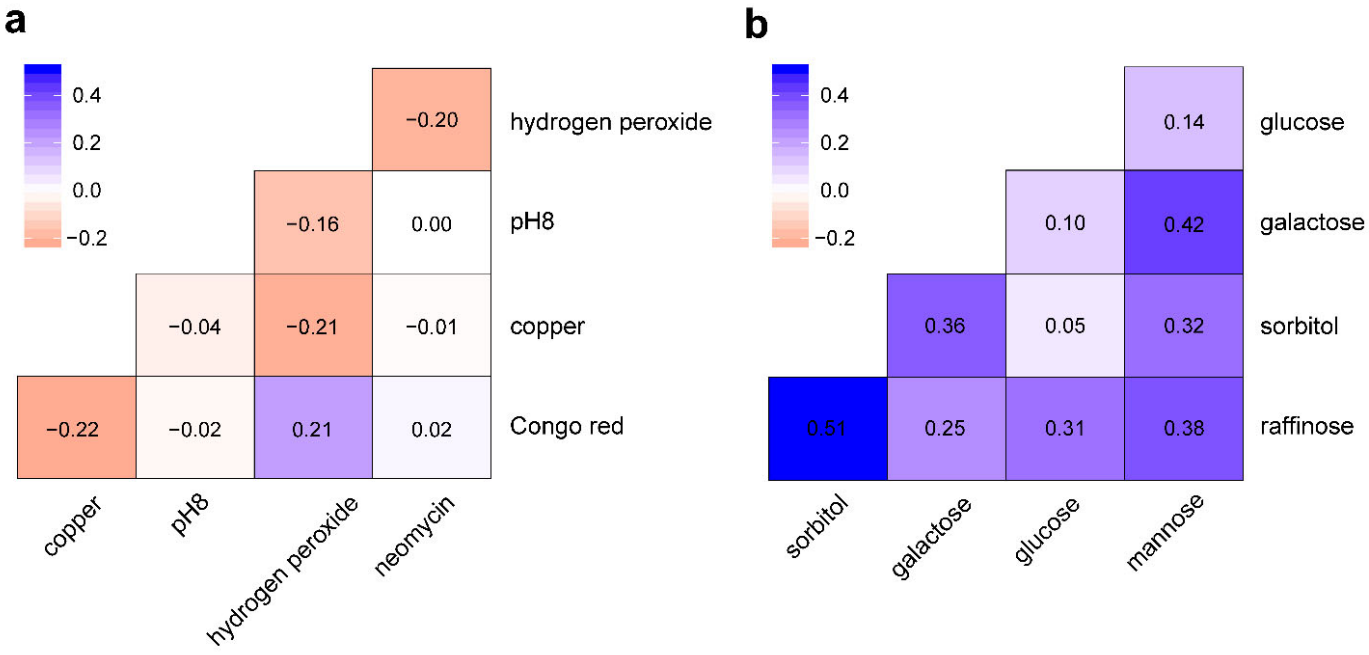
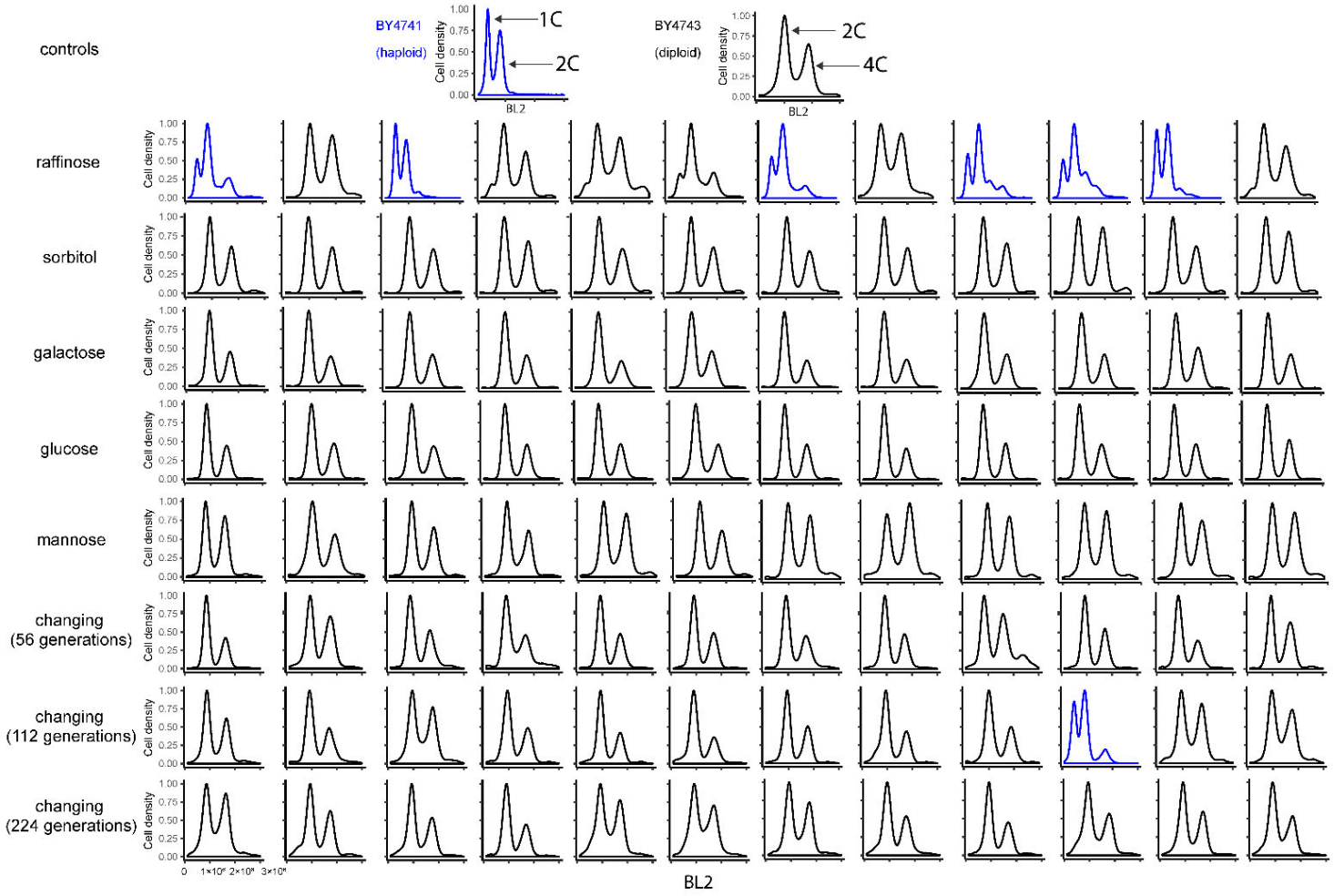
Supplementary materials include:
Supplementary Figures 1-10
Supplementary Table 1
Supplementary Dataset 1 (in a separate Excel file)

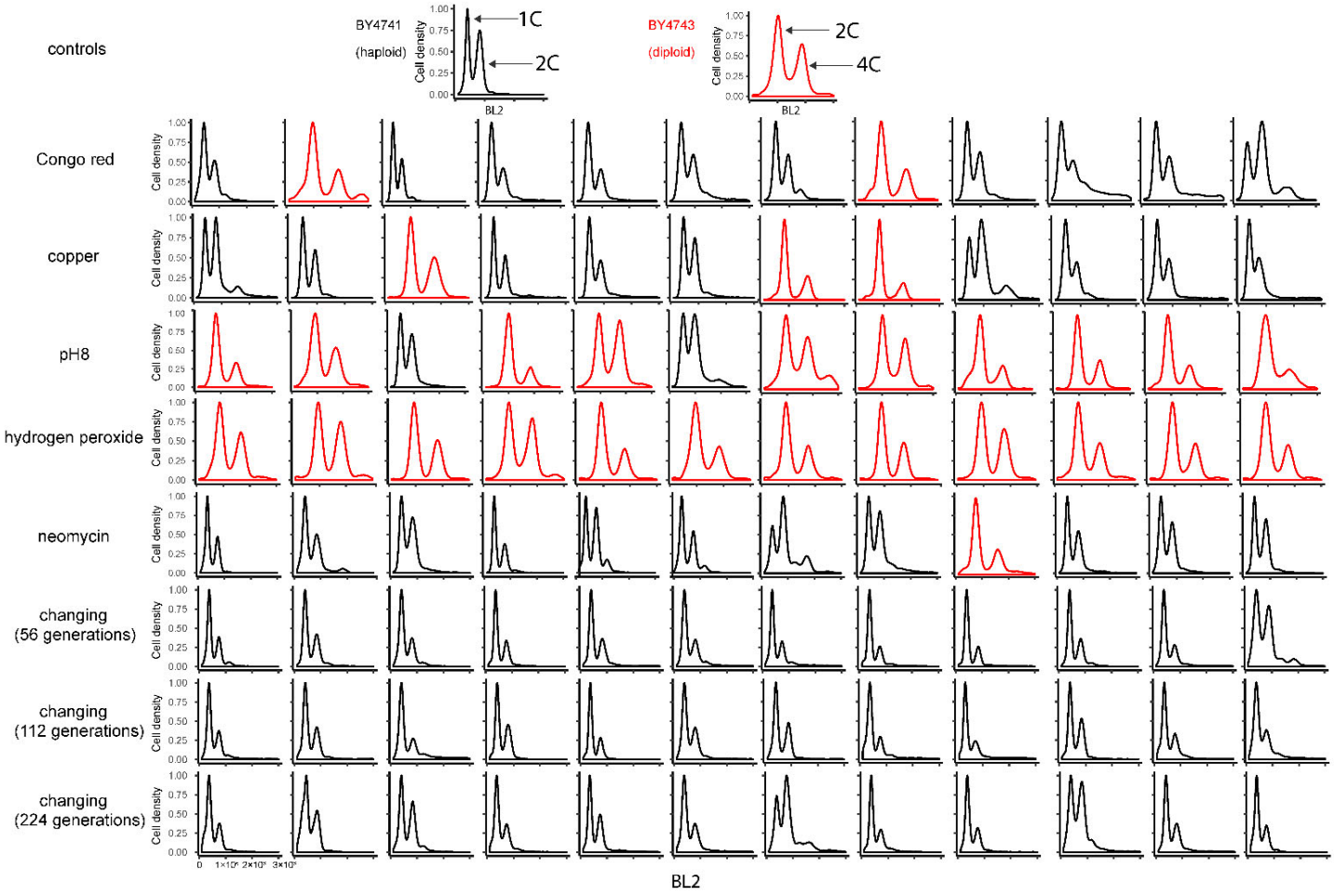
Supplementary Figure 1. Relationships among the five antagonistic conditions chosen and those among the five concordant environments chosen. (**a**) Among-genotype rank correlation in mean colony radius (after growth in solid media for 48 hr) between any two conditions in the set of five antagonistic conditions. (**b**) Among-genotype rank correlation in mean colony radius between any two conditions in the set of five concordant conditions. The correlation is computed among 1008 F1 segregants from a cross between the BY and RM strains of *S. cerevisiae*.



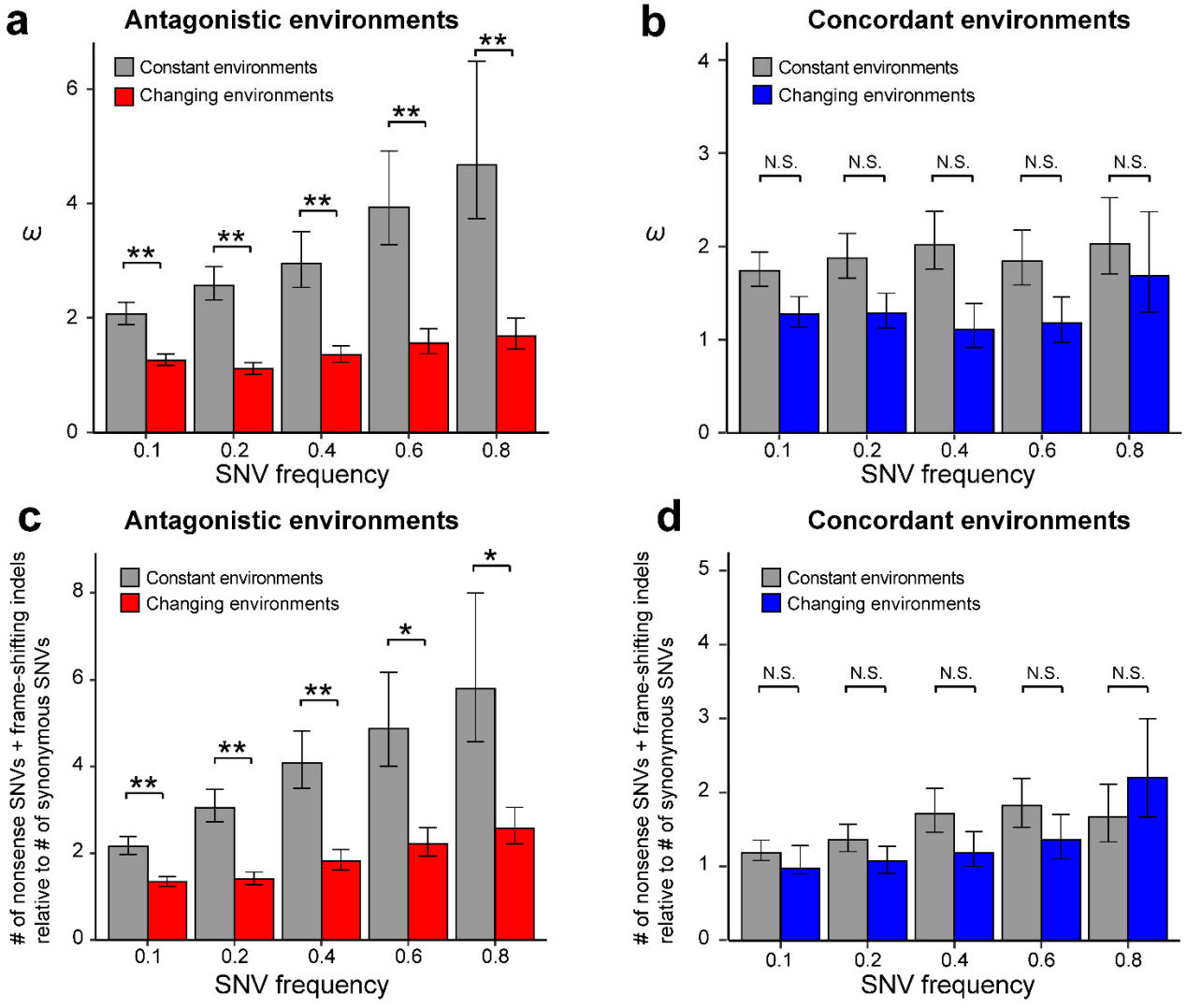
Supplementary Figure 2. Flow cytometry profiles of ploidy for all evolved populations in (constant or changing) concordant environments. SYTOX Green fluorescence was analyzed using the BL2 detector that measured the output from the 488-nm laser (blue). In the control flow cytometry profiles (haploid and diploid cell cultures), the two peaks respectively represent cells in the G1 and G2/M cell-cycle stages (1C and 2C DNA content for haploids while 2C and 4C for diploids). A total of 89 populations became diploid after evolution (shown with black), whereas the rest 7 remained haploid (shown with blue).



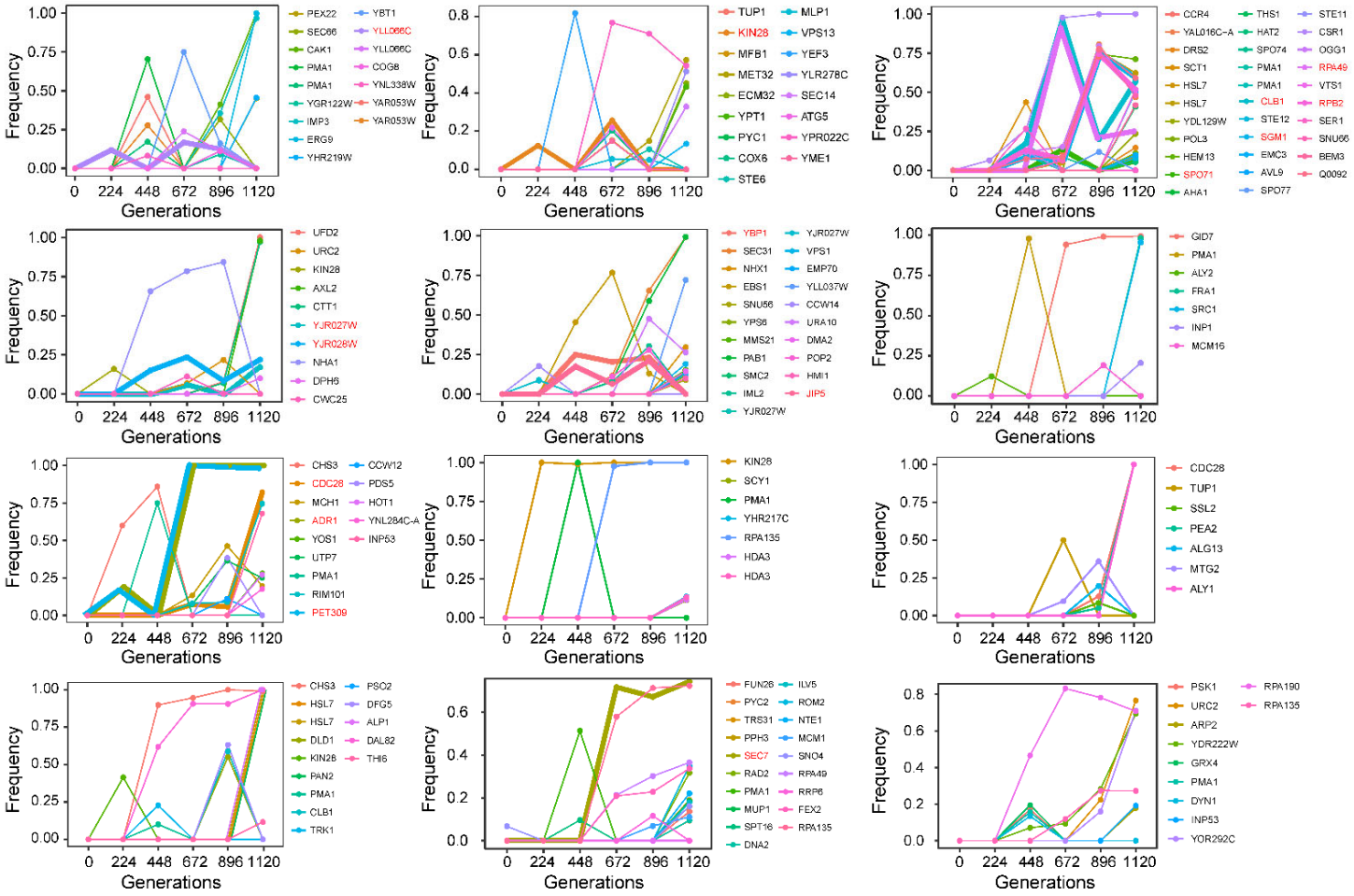
Supplementary Figure 3. Flow cytometry profiles of ploidy for all evolved populations in (constant or changing) antagonistic environments. All populations in changing environments remained haploid, while 28 populations in constant environments became diploid, exhibiting only the 2C and 4C peaks (shown in red).



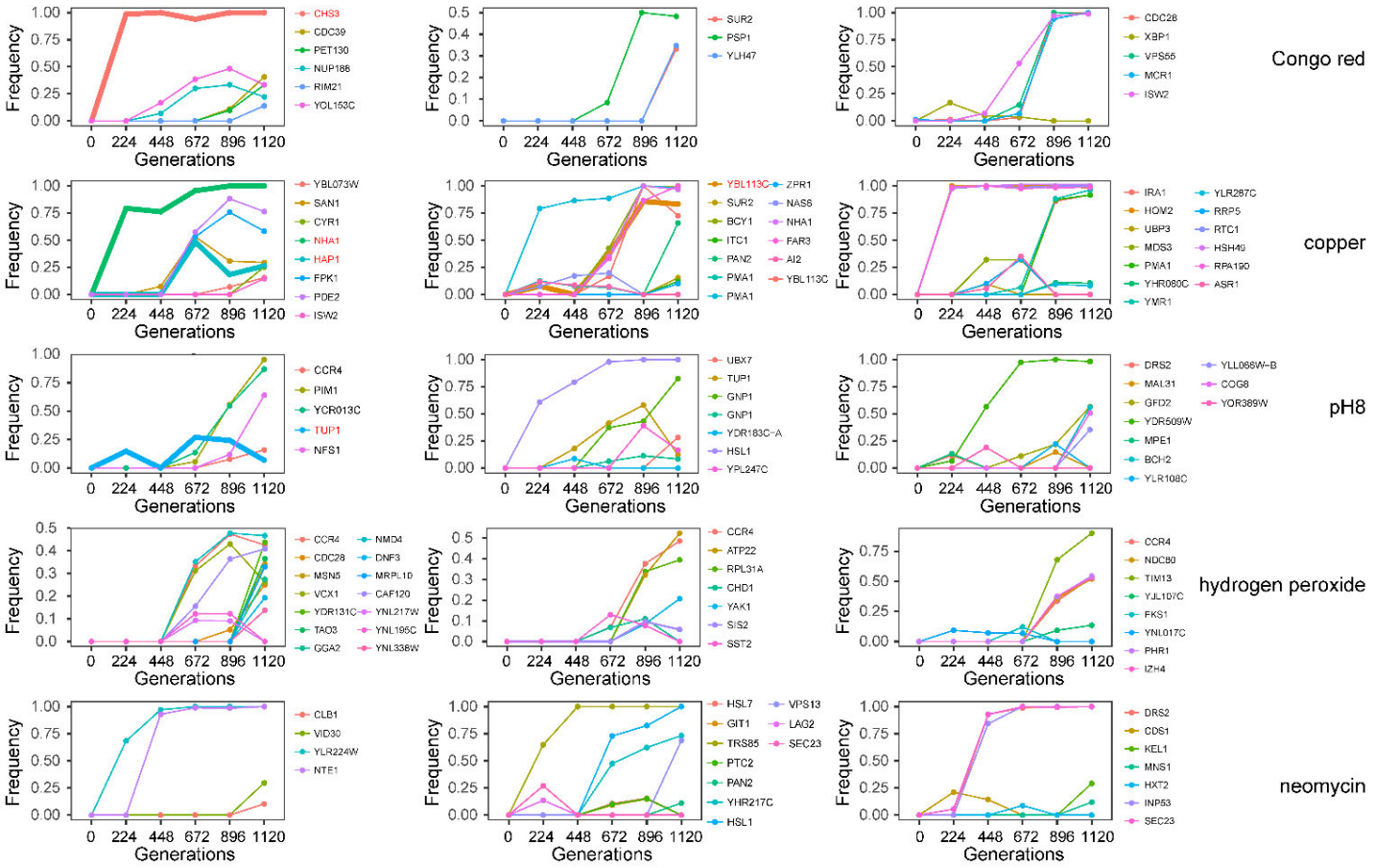
Supplementary Figure 4. The estimated rate of molecular evolution in constant and changing environments when diploidization is treated differently from the analysis presented in Fig. 2. Here, diploid lines are excluded from the analysis of populations in antagonistic (changing or constant) environments, and mutations are considered to have occurred after diploidization for populations in concordant (changing or constant) environments. (**a**) ω is significantly lower in the antagonistic changing environments than in the corresponding constant environments, regardless of the minimum frequencies of the SNVs considered in the evolved populations. (**b**) ω is not significantly different between the concordant changing environments and the corresponding constant environments. (**c**) The ratio of the total number of nonsense SNVs and frame-shifting indels to the number of synonymous SNVs is significantly lower in the antagonistic changing environments than in the corresponding constant environments. (**d**) The above ratio is not significantly different between the concordant changing environments and the corresponding constant environments. *P*-values are determined by bootstrapping the relevant populations 10,000 times and are indicated by * ($P < 0.05$), ** ($P < 0.01$), or N.S. ($P > 0.05$). Error bars indicate the first and third quartiles from bootstrapped data.



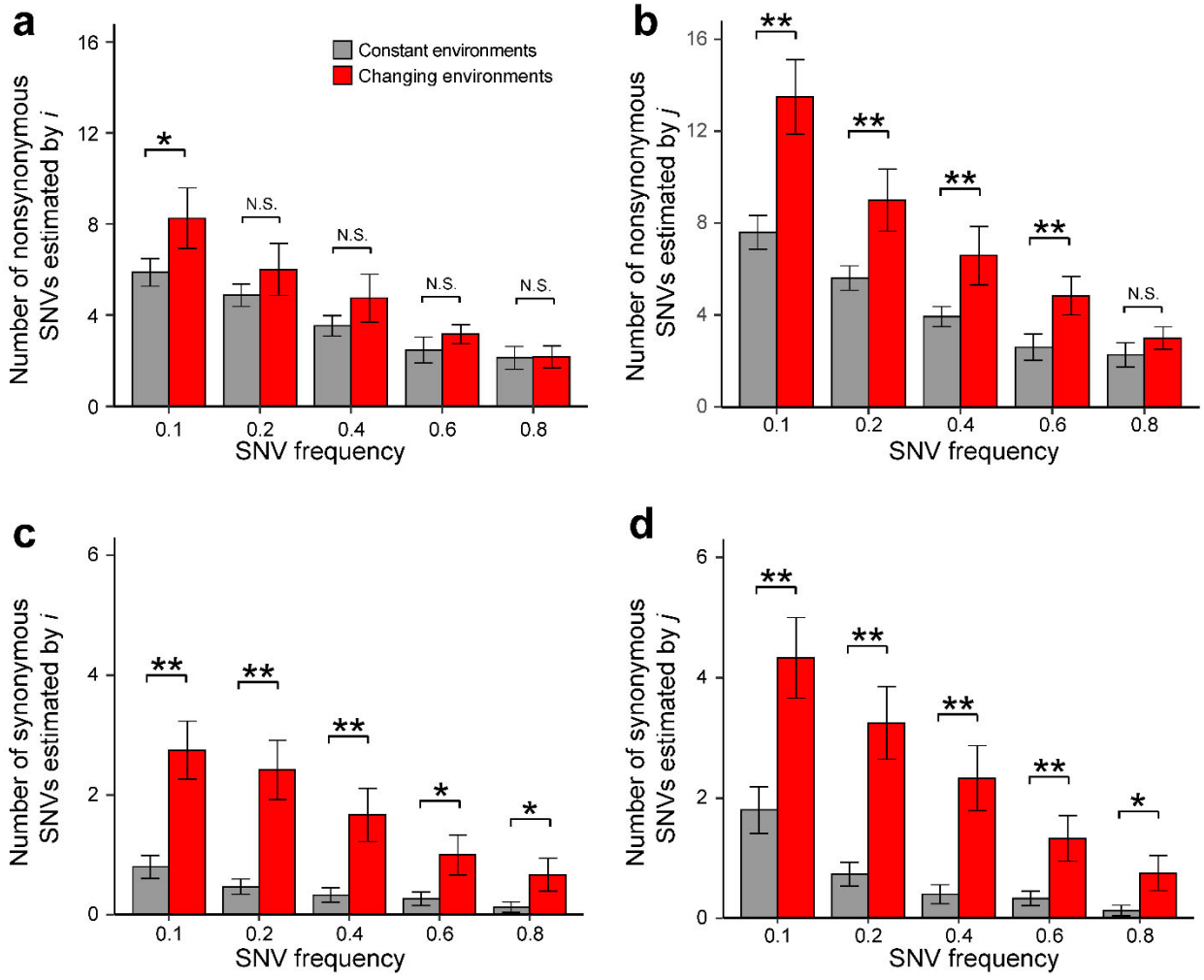
Supplementary Figure 5. Allele frequencies of individual nonsynonymous mutants in changing (antagonistic) environments. Each diagram shows one replicate population. Each line indicates one mutant allele (with a minimum frequency of 0.05 at one or more examined time points) and each dot represents the time right before an environmental change. The name of the gene where the SNV lies is shown on the right of the panel. Each bold line indicates a mutant allele whose frequency increased in a period after a decrease in an earlier period, with the corresponding gene name shown in red.



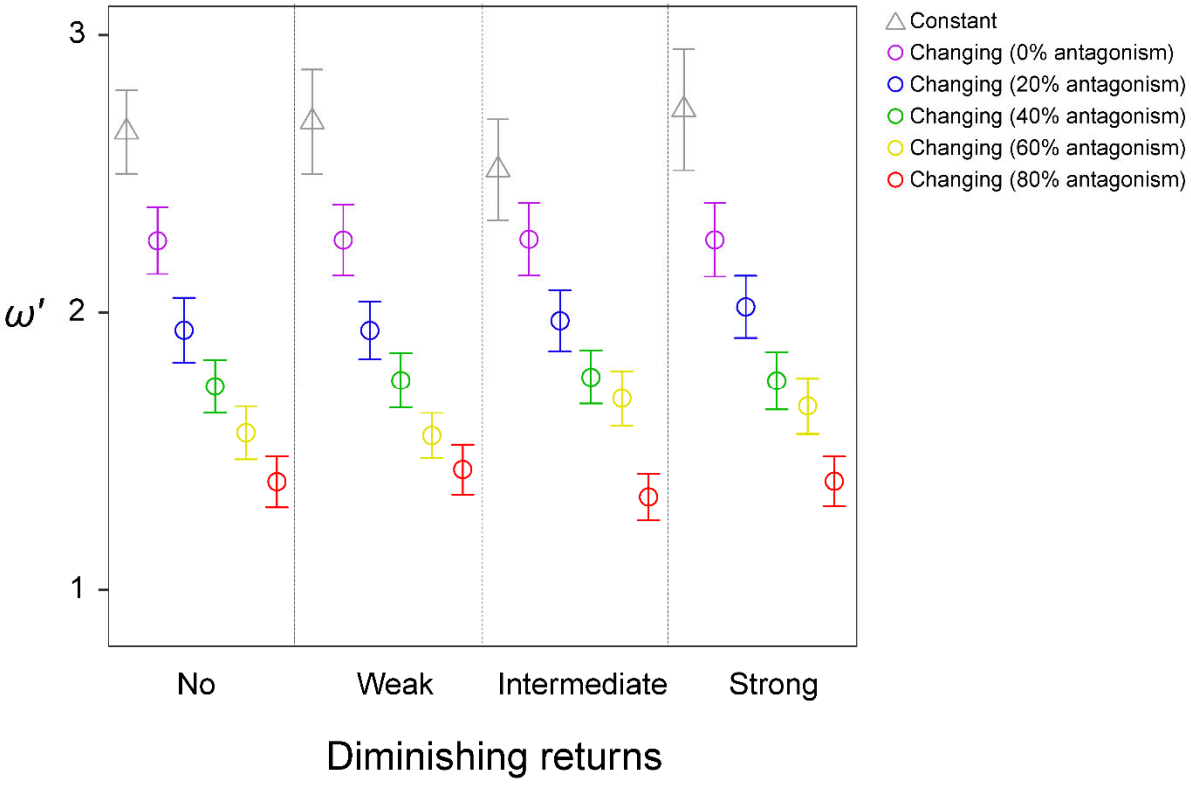
Supplementary Figure 6. Allele frequencies of individual nonsynonymous mutants in constant (antagonistic) environments. Each row shows three examined populations of the 12 replicate populations in a condition. Each line indicates one mutant allele (with a minimum frequency of 0.05 at one or more examined time points) and each dot represents the corresponding time right before an environmental change in changing antagonistic environments. The name of the gene where the SNV lies is shown on the right of the panel. Each bold line indicates a mutant allele whose frequency increased in a period after a decrease in an earlier period, with the corresponding gene name shown in red.



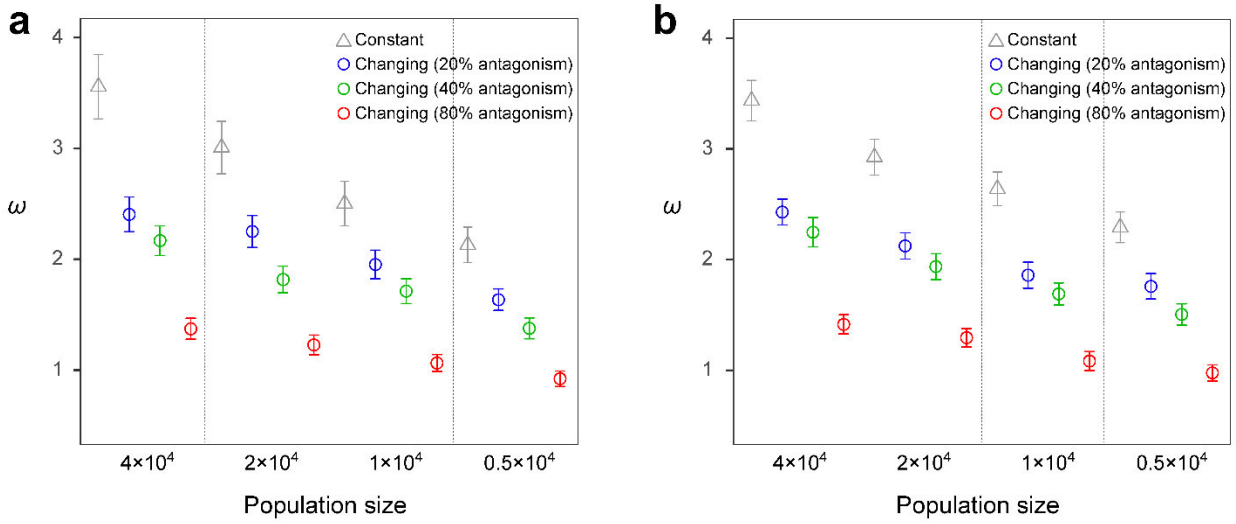
Supplementary Figure 7. Number of SNVs estimated by *i* and *j*, respectively. (a-b) Numbers of nonsynonymous SNVs per population estimated by *i* (**a**) and *j* (**b**) are greater in the antagonistic changing environments than in the corresponding constant environments (see main text for definitions of *i* and *j*). The differences are larger when SNVs are estimated by *j* than when they are estimated by *i*. **(c-d)** Numbers of synonymous SNVs per population estimated by *i* (**c**) and *j* (**d**) are significantly greater in the antagonistic changing environments than in the corresponding constant environments. The data used here are the same as in Fig. 3c and 3d. *P*-values are determined by bootstrapping the relevant populations 10,000 times and are indicated by * ($P < 0.05$), ** ($P < 0.01$), or N.S. ($P > 0.05$). Error bars indicate standard errors estimated by bootstrapping the populations.



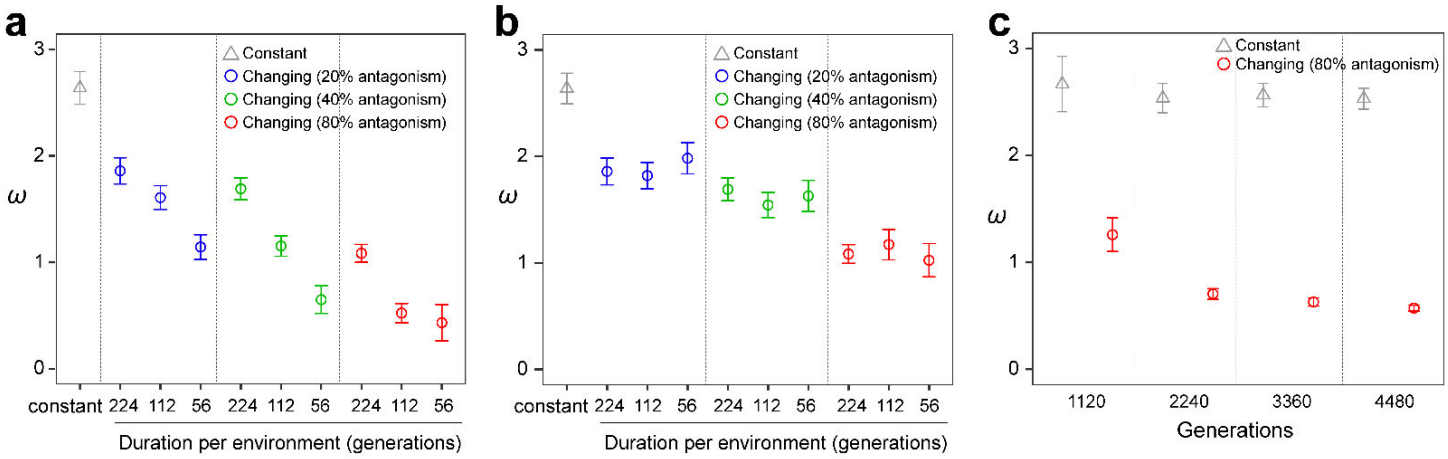
Supplementary Figure 8. Simulation shows that ω' is lower in antagonistic changing environments than in the corresponding constant environments. All parameters are the same as in Fig. 4e except that ω' is calculate instead of ω.



Supplementary Figure 9. Simulation shows that the ω estimate is lower in antagonistic changing environments than in the corresponding constant environments under various population sizes. (**a-b**) ω increases with the population size with (**a**) or without (**b**) diminishing returns epistasis. In (a), the intermediate level of diminishing returns epistasis is considered.



Supplementary Figure 10. Simulation shows that ω could fall below 1 when the environment changes more frequently or the evolutionary time is longer. (**a**) ω decreases as the environment changes more frequently. All parameters are the same as in Fig. 4f except that no diminishing returns epistasis exists here. (**b**) No significant difference in ω exists among three different frequencies of environmental changes when the environment rotates among five fixed antagonistic conditions. All parameters are the same as in Fig. 4g except that diminishing returns epistasis is absent here. (**c**) ω falls below 1 after 2,240 generations in antagonistic changing environments. All parameters are the same as in Fig. 4h except that diminishing returns epistasis is absent here.



Supplementary Table 1. Numbers of synonymous and nonsynonymous SNVs observed in end populations.

Group	Type of mutations	Constant			Changing (56 generations)			Changing (112 generations)			Changing (224 generations)		
		Allele frequency			Allele frequency			Allele frequency			Allele frequency		
		>10%	>40%	>80%	>10%	>40%	>80%	>10%	>40%	>80%	>10%	>40%	>80%
Antagonistic	Total	407	174	92	125	71	49	75	59	47	131	76	34
	Synonymous	60	16	6	20	9	5	15	11	8	33	20	8
	Nonsynonymous	347	158	86	105	62	44	60	48	39	98	56	26
Concordant	Total	224	87	33	59	10	2	31	8	3	49	13	4
	Synonymous	33	12	4	10	2	0	4	0	0	14	3	2
	Nonsynonymous	191	75	29	49	8	2	27	8	3	35	10	2