Yeast Partial Dominance: Effect of Environment and Background Genotype

Abstract. *Growth-rate mutations that were newly induced in haploid yeast by nitrosoguanidine were partially expressed in diploids heterozygous for these mutations only when the diploids were heterozygous elsewhere in the genome, and when both haploids and diploids were grown in a stress environment.*

Partial dominance of growth rate in yeast (that is, partial expression in the diploid) is dependent on the environment (*1*). The strain (Jap-2) employed in that experiment was wild, with a large amount of natural variability. I now report that genetic variability in growth rate induced by the chemical mutagen nitrosoguanidine is very similar in its expression to natural variability. Further, a homozygous background in the diploid masks the effect of the newly induced variability, whereas a heterozygous background allows it to be expressed.

In yeast, any two haploids of mating types *a* and α mate to form a stable diploid (not a heterokaryon), and this diploid can in turn be sporulated to give rise to stable haploid clones. These stable haploids and diploids can be used to study dominance, defined here as the degree to which a haploid character is expressed in the diploid that is formed when that haploid is mated with a haploid of the opposite mating type. Growth rate was selected as the most tractable parameter for measure, with modifications of the methods of James (*2*). Single cells were isolated on a slab of agar with a micromanipulator. The resulting colonies were measured at two different times with a filar micrometer eyepiece, with the growth rates expressed as percentage increase in colony diameter per hour (*1*).

Figure 1 shows the crossing procedure used in the experiment. Strain X2180 arose as the result of a mutation at the mating-type locus of the wild type S288C α. This strain is highly homozygous, and spore isolates from it exhibit very little if any genetic variance in growth rate (*1*). Two such isolates, X2180-1A (*a*) and X2180-1B (α) were cloned. A suspension of the former (10^6 cells per milliliter) was treated for 1 hour with a solution of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (nitrosoguanidine) (1 μg/ml) (*3*). A suitable dilution was plated, and 21 colonies were picked at random. Examination by the tetrazolium-overlay method (*4*) revealed that many of these treated cells were petite; to eliminate the cytoplasmic petites, a cell from each of these clones was mated with X2180-1B, and the resulting diploid was sporulated. Tetrads of spores were dissected, and these in turn gave rise to 42 *a* and 42 α haploids. None of these were petite when tested by the tetrazolium method, although many of them grew slowly. These were in turn mated to X2180-1A and X2180-1B to give diploids with a homozygous genetic background, and to XC-1A and XC-3D, derived from the highly heterozygous strain Jap-2, to give diploids with a heterozygous background. This background was constant throughout each array of 42 diploids, the only difference between them being due to genetic variability induced by the nitrosoguanidine treatment. Twenty-one untreated X2180-1A and X2180-1B clones (controls) were mated to give homozygous and heterozygous diploids by the method used for the treated haploids. The haploids and their corresponding diploids were grown on a variety of media, and correlations were obtained between haploid and diploid growth rates. The group of growth rates obtained from each slab was treated separately to avoid spurious correlations (*5*, *6*). Seven haploids or diploids were grown on each slab, and three replicates of each were measured. I obtained Fisher statistics (*F*) from the ratios of the "among clones within slabs" and the "within clones" or "error" mean squares for the diploids; these ratios give a measure of the variation due to genetic differences between clones. Occasionally one of the three replicate cells failed to germinate, or grew much more slowly than the others; these data were discarded.

Four media were used (*1*). The first two, YEPD and MV, are complete and minimal media, respectively. By lowering the concentration of dextrose in MV from 2 percent to 0.2 percent or by adding ethanol (5 percent) to MV, "stress" media were made. The cells grow much more slowly on them than they do on YEPD and MV.

Two auxotrophs, one for lysine and one for uracil, were produced by the nitrosoguanidine treatment, and the four haploids and eight diploids carrying these mutants were not included in the data since the haploids did not grow at all on MV or on the stress media.

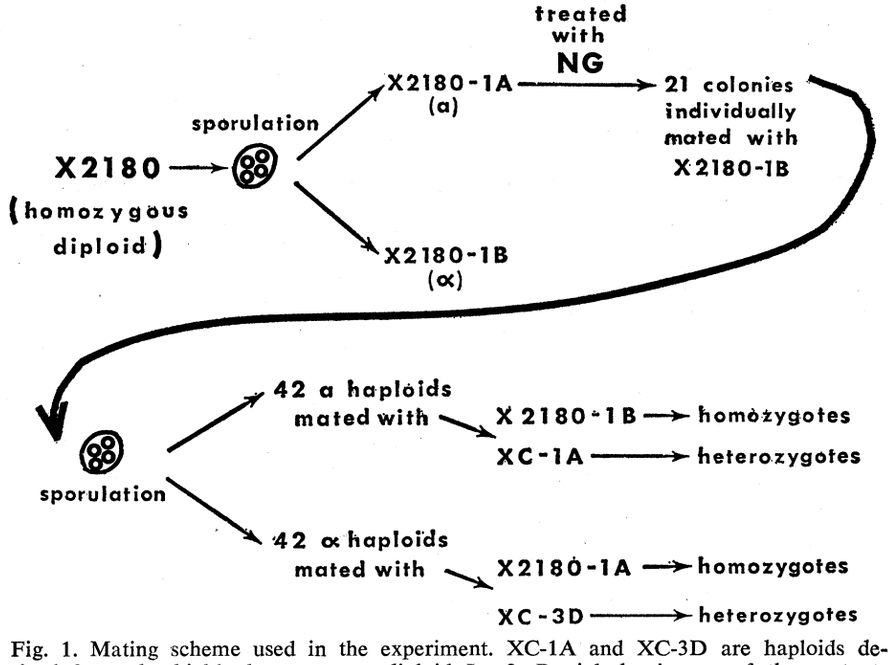

Fig. 1. Mating scheme used in the experiment. XC-1A and XC-3D are haploids derived from the highly heterozygous diploid Jap-2. Partial dominance of the mutants induced by nitrosoguanidine was determined by correlating the growth rates of the 42 *a* and 42 α haploids with their homozygous and heterozygous diploids. NG, nitrosoguanidine.

Table 1. Correlations of haploids with diploids of homozygous and heterozygous background. YEPD and MV are normal and MVDEX and MVETOH are stress media; d.f. = degrees of freedom for the correlations; F represents the ratio of the "among clones within slabs" and the "within clones" or "error" mean squares; r is the correlation coefficient.

Medium	Haploid mating type	Correlations			
		Homozygous diploids		Heterozygous diploids	
		r	F	r	F
		NG-treated haploids (d.f. = 38)			
YEPD	a	−.191	1.17	−.119	2.24†
YEPD	α	+.247	1.34	−.033	3.21†
MV	a	+.074	1.10	+.098	2.46†
MV	α	−.274	1.68*	+.296	2.60†
MVDEX	a	+.022	1.22	+.762†	6.44†
MVDEX	α	−.079	1.70*	+.356*	2.84†
MVETOH	a	+.098	1.53	+.657†	6.35†
MVETOH	α	−.064	1.22	+.617†	4.40†
		Control haploids (d.f. = 19)			
MVDEX	a	−.036	1.16	+.124	1.31
MVDEX	α	−.023	1.41	−.172	1.24
MVETOH	a	+.008	1.09	+.265	1.17
MVETOH	α	−.026	1.09	−.158	1.61

* Significance at the .05 level or less. † Significance at the .01 level or less.

Coincidentally, two of the mutant clones resulting from the crosses with X2180-1B were a and two were α, which reduced the degrees of freedom in all treated-cell correlations from 40 to 38.

The only significant correlations were obtained between the treated haploids and their heterozygous diploids on the stress media (Table 1). Three of these are highly significant, and the fourth is significant at the 5-percent level. The F values are also larger for the heterozygous than for the homozygous diploids. The treated haploids, homozygous diploids, and heterozygous diploids were the same throughout the experiment, the only difference being that cells derived from these clones were grown on different media. The degree and nature of the heterozygosity in the background genotype necessary to bring about the change from no dominance to partial dominance remain to be determined.

Similar phenomena have been observed in *Drosophila melanogaster* and *Tribolium confusum* (*6*, *7*). Crowding, with a presumed increase in environmental stress, increases partial dominance of egg-to-adult viability (*6*). Wallace found a very slight increase in viability from egg to adult of homozygous flies in which one chromosome had been given x-irradiation, but a slight decrease when these same chromosomes were introduced into heterozygous strains (*7*). In my experiment, an untreated diploid clone of the same genotype was included on each slab of treated diploids, and the growth rates of these clones were compared by means of a sign test with the average growth rate of the treated clones on the same slab. With the diploids of homozygous background, the untreated control grew more quickly on 19 slabs and more slowly on 29, giving a nonsignificant chi-square of 1.69. With those of heterozygous background, the control grew more quickly on 38 slabs and more slowly on ten, giving a chi-square of 15.19 (significant at the .01 level), an effect similar to Wallace's second observation. However, overdominance—an increase of heterozygote growth rate over the homozygote—was not detected in the diploids with a homozygous background. This may simply be because of the relatively small amount of data in my experiment.

The effects of newly induced mutations appear similar in both drosophila and yeast. It may therefore be possible to employ yeast to determine the reasons for the striking effect of background genotype on partial dominance.

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References and Notes

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Tritium Movement in Soil of Tropical Rain Forest

Abstract. *Tritiated water applied to the surface of soil in a tropical rain forest was found in free water of the litter and top 18 centimeters of soil as long as 7 months after the application. Plant roots, even in the high-rainfall environment of a tropical rain forest, therefore are exposed to tritiated water for considerable periods of time after release.*

Tritium might be released to the tropical environment through military or peaceful thermonuclear detonations. The behavior of such releases in the tropical ecosystem is not well known, although Koranda (*1*) found tritium in soils and plants of Eniwetok Atoll 12 years after testing of thermonuclear weapons had ceased there. We now report on the residence half-times of tritium in clay soils of the tropical rain forest in the Luquillo Mountains of eastern Puerto Rico.

A soil plot, 0.94 m^2 in area, was prepared by installing a lysimeter (*2*) 18 cm below the soil surface, without disturbing the soil above, from a horizontal tunnel originating outside the plot. Free water that was percolating through the soil was collected in the lysimeter, drained into a plastic collection vessel, and sampled after every rain for 3 weeks and weekly thereafter. The downslope terminus of the plot was fitted with a metal tray that was placed as nearly as possible at the litter-soil interface to collect surface run-off water. Two rain gages were placed at the sides of the plot to measure rainfall at the forest floor. Above, canopy rainfall was measured by a standard tipping-bucket rain gage on a tower. From a sprinkling can, 1 liter of tritiated water (concentration, 20 mcurie/liter) was applied to the plot. The sampling program lasted for 210 days after the tritium was applied. Tritium was determined by standard methods of liquid scintillation counting in 1-ml water samples. We did not convert results to absolute activities because all we required was the variation of count rates with time.

Tritium activity in soil water collected 18 cm below the surface reached a peak in approximately 16 days and declined exponentially during the rest of the experiment (Fig. 1, curve *A*). The effective half-life (*3*) after reaching the peak (uncorrected for tritium decay) in this