

Sex and the Spread of Retrotransposon Ty3 in Experimental Populations of *Saccharomyces cerevisiae*

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ABSTRACT

Mobile genetic elements may be molecular parasites that reduce the fitness of individuals that bear them by causing predominantly deleterious mutations, but increase in frequency when rare because transposition increases their rates of transmission to the progeny of crosses between infected and uninfected individuals. If this is true, then the initial spread of a mobile element requires sex. We tested this prediction using the yeast retrotransposon Ty3 and a strain of *Saccharomyces cerevisiae* lacking Ty3. We infected replicate isogenic sexual and asexual populations with a galactose-inducible Ty3 element at an initial frequency of 1%. In two of six asexual populations, active Ty3 elements increased in frequency to 38 and 86%, due to the spread in each population of a competitively superior mutant carrying a new Ty3 insertion. Ty3 frequencies increased above 80% in all sexual populations in which transposition was induced in haplophase or in diplophase. Ty3 did not increase in frequency when active during both haplophase and diplophase, apparently because of selective sweeps during adaptation to galactose. Repressed Ty3 elements spread in sexual populations, by increasing sexual fitness. These results indicate that active Ty3 elements are more likely to become established in sexual populations than in asexual populations.

MOBILE genetic elements of various types are widespread and abundant in eukaryotic genomes. They have long been hypothesized to be parasitic, spreading because their replication within genomes and dispersal among chromosomes increases their rates of transmission (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980). Because a mobile element may be transmitted to most of the offspring of crosses between individuals with and without the element, it can spread despite reducing fitness to as little as one-half of the fitness of uninfected individuals (HICKEY 1982).

Mobile elements reduce fitness in two ways. First, their insertion into new locations causes mutations, altering either gene products or their patterns of regulation or expression. Second, some elements induce ectopic recombination and chromosomal rearrangements. Mobile elements can produce adaptive mutations and can increase variability in quantitative and metabolic traits (WILKE and ADAMS 1992; CLARK *et al.* 1995), thus accelerating the response of experimental populations to artificial selection (MACKAY 1985; TOKAMANZEHI *et al.* 1992). However, the mobile elements responsible for this variation were already present in most or all of the individuals in the experimental populations. Because only a small fraction of the mutations they produce are beneficial, mobile elements must be abundant to produce adaptive variation and usually re-

duce the mean fitness of individuals (CHARLESWORTH 1987). For example, transposition by Ty1 reduced the mean fitness and increased the variance in fitness of 60 genotypes tested by WILKE and ADAMS (1992). Only one of the 60 mutants had higher fitness, as estimated by density in stationary growth phase, than did the control. Therefore, the effects of established elements on whole populations do not explain their increase in frequency from initial rarity. If mobile elements reduce individual fitness, their spread should occur only in sexual, outcrossing populations, because in clonal or inbreeding populations, biased transmission and the invasion of uninfected lineages cannot occur, and elements that reduce the fitness of a clonal lineage will be eliminated (CAVALIER-SMITH 1980; HICKEY 1982).

The chromosomal distributions of mobile elements in natural populations of *Drosophila* support the view that they produce few if any beneficial mutations: particular insertions are almost always very rare, indicating that they confer no selective advantage (CHARLESWORTH *et al.* 1994). In many cases, what is known of the molecular biology of mobile elements is consistent with sexual parasitism (ZEYL and BELL 1996). For example, the germ-line specificity of transposition by some elements is an attribute that would be expected of a sexual parasite of metazoans. Although the invasion of populations of *D. melanogaster* by *P* elements has been experimentally reproduced (KIDWELL *et al.* 1981; GOOD *et al.* 1989), the obligate association of sex with reproduction in *D. melanogaster* precludes the demonstration that this spread depends on sex. The most direct test

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of molecular parasitism would be to introduce a mobile element at a low frequency into experimental populations of a facultatively sexual eukaryote, with the expectation that the element would spread in sexual populations but decline in asexual populations. FUTCHER *et al.* (1988) used precisely such an experiment to demonstrate the parasitic nature of the 2 μ plasmid of *Saccharomyces cerevisiae*. This plasmid encodes no vegetative functions and reduces fitness by 1%, but invades experimental populations provided that they are sexual and outcrossing.

S. cerevisiae is an ideal test organism for the hypothesis that the spread of mobile elements requires sex, because, in addition to being a facultatively sexual and genetically tractable eukaryote with a very short generation time, it hosts four families of retrotransposons. Retrotransposons are elements several kilobases in length, bounded by direct repeats several hundred base pairs long. They are transcribed and encode structural proteins and enzymes that reverse-transcribe the RNA transcripts and integrate the resulting DNA copies into the chromosomes. The molecular biology of the yeast retrotransposons Ty1 and Ty3 is especially well characterized and has been reviewed by BOEKE and SANDMEYER (1991). We chose Ty3 as a test element because BILANCHONE *et al.* (1993) have constructed a yeast strain with no Ty3 elements by experimentally deleting three endogenous copies from a preexisting strain. Moreover, HANSEN *et al.* (1988) and KIRCHNER *et al.* (1992) have constructed galactose-inducible Ty3 elements by splicing the *GALI-10* upstream activating sequence into the 5' copy of the long terminal repeat, immediately upstream of the site where transcription of wild-type Ty3 elements begins. Transposed copies of this element therefore lack the *GALI-10* sequence, but the galactose-inducible source element permits both the experimental induction of transposition to elevated rates and the repression of Ty3 in control populations cultured on glucose (HANSEN *et al.* 1988; MENEES and SANDMEYER 1994; KINSEY and SANDMEYER 1995). We used this galactose-inducible retrotransposon and yeast strains lacking Ty3 to test the hypothesis that Ty3 spread would occur only in sexual populations in which transposition was induced.

MATERIALS AND METHODS

Strains and plasmids: Yeast strains and plasmids are described in Table 1. Those used to construct the initial experimental populations were a generous gift from S. SANDMEYER, and additional plasmids used as genetic markers in subsequent tests of relative fitness were constructed from the original plasmids or obtained from H. BUSSEY. Plasmids were maintained in *Escherichia coli* strain DH10 α following transformation by electroporation. To construct isogenic yeast strains each carrying single, chromosomally integrated copies of Ty3 and a selectable marker, yVB110 was transformed with the large *EcoRI* fragment of plasmid pEGTy3-1, carrying Ty3 and *URA3*, and strains yVB114 and yVB115 were transformed with

the large *EcoRI* fragment of pJK311AC, carrying Ty3 and *TRP1*, using the lithium acetate method as described by GOLEMIS *et al.* (1994). The Ty3-free strains and these transformed derivatives were used to construct the initial experimental populations, as described below. Additional strains, used in subsequent tests for an effect of Ty3 on mating and sporulation, were constructed by transforming yVB110 and yVB114 with the appropriate restriction fragments of 2 μ -based plasmids carrying *TRP1*, *URA3* or *HIS3* markers, or with the entire plasmids.

All media were prepared as described by GOLEMIS *et al.* (1994), and in addition to standard components included 20 mg/mL uracil and 40 mg/mL tryptophan, except for selective plates used to score genotype frequencies.

Construction of base populations and experimental procedure: Four base populations were established: haploid mating type a (MATa), haploid mating type α (MAT α), diploid MATa/ α , and sexual (both mating types present in equal frequencies). Each base population comprised 99% genotypes lacking Ty3 and 1% isogenic transformants each carrying a single integrated copy of Ty3 and *URA3* or *TRP1*. To construct these base populations, the constituent genotypes were grown overnight in 5 mL liquid YPD at 30° with agitation, and mixtures were prepared using OD₆₀₀ measurements to determine the appropriate volumes of each genotype. From each mixture, diluted aliquots of equal volumes were thin-spread on YPD and on SC -Ura or SC -Trp plates to confirm that the Ty3-bearing genotypes comprised 1 \pm 0.2% of each population.

These four base populations and the experimental lines established from them are outlined in Figure 1. The MATa, MAT α , and diploid mixtures were each used to establish two replicate asexual populations in which transposition was induced by growth on 2% galactose (YPGal medium), and two replicate asexual lines in which transposition was repressed by growth on 2% glucose (YPD medium). For these asexual lines the experimental cycle consisted of culture for 3 days in 5 mL liquid media at 30° with agitation at 250 rpm, spreading 40 μ l aliquots on agar plates of the same media, and 3 days' growth at room temperature on the plates, followed by 6 days' refrigeration while the sexual lines completed their cycle.

From the sexual base population, three replicates each of four treatments were established: transposition repressed by growth on glucose, transposition induced throughout the cycle by growth on galactose, transposition induced during haplophase, and transposition induced during diplophase. In each sexual cycle, mated cultures were presporulated overnight at 30° in 250-mL Erlenmeyer flasks containing 25 mL YPA (1% yeast extract, 2% peptone, 2% potassium acetate) shaken at 150 rpm. The cultures were then pelleted and resuspended in 25 mL sporulation medium and transferred to new 250 mL flasks. Sporulating cultures were shaken at 350 rpm at room temperature for 5 days. Unsporulated cells were killed by digesting the cultures overnight in 100 μ g/mL zymolyase 100T and 0.2% β -mercaptoethanol, as described by GOLEMIS *et al.* (1994). To prevent the inbreeding that results from matings between sibling spores that remain associated (FUTCHER *et al.* 1988), suspensions of the spores in 5 mL 0.25% Triton-X were shaken at 300 rpm for 30 min with 2 mL of glass beads (450–600 μ m; Sigma) to separate sibling spores. Aliquots of 200 μ l from each culture were spread on either YPD (for the lines in which transposition was repressed, and those in which it was induced in diplophase) or YPGal (for lines in which transposition was induced in haplophase or throughout the cycle). After growth on these plates at room temperature for 2 days, the cells covering about a quarter of the plate were thoroughly mixed with a sterile loop to max-

TABLE 1
Strains of *S. cerevisiae* and plasmids

Strain	Genotype	Source
yVB110	<i>MATa ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 trp1-Δ901 gal3 ΔTy3</i>	BILANCHONE <i>et al.</i> (1993)
yVB114	<i>MATα ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 trp1-Δ901 gal3 ΔTy3</i>	BILANCHONE <i>et al.</i> (1993)
yVB115	<i>MATα/MATa ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 trp1-Δ901 gal3 ΔTy3</i>	BILANCHONE <i>et al.</i> (1993)
yVB110-TyU	<i>MATa URA3 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 trp1-Δ901 gal3 Ty3</i>	This work
yVB114-TyT	<i>MATα ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 TRP1 gal3 Ty3</i>	This work
yVB115-TyT	<i>MATα/MATa ura3-52 his3-Δ200 ade2-101 lys2-1 leu1-12 can1-100 TRP1 gal3 Ty3</i>	This work
Plasmid	Relevant markers	Source
pEGTy3-1	2μ, <i>URA3</i> , <i>Ty3-1</i>	HANSEN <i>et al.</i> (1988)
pJK311AC	<i>CEN</i> , <i>TRP1</i> , <i>Ty3-1</i>	KIRCHNER <i>et al.</i> (1992)

imize contact and thus mating between cells of complementary mating types. Since by this stage growth on the plates was usually confluent or nearly so, some mating did occur during this ostensibly haploid stage of the cycle. However, trial sporu-

lation of cultures at this stage confirmed that this premixing mating was very inefficient and contributed few of the diploid cells that later entered the sporulation stage of the next cycle.

After mixing, the cultures were left at room temperature

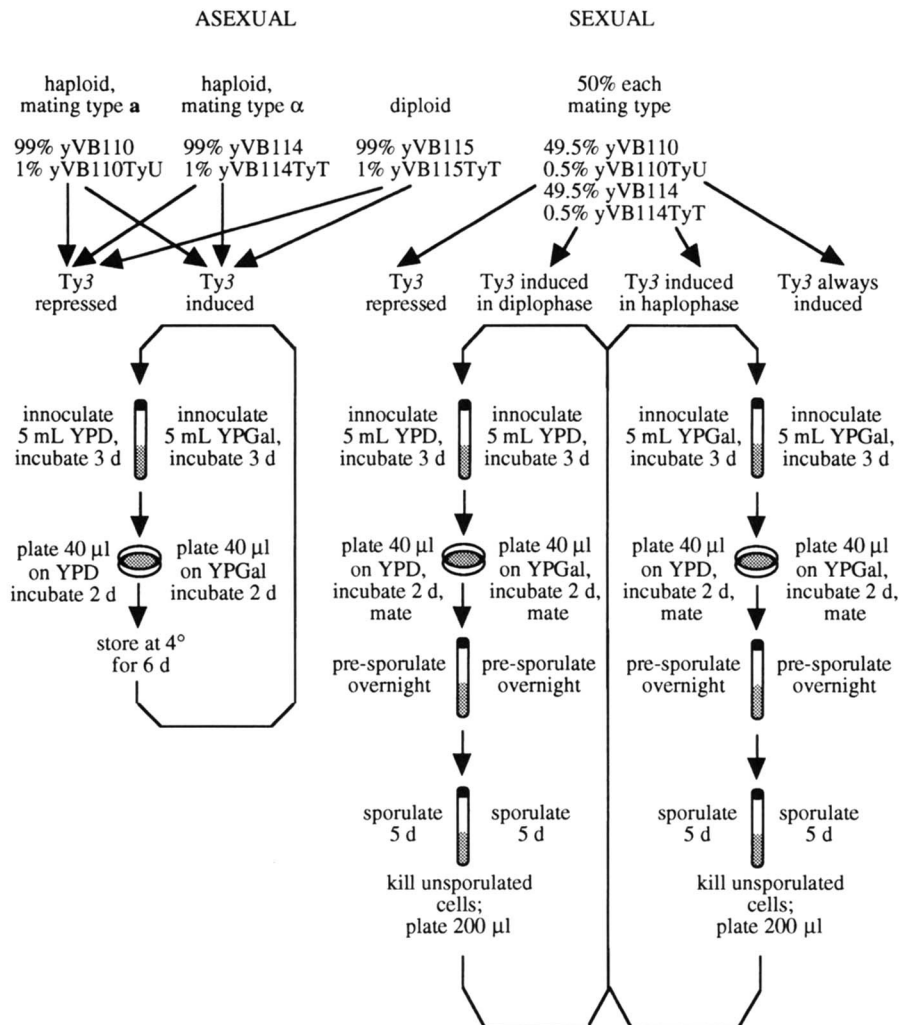


FIGURE 1.—Composition of the base populations and summary of the experimental cycle. The genotypes comprising the base populations were established populations in which Ty3 transposition was induced by culture on galactose and those in which transposition was repressed by growth on glucose. Among the sexual populations, transposition induction during haplophase, during diplophase, or during both were distinct treatments. Each of these treatments was replicated two or three times.

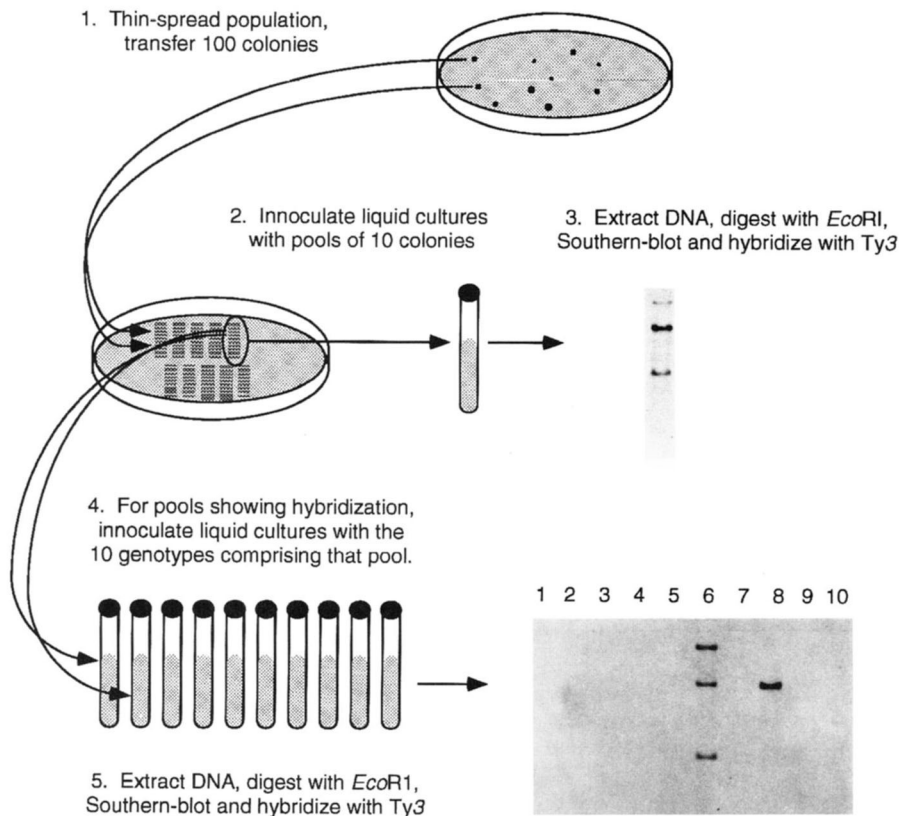


FIGURE 2.—Outline of the procedure used to assay Ty3 frequencies following the fourth and eighth experimental cycles. The Southern blot hybridization of the pooled DNA sample indicates the sensitivity of the procedure to a Ty3 insertion present in one of 10 genotypes and illustrates the effect of differing dosages of each insertion on band intensities.

for 30–36 hr to allow mating to proceed, transferred to 5 mL liquid YPD or YPGal, and grown for 3 days at the same time and under the same conditions as the asexual lines. From these liquid cultures, 40- μ l aliquots were plated, grown for 3 days, and transferred to presporulation media to begin the next cycle. Each sexual cycle lasted 15 days. The experiment was terminated after eight cycles when assays of Ty3 frequency revealed that in most lines Ty3 was either very abundant or undetectable.

Assays of Ty3 frequency: In the base populations, cells with Ty3 also carried *URA3* or *TRP1* markers, and their frequency could have been determined by replica plating. However, in the sexual lines recombination separated the retrotransposon from selectable markers, and in all lines we wished to determine not only the frequency of Ty3 but also copy number frequency distributions. We used Southern blot hybridization to assay Ty3 frequency from each of the 24 populations following the fourth and eighth cycles (Figure 2).

Aliquots from each population were thin-spread on YPD plates and 100 isolated colonies were chosen haphazardly and streaked in groups of 10 onto new plates. In preliminary assays these groups of 10 were screened as units. Tests of the sensitivity of this procedure, using known genotypes, established that one genotype with a single Ty3 copy was reliably detected in DNA samples from mixed cultures containing nine or more Ty3-free genotypes (Figure 2). When Southern blot hybridization revealed the presence of Ty3 in a group of 10, the component colonies were then tested individually, yielding a frequency distribution of Ty3 copy numbers in 100 individuals from each population.

Genomic DNA was isolated by an adaptation of the standard zymolyase (GOLEMIS *et al.* 1994) and glass bead (HOFFMAN and WINSTON 1987) methods. Cultures grown overnight in 5 mL liquid YPD were pelleted, resuspended in distilled water to a total volume of 400 μ l, and transferred to 1.5 mL microfuge tubes containing 0.3 g glass beads (Sigma, 450–600 μ m)

and 100 μ l lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris HCl pH 8.0, and 1 mM EDTA). The microfuge tubes were vortexed at medium speed (setting 6.5 on a Baxter S/P vortex mixer) for 3 min, 200 μ l of 5 M potassium acetate was added, and the tubes were incubated on ice for 1 hr. They were then centrifuged at 25,000 $\times g$ for 5 min, and the supernatants were transferred to new tubes. DNA was precipitated by adding two volumes of room temperature ethanol, pelleted by a 10-sec centrifugation, air-dried 5 min and resuspended in 30–50 μ l water.

Genomic DNA samples were digested with *EcoRI*, which does not cut within Ty3 and thus yields a fragment of unique size for each Ty3 insertion flanked by different genomic DNA sequences. *EcoRI* digests separated by gel electrophoresis and transferred to nylon membranes (ICN) were hybridized with the internal *BglII* fragment of Ty3 (see HANSEN *et al.* 1988), labeled with fluorescein and detected by chemiluminescence using ECL (Amersham) reagents and protocols.

Asexual competition experiments: To determine the relative competitive abilities of Ty3 insertion mutants and Ty3-free genotypes from asexual populations, we prepared three replicate mixtures of these pairs of genotypes. The genotypes to be competed were grown overnight in 5 mL YPD and mixed together. The initial frequencies of each genotype were determined by plating dilutions of the mixtures on YPD plates and replica-plating them to SC –Ura or SC –Trp. Since these genotypes were from asexual populations, those with Ty3 still carried the selectable markers by which the transformants used to construct the base populations had been identified. As in the eight experimental cycles, all media except selective plates contained 40 mg/mL tryptophan and 20 mg/mL uracil. The mixtures were propagated through one asexual cycle performed as described above, and the relative frequencies of the two genotypes were again determined by replica-plating.

Estimation of means and variances of growth rates on galactose: To test hypotheses regarding adaptation in the sexual

lines maintained on galactose for the duration of the experiment, we compared the growth rates of individual colonies with and without Ty3 from the sexual base population and from the sexual lines in which transposition was induced throughout the sexual cycle, sampled after the fourth and eighth cycles from aliquots that had been stored at -80° in 15% glycerol. A sample of each population was spread thinly with a wire loop along one side of a YPGal plate. From each sample, unbudded cells were selected and placed on a 1×1 cm grid on the plate using a Singer MSM System micromanipulator. After 3 days' growth at room temperature, the diameter of each colony was measured with an ocular micrometer. Colonies with and without Ty3 were then distinguished by replica-plating to SC -Trp and SC -Ura. Since Ty3 and a *TRP1* or *URA3* marker were on the same DNA fragments used to construct strains with Ty3, they were very tightly linked and remained associated even in most sexual lineages. Southern blot hybridizations were then used to confirm that *TRP1* and *URA3* colonies carried Ty3 and that *trp1* and *ura3* colonies lacked Ty3.

Sexual competition experiments: To test for an effect of Ty3 on sexual fitness and for gene conversion, we prepared mixtures of three genotypes: a *HIS3*-marked MAT α genotype and two competing MAT α genotypes. The two competing MAT α genotypes in the three mixtures were as follows: (1) yVB114Ty-T and a transformant of yVB114 with a chromosomally integrated *URA3* marker; (2) transformants of yVB114 with plasmids pJK311-AC (see Table 1) and YEp352, a 2 μ plasmid carrying a *URA3* marker; and (3) transformants of yVB114 with chromosomal *TRP1* and *URA3* markers.

Three replicate populations were prepared independently for each of these three trios of genotypes. In each population, the initial frequencies of the three genotypes were determined by replica-plating a thin-spread from YPD to SC -His, SC -Ura and SC -Trp plates. Mating and sporulation were then induced as described above. After mating, the frequencies of the two possible diploid genotypes were determined by replica-plating thin-spreads from YPD to SC -His -Ura and SC -His -Trp plates. After sporulation, vegetative vells were killed and spores dissociated as described above, and the frequencies of each marker were determined again by replica-plating from YPD to SC -His, SC -Ura, and SC -Trp. The mating efficiencies of yVB114Ty-T and of a *URA3* transformant of yVB114 were estimated in separate crosses with a *HIS3* MAT α genotype, using replica-plating to determine the frequencies of the relevant genotypes.

To test for gene conversion, *TRP1* genotypes carrying Ty3 were isolated from the sexual populations in which Ty3 had been repressed and were mated with transformants of yVB110 carrying *URA3* markers. Diploids were selected on SC -Ura -Trp and sporulated. The tetrads were dissected on YPD and replica-plated to SC -Ura and SC -Trp.

RESULTS

Repressed Ty3 elements in asexual populations: No copies of Ty3 were detected in any individuals from any of the asexual populations, diploid or haploid, in which transposition was repressed by growth on glucose (Figure 3). This was true both for the assay that followed the fourth experimental cycle, and after the eighth cycle.

Induced Ty3 elements in asexual populations: For asexual populations with transposition induced by growth on galactose, the results varied among replicate populations. In both MAT α lines, Ty3 was undetected in both assays. In the MAT α and the diploid lines in

which transposition was induced, Ty3 increased in frequency in one replicate but was not detected in the other replicates (Figure 3). In both cases this invasion was driven by the spread of one particular new Ty3 insertion, which was present in almost every one of the colonies carrying Ty3. This contrasts with the invasion of sexual populations with transposition induced either in haplophase or in diplophase, in which a variety of Ty3 copy numbers and insertion sites were observed (Figure 4), as expected when transposition and random segregation have occurred.

The spread of particular transposition mutants in only one replicate of the MAT α (induced) and diploid (induced) treatments suggests that these Ty3 insertions are associated with an increase in vegetative fitness. This explanation was supported by the competitive superiority of these Ty3 mutants over genotypes from the same experimental lines that lacked Ty3. From both experimental lines, three replicate mixtures were prepared, and the frequencies of the Ty3 insertion mutant were determined before and after one cycle of asexual culture, performed as during the long-term experiment (Figure 2). In all three replicates of the haploid MAT α competition, the Ty3 insertion mutant increased greatly in frequency (Table 2). Similar but less consistent results were obtained from the diploid genotypes, of which the Ty3 insertion mutant increased in frequency in two replicates but declined in a third (Table 2). To summarize the results among asexual lines with transposition induced, Ty3 remained at very low frequencies in four lines. The remaining two lines were both invaded by a competitively superior genotype carrying a particular Ty3 insertion.

Ty3 elements induced either in haplophase or diplophase in sexual populations: We predicted that the combination of replicative transposition and cycles of random mating and meiosis would produce an increase in Ty3 frequency. When transposition was induced in haplophase or in diplophase, Ty3 increased rapidly in frequency to over 80% in all three replicates of both treatments (Figure 3). The phase of the life cycle in which Ty3 was induced had no effect either on mean Ty3 frequencies or on the frequency distributions of Ty3 copy numbers (Figure 5).

Ty3 elements induced in both haplophase and diplophase in sexual populations: When transposition was induced throughout the sexual cycle, Ty3 began to spread in one replicate population after four cycles but did not become established, being detected in only a single copy in one individual from one of the three replicates after eight cycles (Figure 3). The strains used to begin this experiment grow slowly on galactose, at least in part because they are *gal3* and thus slow to induce structural genes for galactose catabolism (MENEES and SANDMEYER 1994). Dependence on galactose as a carbon source throughout the experiment would therefore have imposed strong selective pressures, and

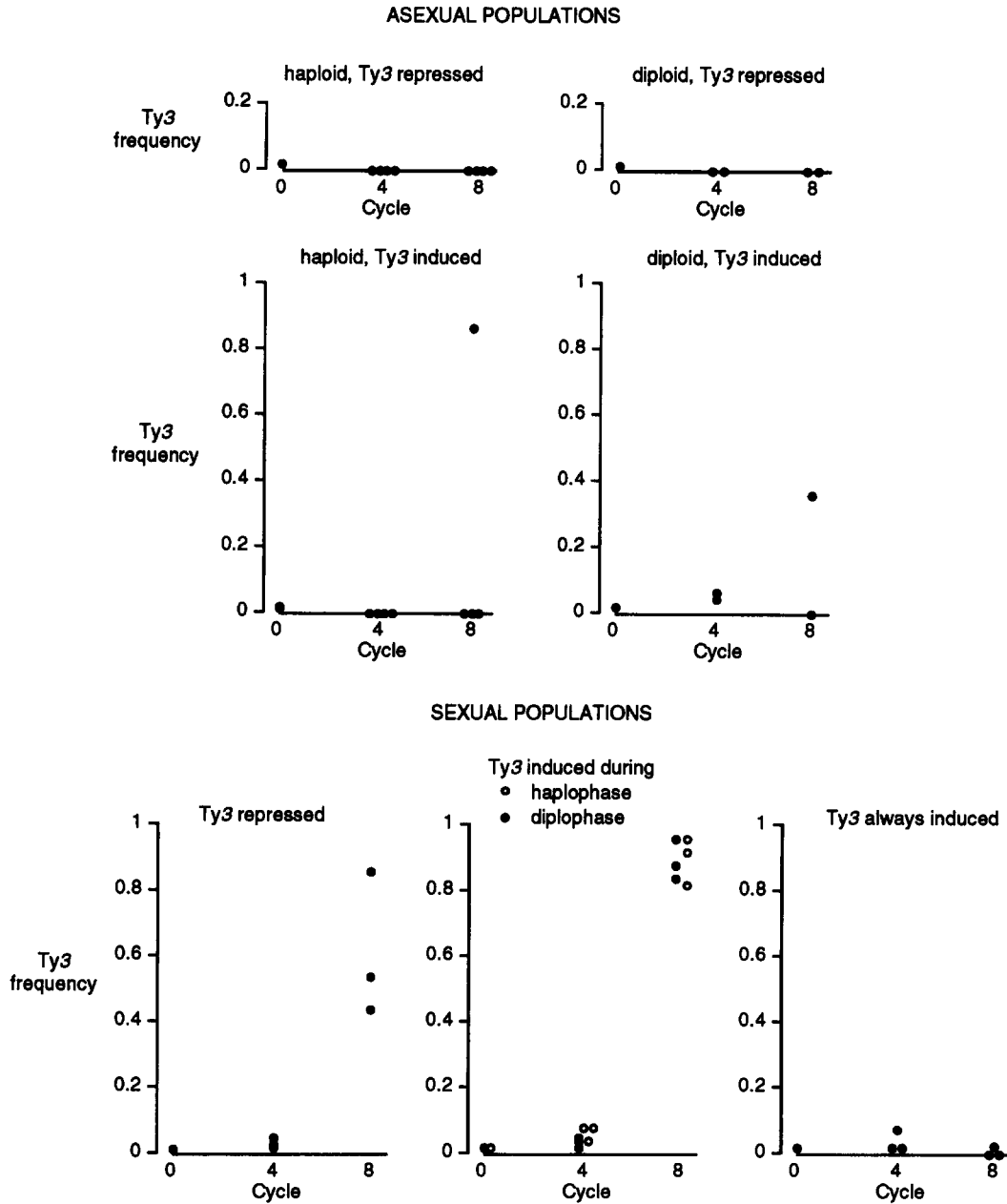


FIGURE 3.—Changes in Ty3 frequencies over eight experimental cycles. Replicate populations were all assayed following four and eight cycles, and identical estimates are shown side-by-side for clarity. Each point represents an estimate of a population Ty3 frequency based on a sample of 100 individuals.

any genotypes bearing a mutation that increased the efficiency of galactose metabolism would quickly spread. If mutations arose while Ty3 was rare, then they would probably occur in genotypes lacking Ty3, removing Ty3 from the population. This explanation is supported by the relative growth rates on galactose of genotypes isolated from fourth- and eighth-cycle populations (Figure 6). In replicate 1, the frequency of Ty3 had risen to 8% after four cycles, the mean growth rate on galactose had increased significantly, and genotypes with Ty3 grew more rapidly on galactose than did those lacking Ty3. After eight cycles Ty3 had decreased in frequency to 2% and growth rates on galactose had

further increased. This suggests that a mutation that increased fitness on galactose arose in one or more genotypes carrying Ty3, which began to increase in frequency and then either was separated from Ty3 by recombination or was replaced in the population by a Ty3-free genotype bearing a superior adaptation to galactose. Similar selective sweeps appear to have doomed Ty3 within the first four cycles in the other two replicates, in which significant adaptation to galactose had occurred and the rare remaining genotypes with Ty3 grew more slowly than did Ty3-free genotypes from cycle four (Figure 6).

Repressed Ty3 elements in sexual populations: The

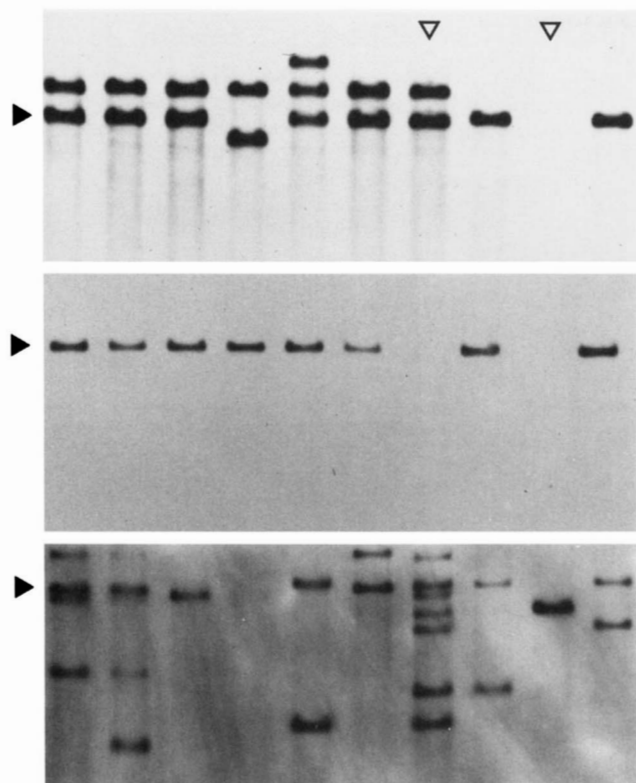


FIGURE 4.—Examples of Southern blot hybridization results for 10 individuals from each of three populations, sampled after eight experimental cycles. Genomic DNA samples were digested with *EcoRI*, which does not cut within Ty3 and generates a fragment of unique size for each copy of Ty3. Bands indicated by filled arrowheads are the galactose-inducible copies of Ty3 introduced at initial frequencies of 1% in each starting population. (Top) Diploid asexual population with transposition induced, in which Ty3 attained a frequency of 38%. The individuals represented in the lanes marked by open arrowheads were used in the competition experiment described in the text and in Table 2. Lane four indicates an individual from which the source Ty3 copy has been lost or partially deleted. (Middle) Sexual population with Ty3 repressed. No transposition was detected by Southern blotting, but the stationary Ty3 element increased in frequency. (Bottom) Sexual population with Ty3 induced during haplophase.

increase in Ty3 frequency above 40% in all three replicates of this treatment is not attributable to a simple failure of glucose repression, because every individual

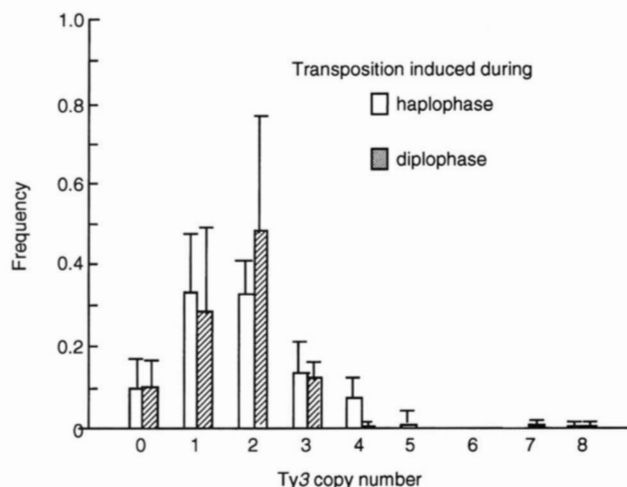


FIGURE 5.—Ty3 frequency distributions in sexual populations with transposition induced in haplophase (□) and in diplophase (▨). Column heights indicate treatment means of three replicates, with error bars above the columns representing one standard deviation. From the similar frequencies of the Ty3-free classes, it is evident that there is no difference between the treatments in the mean frequencies of genotypes with Ty3.

from this treatment with Ty3 carried only the single copy originally introduced at a frequency of 1% (Figure 4). Nor is it the result of an increase in vegetative fitness caused by integrative transformation with a Ty3 element and selectable marker, as indicated by the failure of this same construct to invade asexual populations when repressed.

The copy of Ty3 that spread in all three replicates was that introduced in transformant yVB114-TyT (Table 1). To explain this stationary Ty3 element spread throughout sexual but not asexual populations, we considered three possibilities:

1. Ty3 increases mating efficiency, so that cells carrying it experience greater mating success and increase in frequency with each sexual cycle.
2. Ty3 increases sporulation efficiency with the same effect, or
3. Ty3 was involved in biased gene conversion, so that diploids heterozygous for Ty3 transmitted the ele-

TABLE 2

Outcomes of competition trials between Ty3 insertion mutants and genotypes lacking Ty3

Replicate	Initial Ty3 frequency	Final Ty3 frequency		χ^2	P
		Observed	Expected		
Haploid 1	340/1633	710/1243	258.8/1243	993.49	$P \ll 0.001$
Haploid 2	281/1534	510/1081	198.0/1081	601.88	$P \ll 0.001$
Haploid 3	378/1312	498/936	269.7/936	271.48	$P \ll 0.001$
Diploid 1	68/259	212/725	190.3/725	3.36	$0.05 < P < 0.1$
Diploid 2	99/376	76/376	98.7/375	7.09	$P < 0.01^a$
Diploid 3	45/574	79/301	23.6/301	139.9	$P \ll 0.001$

^a Note that in this replicate the difference is in the opposite direction from that observed in the other two replicates.

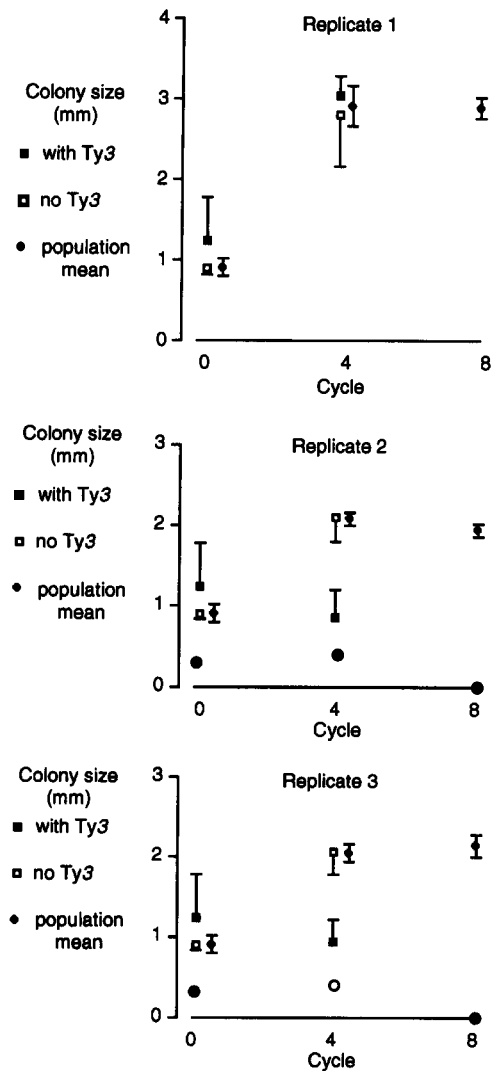


FIGURE 6.—Growth rates on galactose of sexual populations maintained on galactose. ■ and □ indicate colony sizes for genotypes with and without Ty3, respectively, with error bars indicating one standard deviation. ♦ indicate population mean colony sizes. The sample at cycle 0 is the base population from which all sexual populations were established.

ment to more than two of their daughter cells during meiosis.

To test the first two possibilities, we prepared replicate populations containing a *HIS3*-marked MATa genotype and two competing isogenic MATα genotypes. In each case, one of the competing genotypes carried a *URA3* marker. The other was either yVB114-TyT, a transformant of yVB114 with a 2μ plasmid carrying Ty3 and *TRP1*, or a yVB114 transformant with only a chromosomal *TRP1* marker. Ty3 frequencies had increased after mating, both for the chromosomally integrated Ty3 present in yVB114Ty-T and for a plasmid-borne copy, while changes in Ty3 frequency during sporulation were less consistent (Figure 7). Over the entire sexual cycle, Ty3 increased significantly in frequency in two of three replicates, both in genotype yVB114Ty-T

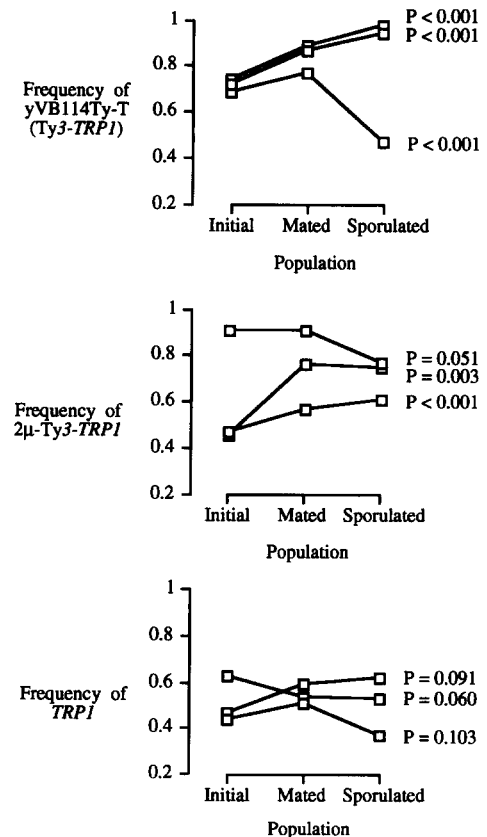


FIGURE 7.—Changes in the frequencies of sexually competing genotypes during mating and sporulation. In all three sets of competitions, a *HIS3*, MATa genotype was present as a mating partner for competing *URA3* and *TRP1* MATα genotypes. In all three tests the *URA3* competitor was a transformant of yVB114. The *TRP1* genotypes were yVB114Ty-T (A), a transformant of yVB114 carrying plasmid pJK311-AC (see Table 1) (B), and a *TRP1* transformant of yVB114 (C). Frequencies of Ty3 and/or *TRP1* marker were calculated from counts of colonies replica-plated to selective plates, as follows: before mating, the ratio of *TRP1* colonies to the sum of *TRP1* and *URA3* colonies; after mating, the ratio of *HIS3-TRP1* colonies to the sum of *HIS3-TRP1* and *HIS3-URA3* colonies; and after sporulation, the ratio of *TRP1* colonies to the sum of *TRP1* and *URA3* colonies. *P* values shown are based on χ^2 tests of the changes in estimated frequency during the entire sexual process in each replicate population. For the two tests in which one genotype carried Ty3, *P* values combined from independent χ^2 tests of each replicate (SOKAL and ROHLF 1981) indicated highly significant increases in Ty3 frequency during mating ($P < 0.01$ in both cases). This calculation could not be performed for the control pair or for changes in frequency during sporulation because the direction of change in *TRP1* frequency varied among replicates; for the two replicates in which *TRP1* increased in frequency, $0.05 < P < 0.10$.

and on a 2μ plasmid, while in the control population the frequency of the *TRP1* marker did not change significantly (Figure 7). The mating efficiencies of yVB114Ty-T and an isogenic *URA3* genotype lacking Ty3 were estimated as 20 and 13%, respectively.

No evidence of gene conversion was observed: all dissected tetrads yielded colonies with and without Ty3 and linked marker in the expected 2:2 ratio. Sporula-

tion of these yeast strains yields many triads in addition to tetrads, and dissections of these triads revealed 1:2 and 2:1 segregation of Ty3 in equal frequencies.

DISCUSSION

The spread of Ty3 was hypothesized to require sex because retrotransposons reduce fitness by causing predominantly deleterious mutations. Only in an outcrossing sexual population can the biased transmission that also results from transposition cause the spread of a retrotransposon. Our results provide qualified support for this prediction: Ty3 increased dramatically in frequency in all six sexual populations in which transposition was induced either in haplophase or during diplophase, and in two of six asexual populations in which transposition was induced.

Ty3 elements in asexual populations: In all six lines in which transposition was repressed, and in four of six lines in which transposition was induced, no copies of Ty3 were detected in the assay following eight experimental cycles. Our samples of 100 individuals are insufficient to conclude that Ty3 frequencies declined from their initial 1% in any one of these populations, but if the three replicates of each treatment are pooled, we can conclude that the final frequency of Ty3 was below 1% in both diploid and haploid asexual lines in which transposition was repressed. Retrotransposons are subject to deletion by recombination between the LTRs of a copy (BOEKE and SANDMEYER 1991). We observed evidence of such a deletion in a genotype recovered from an asexual line in which the source Ty3 copy had been deleted while transposed copies remained (Figure 4, upper panel, lane 4). Typical strains of *S. cerevisiae* carry hundreds of solo Ty1 LTR copies and dozens of solo Ty3 LTR copies thought to be the relics of such deletions. The failure of Ty3 to spread in four of six asexual, transposition-induced lines is therefore not necessarily the result of deleterious mutations. However, in one of these populations, the assay after four cycles yielded several individuals carrying both the original and newly transposed copies of the element. Since no Ty3 copies were detected in these four populations after eight cycles, we conclude that transposition occurred but produced no successful mutations.

In the case of the two asexual lines in which Ty3 mutants spread, these mutants outcompete Ty3-free genotypes from the same populations. Our results do not prove that it was the Ty3 insertion that increased the competitive ability of these genotypes but are certainly consistent with the interpretation that a small fraction of the mutations caused by Ty3 are beneficial. The fact that this spread of a Ty3 insertion occurred in only one of two diploid populations and one of four haploid populations is also consistent with this being a stochastic and relatively unreliable outcome, in contrast with the more deterministic processes implied by the concor-

dance among the replicates of all other treatments (Figure 2).

Cloning and characterizing these particular insertions would probably not be particularly revealing, because it is already well established that Ty3 integrates exclusively adjacent to sites where transcription by RNA polymerase III is initiated, predominantly, if not always, just upstream of tRNA genes (CHALKER and SANDMEYER 1990). This insertion-site specificity is unusual among retrotransposons and has been interpreted as an adaptation by Ty3 to its host genome that reduces the potential for transposition to cause genetic damage (KINSEY and SANDMEYER 1995; ZEYL and BELL 1996). However, this does not preclude effects by some Ty3 insertions on the expression either of the target tRNA gene (KINSEY and SANDMEYER 1995) or possibly of adjacent genes transcribed in the opposite direction.

Another mechanism by which some mobile elements are mutagenic is ectopic recombination, which causes chromosomal rearrangements. The yeast Ty1 retrotransposon causes little ectopic recombination, even when typical copy numbers are experimentally doubled (BOEKE *et al.* 1991). If this is also true for Ty3, then with its insertion site specificity Ty3 is less likely than many other mobile elements to cause genetic damage. This makes our test of the parasitic DNA hypothesis a conservative one: if sex increases the likelihood of Ty3 becoming established in a new population, the same is likely to be true of more virulent elements.

Active Ty3 elements in sexual populations: Ty3 increased rapidly in frequency in sexual lines in which transposition was induced either in haplophase or in diplophase. In contrast with the two asexual lines discussed above, in which specific insertions became abundant, Ty3 spread in these sexual, induced lines was not driven by the spread of a particular insertion. This indicates that Ty3 elements increased dramatically in frequency in all six of these sexual populations in the absence of any linkage to beneficial mutations.

By contrast, induction of transposition throughout the sexual cycle prevented the spread of Ty3 in all three replicates of this treatment. Transposition occurred in at least two of these populations, as indicated by the assay performed halfway through the experiment, when Ty3 appeared to be increasing in frequency (Figure 3) and new insertions were observed in the Southern blots (not shown). Because the genotypes that comprised our base populations were all *gal3* and grow slowly on galactose, we investigated the possibility that adaptation to galactose catabolism occurred in the form of selective sweeps, in which rare genotypes carrying Ty3 were eliminated by the spread of mutations that greatly increased growth rates on galactose and that arose in the much more abundant Ty3-free genotypes. Recombination in sexual populations can reduce the likelihood or extent of selective sweeps by permitting the spread of an adaptive allele independently of the genome in which it

arose. However, episodic selection could still have occurred in our sexual populations during the intervals between meioses. Assuming a generation time of 4 hr, the sexual populations maintained on galactose underwent ~48 mitotic divisions between episodes of sex. A genotype could increase in frequency from 0.01 to 0.99 in 48 generations with a selective advantage of $s = 0.19$, which is not implausible during the first few hundred generations of strong selection on galactose metabolism. Comparisons of the growth rates on galactose of genotypes with and without Ty3 (Figure 6) support the explanation that the dynamics of Ty3 frequency in these populations were determined by periodic selection, rather than by transposition. We cannot rule out the alternative explanation that Ty3 induction was somehow much more deleterious when maintained throughout the experiment than when restricted to haplophase or diplophase. For example, transcription of the genes for galactose catabolism is activated by Gal4p, a protein that binds to the *GAL1-10* promoter situated upstream of regulatory genes (JOHNSTON and CARLSON 1992). The additional *GAL1-10* UAS carried by the galactose-inducible Ty3 element could have hindered the induction of galactose catabolism by titrating Gal4p, to an extent that put cells with the Ty3 element at a greater disadvantage under constant galactose induction than under induction only during haplophase or diplophase.

We were interested in whether the fate of a galactose-regulated Ty3 element would depend on the stage in the sexual cycle at which it was induced, because wild-type Ty3 elements transpose only in the presence of the pheromones produced when haploid cells of complementary mating types are cultured together (KINSEY and SANDMEYER 1995). Artificially inducing transposition during diplophase instead of haplophase had no effect on Ty3 copy number distribution (Figure 4). In an evolutionary sense it is probably not haploidy *per se*, but rather the occurrence of sex to which Ty3 mobility is cued. The restriction of transposition to mating cells is itself a feature of Ty3 that is consistent with molecular parasitism on the yeast sexual cycle (ZEYL and BELL 1996). The experimental galactose regulation of Ty3 is intended in effect to override this adaptation to the yeast genome, yielding a more active element than the wild-type Ty3. However, transcripts from the galactose-regulated Ty3 element lack the galactose promoter and, upon reverse transcription and integration into a chromosome, are wild-type, pheromone-induced Ty3 copies. In our sexual populations, transposed Ty3 copies would be induced by pheromones during the mating stage of each experimental cycle, but pheromone induction of transposed copies could not occur in our asexual populations. Genotypes carrying new insertions may thus have differed in overall transposition rates according to whether or not they were in a sexual population. We consider it unlikely that this significantly affected our results, because in asexual populations trans-

posed Ty3 copies remain in the same genome as their galactose-inducible source Ty3 element. Induction of the galactose-inducible Ty3 probably causes higher transposition rates than pheromone induction of transposed copies: similarly constructed Ty1 elements transpose at 20–100 times their usual rates when induced by galactose (BOEKE *et al.* 1988).

Repressed Ty3 elements in sexual populations: We investigated the possibility that the increase in frequency of stationary copies of Ty3 in the sexual lines in which transposition was repressed resulted from an effect on sexual fitness. An alternative explanation is that repressed Ty3 elements hitchhiked on unrelated mutations that increased vegetative fitness. We consider this unlikely for two reasons: (1) additional data not presented here indicate that little or no adaptation to glucose media occurred in populations maintained on glucose, which is not surprising since lab yeast strains are typically maintained on glucose and already have very efficient glucose metabolism, and (2) this would require that such mutations arose only in the initially rare genotypes carrying Ty3 and did so independently in all three replicate populations.

It was an inevitable limitation of this experiment that the hypothetically parasitic element being used was not invading a foreign genome but has coevolved with it. The increase in frequency of repressed Ty3 elements in sexual populations may have been a result of this previous coevolution. If Ty3 elements are more likely to invade sexual than asexual populations of *S. cerevisiae*, then in the past Ty3 variants that maximized the sexuality of their hosts would have been most likely to become established. It is thus easy to understand why a retrotransposon might increase the mating efficiency of its host. The effect on sexual fitness shown in Figure 7 may vary with the location of Ty3: in other tests in which effects on mating and on sporulation were not distinguished (data not shown), genotypes with Ty3 isolated from sexual populations had higher sexual fitness than isogenic competitors lacking Ty3, but plasmid-borne copies of Ty3 did not increase sexual fitness. In addition to the competitions shown in Figure 7, we estimated the mating efficiencies of yVB114-TyT and an isogenic transformant with *URA3*. We obtained estimates of 20 and 13%, respectively, which qualitatively accounts for the rate of increase in Ty3 frequency observed in the sexual populations with Ty3 repressed.

This effect was observed in the apparent absence of transposition. Because a complete cycle of retrotransposition involves several element-encoded enzymatic functions, and because some of these can be blocked or regulated independently of others (MENEES and SANDMEYER 1994), it is possible that during mating on glucose media, repression of the galactose-regulated Ty3 was overridden by pheromone induction to an extent that permitted an effect on mating efficiency.

In an ideal test of the parasitic DNA hypothesis, an

experimentally inducible mobile element would be introduced into a population of genomes in which it has no previous evolutionary history. Although most mobile elements do not transpose in genomes unrelated to their native host, the activity of the maize transposon *Activator* in transgenic plants of several species suggests that with the right element, this obstacle could be overcome.

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