It is possible that mutations in genes other than *inhA* and *katG* may also cause INH resistance. Characterization of the full spectrum of mutations that confer resistance to INH could allow for the rapid identification of all INH-resistant strains by ligase chain reaction (*26*) or PCR single-strand conformational polymorphism strategies (*27*).

The ability to produce large quantities of the InhA protein will facilitate studies of the nature of InhA activity and the mode of action of INH and ETH in M. *tuberculosis*. Further investigation of this target may lead to the development of new agents for treating tuberculosis, including that caused by INH-resistant mutants of M. *tuberculosis* and disseminated M. *avium* infections, found frequently in late stage acquired immunodeficiency syndrome.

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Degeneration of a Nonrecombining Chromosome

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Comparative studies suggest that sex chromosomes begin as ordinary autosomes that happen to carry a major sex determining locus. Over evolutionary time the Y chromosome is selected to stop recombining with the X chromosome, perhaps in response to accumulation of alleles beneficial to the heterogametic but harmful to the homogametic sex. Population genetic theory predicts that a nonrecombining Y chromosome should degenerate. Here this prediction is tested by application of specific selection pressures to *Drosophila melanogaster* populations. Results demonstrate the decay of a nonrecombining, nascent Y chromosome and the capacity for recombination to ameliorate such decay.

Once the Y chromosome stops recombining with the X chromosome, it becomes an asexual (nonrecombining) component in an otherwise sexual genome (*1*, *2*). Asexual transmission of a chromosome leads to increased amounts of sampling error–induced linkage disequilibrium in finite populations. This interferes with the process of purifying selection and thereby causes chronic accumulation of small effect, deleterious mutations by a variety of mechanisms (*3*–*9*). The rate of accumulation increases with increasing mutation rate (U, the average per locus mutation rate multiplied by the number of loci per chromosome) and decreasing effective population size (N_e, essentially the number of successfully reproducing individuals). Population genetic theory predicts that recombination can stop or substantially slow the decay process (*4*–*9*). Studies have demonstrated that mutations accumulate when populations are repeatedly propagated through bottlenecks of a single individual (*10*–*12*). This report analyzes the effect of recombination on a simulation of a primitive Y chromosome without the use of recurrent single individual bottlenecks, thereby permitting natural selection to operate continuously.

Drosophila melanogaster populations were used to test for mutation accumulation in large autosomal haplotypes made to segregate like nascent Y chromosomes, that is, being always heterozygous and transmitted without recombination from father to son. Two things

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were done to speed the decay process to the point where it should be discernible during a microevolutionary study. First, the mutation rate (U) was made large by making the haplotype large: both large autosomal chromosomes, together accounting for 80% of the total genome, were selected such that they cosegregated as if they were one large, nonrecombining Y chromosome. Second, population size (N_e) was kept small by limiting the number of mating pairs per population so that only 32 haplotypes were transmitted each generation (Fig. 1).

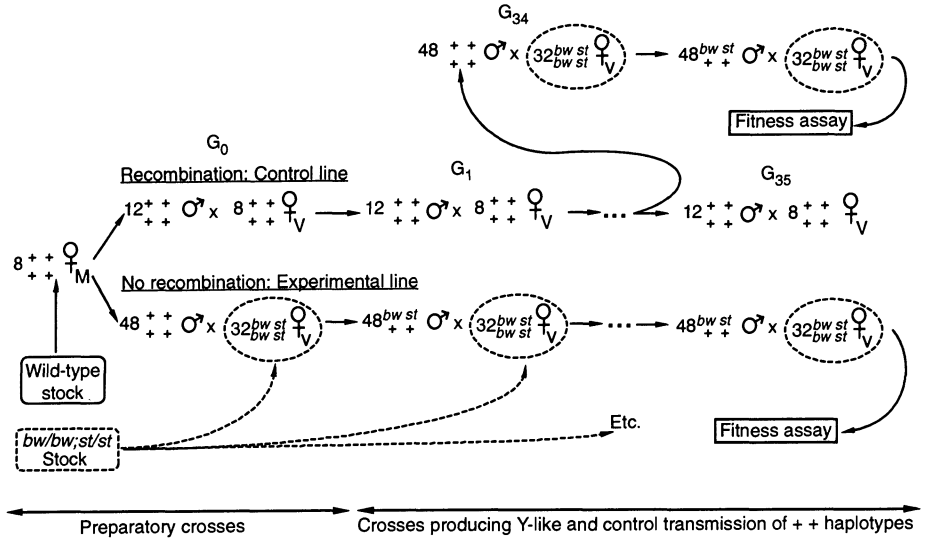

Fig. 1. The crosses used in the experimental protocol. For clarity, genotypes produced from a cross but not retained for mating are not shown. The subscripts M and V denote mated and virgin females, respectively, and all crosses are mass matings. The wild-type stock was derived from wild flies captured 2 years earlier near Davis, California (*18*). The *bw/bw;st/st* stock was homozygous for the recessive eye color markers *brown* (*bw*, located at II-104.5) and *scarlet* (*st*, located at III-44.0) and was produced by means of three sequential iterations of the crosses r/r (males) × +/+ (females) → $F_1 \times F_1$ → r/r (r/r denoting either *bw* or *st* homozygotes), with the final products from these crosses being mated to each other: *bw/bw;+/+* × *+/+;st/st*, → $F_1 \times F_1$ → *bw/bw;st/st*. These initial crosses minimize the potential operation of hybrid dysgenesis by placing the marker and wild-type chromosomes into the same wild-type cytoplasmic background without any intervening dysgenic crosses. The experiment was begun with five groups of eight mated females each taken from the wild-type stock. Offspring from each group of eight females were used to produce two paired lines in generation 0 (G_0). In the control line eight females were randomly chosen each generation, mixed for mating with 12 randomly chosen males, and then cultured in an 8-dram vial containing 15 ml of standard cornmeal-molasses medium seeded with live yeast. In the experimental line beginning in G_1, 32 virgin *bw/bw;st/st* stock females taken from a separate stock were mixed for mating with 48 *+/bw;+/st* males from the experimental line and cultured as described for the controls, with the exception that half-pint plastic jars containing 75 ml of medium were used. Larval density on the food was similar in both lines. A measure of the fitness (Fig. 2) of the experimental and control haplotypes was taken in G_{35}. The additional crosses made for each control line (top right of figure) in G_{34-35} were in preparation for the fitness assay in G_{35} (Fig. 2).

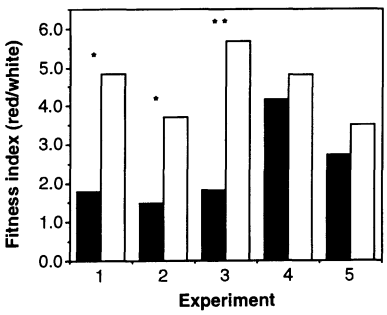

Fig. 2. Experimental lines consistently displayed lower fitness than control lines. In generation 34, males from the control line were crossed to doubly marked, *bw/bw;st/st*, stock females to generate a sample of heterozygous males with a genetic background matching that of the experimental line (top and right, Fig. 1). When these males, or males from the experimental lines, were crossed to the doubly marked stock females in generation 35, four genotypes with unique phenotypes were produced in equal zygotic frequencies. A relative fitness index was measured by first counting the number of rapidly developing adult males that emerged during the first 2 days post-eclosion. Because it is common for nonlethal mutations to retard development (*19*), this measure is sensitive to a wide diversity of deleterious mutants. The fitness index was defined as the ratio of the number of red-eyed flies, carrying a heterozygous wild-type haplotype (++), to the number of white-eyed flies, carrying two haplotypes (*bw st*) from the marker stock. Significance of paired differences between experimental (filled bars) and control (solid bars) was calculated with two-tailed conditional binomial exact tests (*20*). Total red and white males counted and used to generate the histograms are 99, 109, 147, 126, and 187 for experiments 1 through 5, respectively. $^*P < 0.05$ and $^{**}P < 0.01$.

A direct assessment of individually accumulated mutations was impractical because of the thousands of loci contained in each haplotype. Genetic deterioration of entire haplotypes should be reflected by the number of accumulated mutations, however, and this was assayed by the measurement of a multifarious fitness index (a measure reflecting simultaneously several different fitness characteristics) of heterozygous haplotypes relative to a marked, reference genotype.

There were five 35-generation experiments (Fig. 1). Initially, a minimum of 32 autosomal haplotypes, containing chromosomes II and III, were sampled from a wild-type population by random selection of eight mated females, some of which may have been multiply mated. Offspring from the eight mated females were used to generate paired experimental and control lines.

In the experimental line, chromosomal markers and artificial selection were used to keep haplotypes limited to males, permanently heterozygous, and to make 32 pairs of chromosomes II and III that cosegregated as a single chromosome. This experimental design takes advantage of the fact that intrachromosomal recombination does not occur in meiosis of *D. melanogaster* males. Thus, none of the haplotypes selected to persist had recombination. To ensure that sexual selection, in addition to natural selection, operated during the study, 48 males were available for mating with the 32 females of each generation.

In the control line the opportunity for sampling error (chance variation in gene frequencies between generations which can interfere with natural selection) and sexual selection was made similar to that in the experimental lines by mixing 8 females with a pool of 12 males. Each sex carries two wild-type haplotypes so that eight mated females transmit a total of 32 haplotypes to their offspring, as occurred in the experimental population (Fig. 1). The opportunity for sampling drift to fix nonrecessive deleterious mutations was therefore the same in experimental and control populations. In the control population, however, haplotypes that had segregated or recombined, or both, were allowed to persist, as normally occurs.

After 35 generations with natural and sexual selection operating in both lines, a fitness index was measured in the control and experimental males (Fig. 2). The fitness index directly measures relative viability and development time and was only measured for males, because selection on female-specific fitness characteristics was absent in the experimental but present in the control lines over the course of the 35-generation experiment.

In all five replicated populations, experimental and control, the fitness index was lower for the experimental line than its paired control. Within each of experiments 1, 2, and 3, this difference is statistically significant (Fig. 2). Furthermore, across the five paired experiments there is a strong consensus that 35 generations without recombination leads to lowered fitness [$P < 0.0006$, two-tailed consensus combined probability test (*13*)]. The estimated mean and standard deviation of the magnitude of fitness index reduction, 47 and 25%, respectively, are large and may substantially overestimate net fitness reduction because sample sizes in the fitness assays were small (Fig. 2) and a fitness index rather than net fitness was measured. It remains clear, however, that fitness declined in experimental relative to control populations.

Because reduced fitness was manifest in heterozygous individuals, these experiments extend prior work (*14*) by demonstrating genetic decay by means of the accumulation of nonrecessive mutations in response to the elimination of recombination. Studies of asexual genomic haplotypes in natural populations (*15*) also support the conclusion that once a haplotype stops recombining it becomes susceptible to deterioration through mutation accumulation.

The mechanism by which mutations accumulated in the experimental, nonrecombining populations cannot be unambiguously determined, but Muller's ratchet (*3–6*) and changes in the equilibrium mutational load (*8, 16, 17*) are likely candidates. Although the opportunity of sampling drift to fix deleterious mutations was the same in the control and experimental populations, the latter could not express mutations in the homozygous state, and this may have contributed to faster accumulation of partially recessive mutations. Part of the evolutionary advantage of sexual species may be due to the lack of recombination in asexual species, which therefore accumulate more deleterious mutations than do their sexual counterparts.

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Innervation of the Heart and Its Central Medullary Origin Defined by Viral Tracing

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The vagus nerve exerts a profound influence on the heart, regulating the heart rate and rhythm. An extensive vagal innervation of the cardiac ventricles and the central origin and extent of this innervation was demonstrated by transynaptic transport of pseudorabies virus with a virulent and two attenuated pseudorabies viral strains. The neurons that innervate the ventricles are numerous, and their distribution within the nucleus ambiguus and dorsal motor nucleus of the vagus is similar to that of neurons innervating other cardiac targets, such as the sino-atrial node. These data provide a neuroanatomical correlate to the physiological influence of the vagus nerve on ventricular function.

The innervation of the heart by the vagus nerve has been investigated since profound vagal cardiac influences were demonstrated by the Weber brothers in 1845 (*1*). The vagus nerves exert important control over cardiac rhythm and rate, and the baroreceptor vagal reflex is crucial for normal blood pressure regulation (*2*). In addition, vagal stimulation elicits an appreciable frequency-dependent negative inotropic effect on the left ventricle (*3*). However, the vagal cardiac innervation has been a continuing source of controversy, and uncertainties remain as to whether the cardiac ventricles receive a significant vagal innervation. Vagal innervation of the ventricles has been thought to be absent or at least greatly less than that of the atrium (*4*). Further, questions remain as to the location and distribution of central medullary vagal cardiac preganglionic motoneurons innervating specific cardiac targets. Neurons of the dorsal motor nucleus of the vagus (DMV) and of the nucleus ambiguus (NA) innervate the heart as shown by various physiological, traditional tract tracing, and degeneration techniques. Cardiac vagal preganglionic neurons located in both the DMV and NA (*5, 6*), mostly in the NA (*7*), or primarily in the DMV (*8*) have been reported. Species variations may account for some of these differing results. However, conventional tracing studies with cholera toxin–conjugated horseradish peroxidase (CT-HRP) as a tracer have suggested a rather minor involvement of the DMV in the rat (*9, 10*), and recent reviews have reached a consensus that cardiac neurons arise mainly in the NA, with little contribution from the DMV (*11, 12*). This conclusion is consistent with the evidence that most, if not all, cells of the DMV appear to project to subdiaphragmatic, not cardiac, structures (*11*).

It has been difficult to resolve questions concerning cardiac, particularly ventricular, innervation by the usual retrograde tracing techniques because the postganglionic neurons at the heart break the link between the medullary vagal motoneurons and their cardiac targets. The postganglionic neurons are widely scattered at the heart (*13*). We have been able to circumvent these problems by using pseudorabies virus (PRV), a neurotropic herpesvirus, as a transynaptic tracer. Herpesviruses have been used to map synaptically linked neuronal circuits both centrally and peripherally (*14*). PRV can be injected directly into a peripheral organ, in this case the myocardium, and the virus transported from the specific cardiac site to postganglionic neurons that innervate that target. PRV replicates in these postganglionic neurons, then retrogradely crosses synapses and is transported to the specific vagal preganglionic neurons innervating the postganglionic neurons. Transneuronal spread of virus in

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