age of the upper 40 cm of the core). The calculation shows that in the transition from full glacial conditions to the Holocene, the $\delta^{18}O$ value of seawater decreased by 1.15 ± 0.18 per mil (1σ) while SST increased by 2.0° ± 0.8°C. Because we averaged data from intervals that represent about 1000 years, time longer than the mixing time of the ocean, the calculated change in $\delta^{18}O_w$ is the global effect and not a local feature of the APF zone. This observation suggests that, in the absence of local variability, the coupling of silica and carbonate $\delta^{18}O$ can be used to predict the global ice volume effect. If so, the method represents one possible means for resolving ice volume changes over intervals poorly constrained by coral reef terraces.

Short-term fluctuation can be analyzed similarly by taking segments of the curve. The sharp transition into the LGM corresponds to an enrichment of 1.3 per mil in seawater $\delta^{18}O$ composition. The same applies for the transition of 0.6 per mil in $\delta^{18}O_w$ at 420 cm. The shift around 1200 cm corresponds to a cooling of 0.8°C and a negative shift of 1.38 per mil in $\delta^{18}O_w$. The migration of the APF north of its present position and the presence of meltwater would generate such a change in isotopic composition of silica and carbonate. Such an effect cannot be deduced from analysis of a single phase and requires the simultaneous measurement of two phases.

More $\delta^{18}O_{Si}$ records from other Southern Ocean cores are needed to assess the significance of the local variability recorded in core RC13-271. In order for the coupled $\delta^{18}O_{Si}$-$\delta^{18}O_c$ approach to be generally useful, the relation between diatom blooms and the seasonal growth of surface-dwelling planktonic foraminifera must be established in other regions of the ocean. Our results provide the basis for extending research to regions of the ocean floor void of carbonate, and isotope stratigraphies may be constructed for records where previously they seemed impossible.

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Sexually Antagonistic Genes: Experimental Evidence

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When selection differs between the sexes, a mutation beneficial to one sex may be harmful to the other (sexually antagonistic). Because the sexes share a common gene pool, selection in one sex can interfere with the other's adaptive evolution. Theory predicts that sexually antagonistic mutations should accumulate in tight linkage with a new sex-determining gene, even when the harm to benefit ratio is high. Genetic markers and artificial selection were used to make a pair of autosomal genes segregate like a new pair of sex-determining genes in a *Drosophila melanogaster* model system. A 29-generation study provides experimental evidence that sexually antagonistic genes may be common in nature and will accumulate in response to a new sex-determining gene.

Sexually antagonistic genes (SA genes) are those that are favored by selection in one sex but disfavored in the other. Such genes have been hypothesized to play an important role in the evolution of karyotypes, sexual dimorphism, sex chromosomes, and genetic polymorphisms (*1–5*), but there is little evidence for an adequate pool of SA genes in nature.

One of the major problems that interferes with a search for SA genes is that theory predicts only a very narrow range of circumstances under which they are expected to be maintained in the polymorphic state (*2, 3*). As a consequence, SA genes may be rare at individual loci despite their potential abundance when summed over whole genomes or populations.

One circumstance where the level of polymorphism is expected to be high is that of primitive sex chromosomes, where a single Mendelian gene (or cluster of tightly linked genes) determines gender. Theoretical work predicts that the chromosomal region located within a few centimorgans (cM) of a sex-determining gene will be a "hot spot" for the accumulation of SA genes favoring the heterogametic sex, even when the disadvantage to the homogametic sex is large (*2, 4*).

The rationale for the theoretical prediction is intuitively straightforward. First consider the requisite conditions for an SA gene to accumulate at an ordinary autosomal locus. A new SA mutation favoring females will be transmitted with equal frequency to sons and daughters. When in females (males) it experiences a gain (loss) in gene frequency due to the action of sex-specific selection. To accumulate in the gene pool, gain must exceed loss, requiring the mutation to have a net advantage when averaged across the sexes. A similar SA mutation introduced at a position 1 cM away from a female-determining gene will be transmitted 99% (1%) of the time to daughters (sons) where it is favored (disfavored). This sex-specific gene transmission will permit virtually any female-benefit–male-detriment mutations to accumulate when rare, even when highly harmful to males. The constraint for the accumulation of SA genes is far less stringent at loci near a sex-determining locus, hence the SA "hot spot" prediction.

Our experiments take advantage of this prediction by creating a new sex-determining locus in a *Drosophila melanogaster* model system and then testing for the accumulation of SA genes in response to this experimental treatment. Details of the stock construction and protocol for maintaining the flies are given in Fig. 1. The basic strategy was to use artificial selection to make a dominant eye-color gene segregate as if it were a new female-determining gene. In each generation, artificial selection

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makes females heterozygous for the dominant gene while males are homozygous for its recessive allelomorph. Genetic variation, which is tightly linked to the dominant gene, will be transmitted almost exclusively from mother to daughter and this facilitates the accumulation of female-benefit–male-detriment genes (*2*, *4*).

Experimental power was increased in two ways. First, it was doubled by causing gender to be determined by a pair of dominant eye-color genes located on different chromosomes. Second, we used eye-color loci that were positioned in the euchromatin within a few centimorgans of the centromere, where studies of visible mutations indicate that the density (autosomal loci per centimorgan) of genes is about eight times as high as in other chromosomal regions (*6*). This increases the number of genes that are tightly linked to (under the influence of) the new female-determining genes. By using two centromere-proximate loci to determine gender, the degree of accumulation of SA genes in our 29-generation experiments should approach what would have occurred in a 464-generation experiment ($2 \times 8 \times 29$) using a single sex-determining locus placed at an ordinary chromosomal position.

At the start of the experiments, two of the four founding populations were randomly assigned to be the experimental lines; the other two were controls. The experimental lines were propagated each generation by randomly selecting 100 *pr*/*pr*$^+$;*st*/*st*$^+$ virgin females (red eyes) and 150 *pr*/*pr*;*st*/*st* males (orange eyes) from the offspring pool and mixing them when 4 days old (Fig. 1). All other individuals lacking the appropriate sex-specific genotypes were discarded. The protocol caused the *pr*$^+$ and *st*$^+$ genes to behave as if they were a pair of major sex-determining genes that are fully epistatic to the naturally occurring chromosomal sex-determination system in *D. melanogaster*. The *pr*$^+$ and *st*$^+$ genes were sex-limited in their transmission, being confined entirely to females in the pool of offspring used to generate successive generations. Genes that were tightly linked to these genes were therefore transmitted predominantly from mother to daughter which facilitates the accumulation of female-benefit–male-detriment SA genes (*2*, *4*).

In odd-numbered generations the two control populations were cultured as described above for the experimentals, but in even-numbered generations the sex-specific genotypes were switched so that 100 *pr*/*pr*;*st*/*st* virgin females (orange eyes) and 150 *pr*$^+$/*pr*;*st*$^+$/*st* males (red eyes) were selected at random from the offspring pool and mixed when 4 days old to generate the next generation (Fig. 1). In this treatment the *pr*$^+$ and *st*$^+$ genes spent half of the generations in males and half in females, as would occur on average for normal autosomal genes. This protocol does not generate sex-biased transmission of the genes tightly linked to the *pr*$^+$ and *st*$^+$ genes.

Theory (*2*, *4*) predicts that SA genes (female-benefit–male-detriment) that were previously held at low frequency due to mutation-selection balance should accumulate in tight linkage with the wild-type marker genes in the experimental but not the control populations. The prediction was tested by measuring the day 1 sex ratio (that is, sex ratio on the first day after the flies began to eclose) of *pr*$^+$/*pr*;*st*$^+$/*st* flies (red-eyed) four times over the course of 29 generations. Red-eyed flies were measured because these are the flies that were expected to carry the highest dose (both chromosomal regions proximate to the new sex-determining genes) of SA genes in the two experimental lines. Sex ratio (expressed as percentage of males) is expected to decline as female-benefit–male-detriment genes accumulate. The flies were measured on the first day post-eclosion to help resolve genetic effects that were harmful yet nonlethal to males, because it is common for nonlethal mutations to retard development (*6*).

Figure 2 illustrates how, over the 29 generations of the experiment, the day 1 sex ratio became progressively more female-biased in the two experimental treatments compared to the two controls. This result is statistically significant (Fig. 2B; $P < 0.02$; two-tailed Pearson's product-moment correlation test, df = 2), supporting the conclusion that SA genes had accumulated in the experimental lines.

To obtain more direct evidence that SA genes that were detrimental to males had accumulated, we compared an index of the net fitness of males between the experimental and control populations in generation

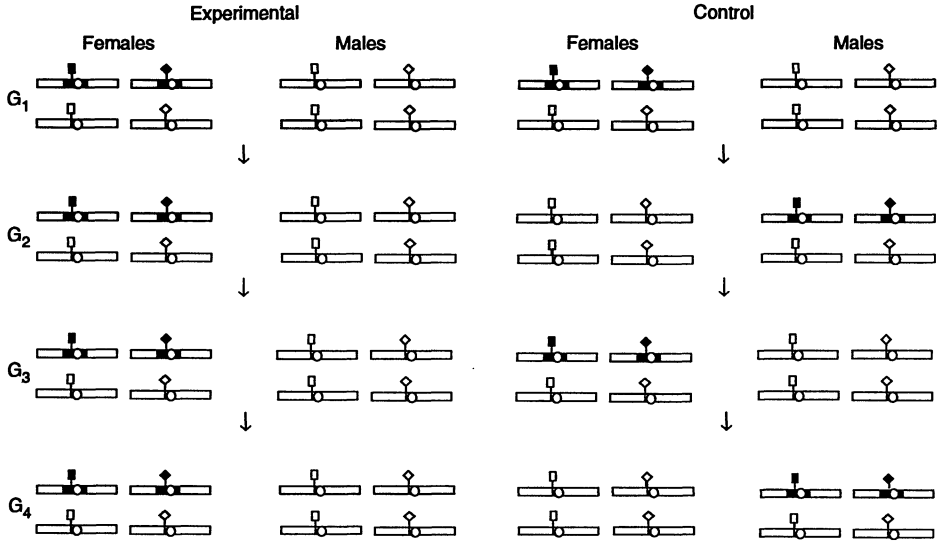

Fig. 1. The selection protocol used to propagate the control and experimental populations. The rationale for the protocol is that two unlinked dominant eye-color genes (illustrated by filled symbols above chromosomes) are made to co-segregate and remain in females (by manually removing all of the inappropriate genotypes each generation before mating) in the experimental lines and to alternate between the sexes in the controls. The surviving dominant markers therefore segregate like a pair of new female-determining genes only in the experimental lines and the chromosomal regions tightly linked to these markers (shaded portions of chromosomes) are expected to accumulate female-benefit–male-detriment genes. More specifically, genotypes produced by a cross but not shown in the next generation as parents were discarded before mating. In the figure homologous pairs of chromosome 2 (or 3) are positioned to the left (or right); centromeres are illustrated by open circles and the marker loci by squares (chromosome 2) or diamonds (chromosome 3); filled marker symbols denote the dominant wild-type alleles that acted as female-determining genes in the experimental lines and the filled portions of the chromosomes (not to scale) represent the regions expected to accumulate female-benefit–male-detriment SA genes only in the experimental lines. To begin the experiments, the recessive eye-color genes *purple* (*pr*, located at II-54.5) and *scarlet* (*st*, located at III-44.0) were recombined into a recently established wild-type strain of *D. melanogaster* (derived from San Carlos, Mexico). Males from this strain homozygous for *pr* and *st* genes were crossed to a second recently established wild-type strain (derived from Davis, California) to produce 400 doubly heterozygous *pr*/*pr*$^+$;*st*/*st*$^+$ virgin females. The second wild-type strain was used in order to further increase the genetic variation in the founding population. These virgin females were divided into four populations of 100 individuals (*9*), each of which was mixed with 150 *pr*/*pr*;*st*/*st* males (males and females were 4 days post-eclosion when mixed). This cross produces four phenotypic classes in equal zygotic frequencies: red eyes (*pr*/*pr*$^+$;*st*/*st*$^+$), scarlet eyes (*pr*/*pr*$^+$;*st*/*st*), purple eyes (*pr*/*pr*;*st*/*st*$^+$), and orange eyes (*pr*/*pr*;*st*/*st*).

29. This was accomplished by culturing the flies onto an additional set of food in generation 28 and then permitting the replica set of generation 29 flies to eclose and mate without human disturbance in all four populations. Four days after the first flies eclosed, 100 *pr/pr;st/st* females (orange-eyed, most of which had mated) were removed and cultured individually in 8-dram vials supplied with standard molasses-cornmeal medium. These females were chosen because the identity of the males to which they had mated could be determined from the distribution of their offspring. On rare occasions the phenotypic distributions of offspring indicated a double mating. A mating by both male types was scored in these cases unless a clear majority of offspring were assignable to one or the other male type.

Because our mating design causes the zygotic frequencies of the four male genotypes (that is, those carrying both, neither, or only one or the other of the female-determining chromosomal regions) to be equal each generation, their relative representation in the pool of successfully mating males is a multifarious index of net fitness—that is, many fitness components (for example, viability, development time, and mating behavior) collectively determine this measure. Percentages of orange females mated to the four male genotypes are presented in Table 1.

There are a variety of comparisons that could be made to determine whether the female-determining chromosomal regions reduce male fitness in the experimental populations. To avoid the diminished statistical power associated with a posteriori testing among many possible comparisons, I decided a priori to make only two comparisons.

First, a comprehensive comparison was made by dividing the males into two groups: SA males [those carrying one or both of the new sex-determining chromosomal regions (red, scarlet, and purple)] versus doubly marked males [those carrying neither of the sex-determining chromosomal regions but expressing both eye color markers (orange)]. The higher the percentage of doubly marked (orange) males in the mating pool of the experimentals compared to the controls, the greater the evidence for the accumulation of SA genes in response to the new sex-determining loci. The percentage of doubly marked males (orange-eyed) was nearly double in the experimental lines compared to controls (Fig. 3A, $P < 0.001$, two-tailed t test, df = 2), indicating a substantial reduction in net fitness associated with carrying one or more of the chromosomal regions that were tightly linked to the new female-determining genes.

Second, I made a more restrictive comparison between the two extreme male genotypes, that is, between double-SA males [carrying both female-determining chromosomal regions (red)] and doubly marked males carrying neither (orange). The ratio (red/orange) of the numbers of the two types of males in the mating pool of the experimental lines represents the net fitness of males expressing both female-determining chromosomal regions relative to those expressing neither. The same ratio in the control lines measures the marker effects alone. The net fitness of red males is expected to be lower in the experimental populations owing to the accumulation of female-benefit–male-detriment genes. This expectation was realized with the red to orange ratio of the experimentals being less than one-half the value observed in the controls (Fig. 3B, $P = 0.045$, two-tailed t

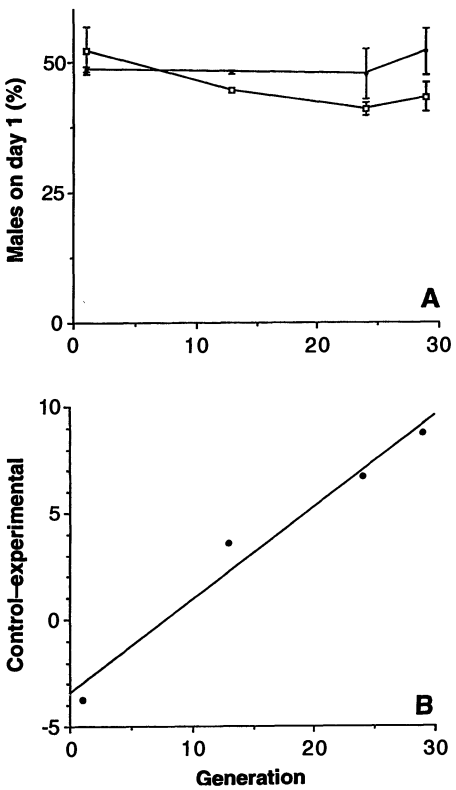

Fig. 2. Sex ratio fitness assay of control and experimental lines. (**A**) The percentage of males, from the pool of red-eyed flies (those carrying both chromosomal regions expected to accumulate SA genes in the experimentals; $pr^+/pr;st^+/st$), on the first day after the onset of eclosion as a function of the number of generations since the beginning of the experiment. Filled (open) symbols depict mean values for the controls (experimentals), the error bars depict the range of the two replicates, and lines connect mean values. (**B**) A plot of the difference between experimental and control mean values as a function of generations. The line is the least squares fit to these differences.

Table 1. Percentages of orange-eyed females mating to the four male phenotypes. R, red; P, purple; S, scarlet; and O, orange.

Treatment	Male phenotype selected for mating (%)				Total*
	R	P	S	O	
	Control				
Replicate 1	42	26	22	10	90
Replicate 2	36	21	33	9	75
	Experimental				
Replicate 1	40	20	22	17	90
Replicate 2	17	40	26	17	70

*These total numbers of females assayed vary because some of the 100 individually cultured females from each population failed to produce offspring (reasons are unknown but they may have become stuck in food and died, were sterile, or failed to mate before being cultured).

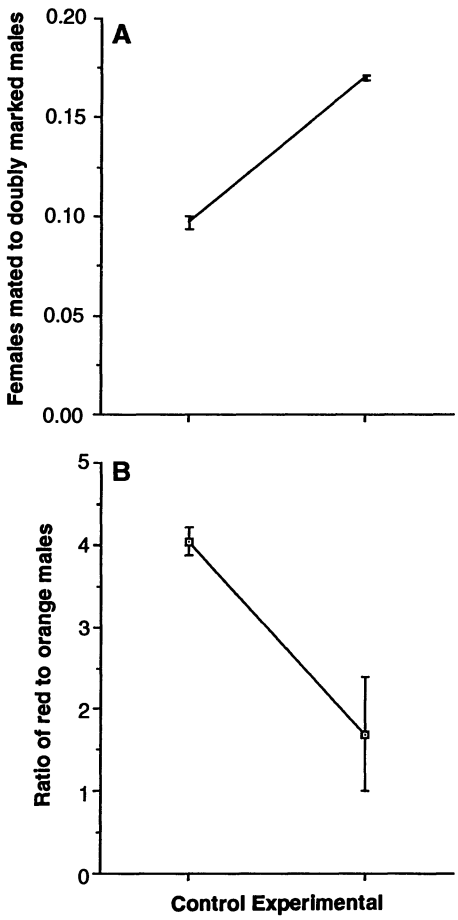

Fig. 3. Net fitness assay of control and experimental lines. (**A**) The percentage of females mating to doubly marked males (orange-eyed; those carrying neither of the chromosomal regions predicted in the experimentals to accumulate SA genes) in control versus experimental treatments. (**B**) The ratio of red (double SA; carrying both chromosomal regions predicted to accumulate SA genes in the experimental lines) to orange (doubly marked) males in the mating pools of the control and experimental populations. In both panels the line connects the mean values and the error bars denote the range of the two replicate populations.

test, df = 2), further supporting the conclusion that SA genes have accumulated in the experimental lines.

Replicate 2 of the experimentals is especially noteworthy because of the parity in fitness observed between wild-type red-eyed males and the orange-eyed males that expressed two recessive eye-pigment mutations that reduce visual acuity and thereby substantially reduce male mating success (8). I have used the net fitness assay in numerous other unrelated applications with the same or similar starting genetic backgrounds, and never have the wild-type flies performed so poorly against the double mutant standard, as was observed in replicate 2.

Owing to the lack of recombination in males, there was twice as much opportunity in the experimental lines for recombination to breakdown linkage disequilibrium (in the vicinity of the eye-color loci) that existed at the beginning of the experiments. This difference could elevate the fitness of orange-eyed males in the experimentals relative to controls if the chromosomal regions flanking the recessive markers were considerably less fit than the homologous regions flanking the wild-type alleles.

The observed pattern of genotype-specific fitness in replicate 1 of the experimentals might be explained by this interpretation, but not the pattern observed in replicate 2 (Table 1). The day 1 sex ratio assay (Fig. 2), however, demonstrates that SA genes accumulated in both experimental replicates; the correlation test (Fig. 2B) is statistically significant ($P < 0.05$) when each experimental replicate is individually compared to the two controls. The day 1 sex ratio assay controls for any confounding effects associated with differing rates of decay in initial linkage disequilibrium by comparing the fitness (viability and development time) of red-eyed males relative to females with identical levels of disequilibrium.

The first conclusion from these experiments is that we now have experimental support for the hypothesis that the chromosomal region proximate to a new sex-determining gene can act as a hot spot for the accumulation of genes that are detrimental to the homogametic sex. Such accumulation is vital to the operation of the current models proposed for the evolution of suppressed recombination between primitive sex chromosomes (*2*, *4*, *8*).

The second, more general, conclusion is that SA genes may be common in natural gene pools. The fact that SA genes accumulated in a small portion of the genome over the course of a microevolutionary study suggests that these genes may be present at low frequency at many loci that are widely dispersed throughout the genome. This would lead to a considerable conflict between the sexes being manifest at the level of the genome and adaptation by each sex would be compromised (sexual dimorphism load), owing to sex-specific selection and the fact that the sexes must share a common gene pool.

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9. To avoid overcrowding the males and females were separated before mating into five equal groups (pairs of 20 females and 30 males) placed into five half-pint plastic containers containing approximately 75 ml of standard molasses-cornmeal medium. Flies were allowed to lay eggs for 24 hours before being removed, and then, starting 9 days later, offspring were collected and pooled across the five bottles until sufficient numbers were obtained to begin the next generation.

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Lyme Disease in California: A Novel Enzootic Transmission Cycle of *Borrelia burgdorferi*

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Knowledge of zoonotic transmission cycles is essential for the development of effective strategies for disease prevention. The enzootiology of Lyme disease in California differs fundamentally from that reported from the eastern United States. Woodrats, not mice, serve as reservoir hosts, and *Ixodes neotomae*, a nonhuman-biting tick, maintains the agent of Lyme disease, *Borrelia burgdorferi*, in enzootic cycles. The western black-legged tick, *Ixodes pacificus*, is the primary vector to humans, but it appears to be an inefficient maintenance vector. Isolates of *B. burgdorferi* from California exhibit considerable antigenic heterogeneity, and some isolates differ strikingly from isolates recovered from this and other geographic regions.

Vector-borne zoonotic diseases are often maintained in complex transmission cycles involving several arthropod vectors and their wild vertebrate hosts. Comprehensive studies of the relative importance of potential reservoir hosts and their associated vectors provide insight into the basic mechanisms maintaining a zoonotic disease agent and may yield the knowledge necessary to avoid or control human disease.

Reservoirs of Lyme disease are defined as host species whose individuals are commonly infected, that perpetuate borrelial infections for prolonged periods, and that remain infective to vectors. Vector competence describes the inherent ability of an arthropod to become infected, to perpetuate, and to subsequently transmit the disease agent. The relative importance of competent reservoirs and vectors depends on the interaction of many variables and may differ between populations or communities of hosts and vectors.

The etiologic agent of Lyme disease, *Borrelia burgdorferi*, is maintained in enzootic cycles involving wildlife hosts and ticks throughout much of the temperate world. In the United States, ticks in the *Ixodes ricinus* "complex," including *Ixodes dammini* in the Northeast and Midwest and *Ixodes pacificus* in the West, have been shown to be the primary vectors to humans (*1*). *Ixodes dammini* is also the principle maintenance vector within cycles involving wild reservoirs, primarily the white-footed mouse, *Peromyscus leucopus*, in the East (*1*, *2*), and it has been generally assumed that ticks in the *I. ricinus* "complex" are serving similar roles in other geographic areas. However, although *Ixodes pacificus* and *Peromyscus* spp. mice are common, they appear relatively unimportant in transmission cycles of *B. burgdorferi* in north coastal California. Rather, dusky-footed woodrats (*Neotoma fuscipes*, hereinafter referred to as woodrats) and a non–*I. ricinus* complex tick, *Ixodes neotomae*, support an enzootic cycle that maintains levels of *B. burgdorferi* in nature. The known geographic range of *I. neotomae* overlaps the distributions of *I. pacificus* and *N. fuscipes* in California; these species co-occur

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