hybridization signal detectable with RNA from cyc$^-$ S49 cells, even though equivalent amounts of RNA were present on the nitrocellulose; this is shown by the similarity of signal obtained with a chicken actin cDNA probe (Fig. 4B). The presence of an RNA in wild-type S49 cells that hybridizes to the bovine brain cDNA clone and the lack of a hybridization signal in RNA of cyc$^-$ cells confirms the identity of the cDNA clone as one that encodes bovine brain $G_{s\alpha}$. The bovine brain $G_{s\alpha}$ clone, designated pBBG$_{s\alpha}$, was then used as a probe to investigate the gene encoding the message for $G_{s\alpha}$.

Genomic DNA was prepared from bovine liver (*28*) and 5 μg was digested with Kpn I, Xba I, or Hind III; resolved on 0.8 percent agarose gels; and transferred to nitrocellulose (*18*). Blots were hybridized to uniformly labeled, single-stranded probes corresponding to the 3′ or the 5′ end of pBBG$_{s\alpha}$. The 3′ probe probably corresponds to an untranslated region of $G_{s\alpha}$ messenger RNA (mRNA), while the 5′ probe includes the region where amino acid sequence is strongly conserved among G_s, G_o, and transducin. A single band was obtained in each of the three restriction digests analyzed with the 3′ probe, while two bands were obtained with the 5′ probe (Fig. 5). It thus seems likely that one or at most two genes contain sequences that are revealed by such hybridization analysis. This is consistent with the observation that the cDNA sequence for $G_{s\alpha}$ differs significantly from that for transducin in the region where the amino acid sequence is identical (*8*).

The oligonucleotide probe corresponding to a region of shared amino acid sequence among G proteins detected only $G_{s\alpha}$ in the bovine brain cDNA library and, surprisingly, did not detect bovine brain $G_{o\alpha}$ or $G_{i\alpha}$. A bovine adrenal cDNA library was also screened with the mixed 36-base oligonucleotide probe, and again clones corresponding to $G_{s\alpha}$ were the only ones that were identifiable under the conditions utilized. The limited sequence redundancy allowed in the synthesis of the oligonucleotide probe may have caused it to be more "$G_{s\alpha}$-like" than "$G_{o\alpha}$- or $G_{i\alpha}$-like." Alternatively, it may be difficult to synthesize long cDNA's for $G_{o\alpha}$ or $G_{i\alpha}$ with reverse transcriptase, and oligonucleotide probes corresponding to carboxyl terminal regions of these proteins may have advantages for cDNA cloning.

The immunoblot of S49 cell membrane extracts with $G_{s\alpha}$-specific antibody (Fig. 3) and analysis of RNA from these cells (Fig. 4) address the nature of the S49 cyc$^-$ mutant; this phenotypic variation occurs at a high frequency (*29*). The absence of a detectable amount of protein that is immunologically reactive at this NH_2-terminal epitope does not support the idea that an altered $G_{s\alpha}$ molecule contributes to low basal adenylate cyclase activity in the cyc$^-$ mutant. Because no RNA was found in the cyc$^-$ cells that would hybridize to a $G_{s\alpha}$-specific probe, it is likely that the gene encoding $G_{s\alpha}$ is not transcribed in these cells or that the mRNA is unable to accumulate to levels detectable by hybridization to total RNA.

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Genetic Damage, Mutation, and the Evolution of Sex

Abstract. *The two fundamental aspects of sexual reproduction, recombination and outcrossing, appear to be maintained respectively by the advantages of recombinational repair and genetic complementation. Genetic variation is produced as a by-product of recombinational repair, but it may not be the function of sexual reproduction.*

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Why organisms reproduce sexually is a major unsolved problem in evolutionary biology (*1, 2*). Evolutionary explanations have usually appealed to the genetic variation produced by sex (*1–3*). In a recent review of the problem Bell concluded that the consequence of increased genetic diversity of progeny is "a category which includes all hypotheses of interest" (*3*). There is, however, widespread skepticism that any particular variation argument can provide a general explana-

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tion for the evolution of sex (*1, 2*). Here we propose an alternative, the "repair hypothesis," which is based on a selective advantage arising from recombinational repair of genetic damage. That repair is as directly correlated with recombination as variation is indicated by the fact that mutations in genes known to be responsible for general recombination increase sensitivity to DNA damaging agents (*4, 5*). Damage appears to be a fundamental problem for living systems (*4*), and we have argued that repair of genetic damage in conjunction with the costs of redundancy are the selective forces that lead to the origin of sex (*6*). We now propose that repair and complementation are the selective forces that maintain sex.

Sexual reproduction has two aspects: (i) recombination, in the sense of the breakage and exchange of DNA between two homologous chromosomes, and (ii) outcrossing, in the sense that homologous chromosomes from two different individuals come together in the same cell. Recombination is clearly the more basic aspect of sex as evidenced by the various reproductive systems that have abandoned outcrossing but have retained recombination. We propose that the maintenance of these two aspects of sex is a consequence of selective forces resulting from the two main categories of problem for transmission of genetic information, DNA damage and mutation. Recombination is maintained because it is needed for repair of damage, and outcrossing is maintained because it promotes complementation, or the masking of deleterious mutations, in reproductive systems with recombination. Thus, damage selects for recombination, and mutation in the presence of recombination selects for outcrossing.

Organisms contain elaborately designed structures for rapidly replicating their genetic material (*7*), usually DNA, while at the same time avoiding mutations and repairing damages. Mutations and damages pose distinct problems that are handled differently and lead to different consequences. Genetic damages are physical alterations in the structural regularity of DNA such as breaks, depurinations, depyrimidinations, cross-links, thymine dimers, and modified bases. Damages usually interfere with replication (*8, 9*) and transcription (*10*). They are neither replicated nor inherited. By contrast, mutations are changes in the base-pair sequence of DNA that result from substitution, addition, deletion, or rearrangement of the standard base pairs. Mutations do not generally alter the physical regularity of the molecule, although they may occasionally affect structure by, for example, preventing or allowing methylation at a mutated site or by altering the stability of hairpin loops. Mutations are replicable and thus can be inherited.

The aspect of damage that is crucial for the evolution of sex is that damage can be recognized directly by enzymes and can be repaired, so long as a redundant template is available to replace the information lost through damage. Mutations may be prevented from arising by enzymes that recognize and remove a mispaired base in DNA, an intermediate in the formation of a mutation. However, if the mispairing is resolved by replication, there is no mechanism for determining which daughter DNA is correct, that is, which carries the original information. Mutations are only "recognized" after phenotypic expression, and are thus only "removed" by natural selection. In general, damages can be repaired, but mutations cannot.

There are numerous instances in different organisms of repair enzymes that have no known function other than to repair DNA (*11*). This implies that the damages they repair are prevalent in nature and would significantly reduce fitness if left unrepaired. Excision repair enzymes remove damages to only one strand of DNA, that is, single-strand damages. In such cases the undamaged strand serves as a template for replacing the excised DNA and recombination does not occur. Damages affecting both strands at nearby positions, that is, double-strand damages, cannot be repaired by such mechanisms, since there is no intact strand to serve as a template. These double-strand damages are critical for our hypothesis since they can only be repaired by recombination.

Evidence exists for the prevalence of double-strand damages in DNA due to the highly reactive superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2). These probably occur frequently in cells as a by-product of cellular respiration (*12*), and the DNA damage produced by these agents has been linked to aging (*13*) and cancer (*14*). The relative rates of formation of various types of DNA damage produced by H_2O_2 have been estimated (*15*). About 60 modified bases are produced for every double-strand damage, and two types of double-strand damage, cross-links and double-strand breaks, occur with roughly equal frequency. The modified bases produced by oxidative damage can be removed from DNA by excision repair enzymes that deal with specific types of altered bases (*16*). In nature, oxidative reactions are probably the major cause of these modified bases.

It has recently been estimated (*17*) that about 320 thymine glycol residues are removed by repair from the average human cell per day, and that a comparable number of hydroxymethyluracil residues are also removed. If we assume that at least 600 modified bases occur per human cell per day, then an additional ten double-strand damages should also occur (since modified bases and double-strand damages are produced in a ratio of about 60 to 1). About 90 percent of oxidative reactions occur in the cell's mitochondria suggesting that about 90 percent of DNA damages are in mitochondrial DNA and 10 percent are in DNA of the cell nucleus. To be conservative, if we assume that only 1 percent of oxidative damages occur in the nucleus, the number of double-strand damages per cellular genome per day would still be 0.1. This can substantially reduce fitness since a single unrepaired double-strand damage may block replication and cause cell death (*18*). Studies in *Escherichia coli* (*19*) and yeast (*20*) have shown that double-strand breaks and cross-links can be efficiently repaired but only if the cell contains at least two homologous chromosomes and a gene essential for recombination. Consequently, repair of DNA double-strand breaks and cross-links in these organisms depends on recombination. The above evidence supports the view that double-strand damages are prevalent in nature, have a significant effect on fitness, and can only be repaired by recombination between homologous chromosomes.

Since passing on genetically intact gametes is an essential component of fitness, the selective advantage of overcoming DNA damage is clear. As discussed above, specific types of double-strand damage are probably common enough in nature to reduce fitness if left unrepaired.

For higher organisms it is likely that recombinational repair is primarily associated with meiosis, although a low level of recombination, and hence probably also recombinational repair, occurs during mitosis (*21*). Therefore, in vegetative reproduction and apomixis, recombinational repair is largely unavailable, leaving somatic selection as the main strategy for dealing with double-strand damage. A population of cells could survive so long as there are resources to support division of undamaged cells and the number of double-strand damages per genome, m, is sufficiently small to prevent the frequency of undamaged cells, e^{-m}, from being small. Because vegeta-

tive reproduction commits many somatic cells to the production of each offspring, it is not as vulnerable to genome damage as is apomixis.

There are two potential sources for the two chromosomes needed for recombinational repair: (i) in a closed-system strategy for repair, they come from the same parent; (ii) in an open-system strategy for repair, they come from different individuals. If the only selective advantage of sexual reproduction is the recombinational repair of genetic damage, the most effective strategy would be a closed system such as self-fertilization or automixis (uniparental production of eggs through ordinary meiosis followed by some internal process for restoring diploidy), which would avoid the major costs of sex, namely, the cost of mating (*22, 23*), the cost of males (*2*), high recombinational load (*24*), and the lower genetic relatedness between parent and offspring (*25*). We need, then, to explain why the common recombinational repair strategy is outcrossing sex, an open system.

We have argued (*6*) that the costs of maintaining redundant genetic information (that is, extra chromosomes) in simple protocells was responsible for the origin of outcrossing between haploid protocells. However, as organisms became more complex, the relative costs of maintaining extra chromosomes in each cell decreased. Diploidy probably emerged as the predominant stage of the life cycle because of complementation, the masking of recessive deleterious mutations (*26*). We now argue that complementation is also the key factor in the maintenance of outcrossing in reproductive systems which have recombination (*27*).

Mutations are a result of replication errors. Since improvements in replication accuracy have costs (*28*), it is likely that there are cost-effectiveness barriers to indefinite improvement in accuracy. Thus a finite spontaneous mutation rate is probably intrinsic. As noted by Felsenstein (*29*), deleterious mutations have a much greater effect on fitness in the short run than do favorable mutations because of their much higher rate of occurrence. Haldane (*30*) argued that at equilibrium deleterious mutations are removed from the population at the same rate as they arise. He showed that the average equilibrium survivorship is a function only of u, the rate of mutation per haploid complement, and is not affected by how deleterious the mutations are.

We have generalized Haldane's argument, and have found in multilocus models that recessives give an equilibrium survivorship of e^{-u} for the diploid reproductive systems listed in Table 1 (*31*). This survivorship applies also to haploids, but they present a special challenge to our theory, which is discussed later. Since the effect of u on survivorship is the same for all reproductive systems, we do not expect different reproductive systems to have different u. Consequently, in our arguments below we fix u and consider the effect of changing the reproductive system on the expression of mutant alleles.

Table 1. Classification of reproductive systems (all systems refer to diploids).

Reproductive system	Masking ability (lethals at equilibrium)	Recombinational repair	Source of homologous chromosome
Automixis	Low ($\sim 2u$)	Yes	Self
Selfing	Low ($\sim 2u$)	Yes	Self
Outcrossing*	Intermediate (~ 1 to $\sqrt{N}$)†	Yes	Another individual
Panmixia	Intermediate ($\sim \sqrt{N}$)	Yes	Another individual
Endomitosis	High ($\sim N$)	Limited‡	Self
Apomixis	High ($\sim N$)	No	Not applicable
Vegetative	High ($\sim N$)	No	Not applicable

*With some mating between relatives as occurs in nature. †N denotes the number of functional genes per genome, which, in higher organisms, is approximately 40,000. ‡See text.

Data from *Drosophila* (*32*) suggest $u = 0.3$ for lethal and nonlethal mutants combined. This yields a survivorship $e^{-u} \simeq 0.7$. The mutational load is not so large as to be overwhelming. If it were much smaller, we expect that selection would generally favor an increase in the number of gene functions, N. Insofar as u should be proportional to N, selection for increased N would increase u. We therefore take 0.3 as an approximate general value for u.

The second column of Table 1 gives the number of accumulated lethals at equilibrium. There are substantial differences among reproductive systems in the number of accumulated recessive mutations. Those systems that are effective at masking recessives accumulate many. Those which are ineffective at masking accumulate few. While mutational load makes all systems equally competitive at equilibrium, it creates a transient selective advantage to moving downward on Table 1 and a transient selective disadvantage to moving upward (*33*). Consider, for example, a population fixed for selfing. Although it will have accumulated few recessives (Table 1), new ones occur each generation at a frequency u. In an equilibrium population of selfers with survivorship e^{-u}, a new mutant outcrosser will mask these recessives in its offspring, and it therefore has a survivorship of unity. The outcrossers must, however, pay the costs described above. Let C be the reduced fitness of outcrosses due to these costs ($C > 1$), so that the overall fitness of outcrossers is $1/C$. Consequently, if $C < e^{u}$, then a gene for outcrossing will expand. Using the value $u = 0.3$ given above, $e^{u} = 1.4$, which allows for fairly large costs of sex.

When outcrossing becomes fixed, the number of recessive mutant alleles increases, and eventually the outcrosser has an equilibrium fitness that is reduced both by the costs of outcrossing and the mutational load, that is, the net effect of the transition to outcrossing is a reduction of individual fitness. However, the reverse transition to selfing or inbreeding is now inhibited by the unmasking of the many accumulated recessive mutations. Passage from outcrossing to selfing will only be advantageous when the costs of outcrossing become very large. Hence, among the first four cases in Table 1, which are those with complete recombinational repair, outcrossing is favored. An intermediate level of outcrossing may be preferred over panmixia because of the need to preserve coadapted gene complexes (*24*).

The transient advantages associated with complementation favor asexual systems in which the maternal genome is passed down intact from mother to daughter, since this gives maximal masking of deleterious recessives. However, we now need to consider the effect of recombinational repair which is listed in column 3 of Table 1. In apomixis, meiosis is suppressed, and there is a single mitotic maturation division. This largely abandons recombinational repair of double-strand damage and is thus a costly strategy. In endomitosis there is a premeiotic doubling of the chromosomes, followed by meiosis (*34*). If pairing, and presumably recombination, should occur between nonsister homologs, in either apomixis or endomitosis, the transient advantage is lost because of the immediate expression of accumulated deleterious recessives. Since recombination occurs only between sister homologues, the recombinational repair allowed by endomitosis is equivalent to normal meiosis only with respect to damages that occur after the first premeiotic replica-

tion. Double-strand damage occurring before the first premeiotic replication cannot be repaired, since all the four chromatids that pair during meiosis are derived from a single premeiotic chromosome and would be damaged at the same site (no redundant template).

In conclusion, shifting from outcrossing to any of the reproductive systems considered reduces fitness. This reduction is transient for selfing or automixis and permanent for apomixis and endomitosis, since the latter have reduced capacity for recombinational repair. Lynch (*35*) has discussed costs of parthenogenesis and has summarized the substantial literature indicating that parthenogens most often have lower reproductive rates than their sexual relatives, often less than 50 percent. This lower rate frequently stems from poor hatching success of eggs resulting from developmental abnormalities, and in some cases reduced egg production. The magnitude of the decrease in fecundity seems to be greater for newly arisen parthenogens than for established ones. This reduction in fecundity plus the other costs imply that parthenogens should be found in nature in those situations in which the costs of outcrossing are large. In our review (*22*) we considered the cost of mating, and find that this result is a valid generalization for parthenogenesis in nature.

Our arguments concerning the role of the masking of mutations in maintaining outcrossing apply to those gene functions that are expressed only in the diploid stage of the sexual life cycle and do not apply to genes expressed during the haploid stage. Thus there is a problem in explaining the existence of outcrossing multicellular haploids. For unicellular, as opposed to multicellular, haploids outcrossing is needed simply to bring homologous chromosomes together for recombinational repair. However, in multicellular haploid organisms deleterious mutations expressed during the haploid stage are removed by natural selection. Thus the advantages of outcrossing may be small, and there should be little barrier to switching to or maintaining a closed repair strategy. A variant on this argument concerns haplodiploid insects. Recessives are weeded out in the male line, and are largely masked in the female line independently of outcrossing. At first glance, these cases suggest that the primary function of outcrossing is not complementation.

To counter the above reasoning we point out that, for haploid organisms, there are key gene functions that are only expressed during the diploid stage, such as the genes responsible for meiosis. For simpler organisms, such as fungi, meiosis is one of their more complicated functions, since the multicellular haploid stage, although dominant in size, often has a modular construction with little differentiation. Furthermore, in mushrooms and bracket fungi, for example, the conspicuous structures are often dikaryons (heterokaryons in most other fungi) containing nuclei from different parents (*36*). These are functionally diploid (or polyploid) since they are capable of masking deleterious recessive alleles. This masking gives a selective advantage to outcrossing.

In haplodiploid insects, we expect a smaller, but significant, fraction of deleterious mutations to be expressed only in the diploid phase (females), and not in the haploid phase (males). Consequently, the repair hypothesis predicts in accord with data (*37*) that haplodiploid insects should be more inbred, since there are fewer benefits to complementation.

Premeiotic replication, a general feature of meiosis leading to four homologous chromatids which then recombine in pairs, has a definite function under the repair hypothesis. Studies in *Escherichia coli* show that replication of DNA with single-strand damages leads to gaps in the new strands opposite the damages (*8*), and that these gaps promote recombinational repair (*38*). Such gaps have a molecular structure that is independent of the molecular structure of the original damage and can serve as a universal initiator of recombinational repair. This avoids the need of evolving a specific enzyme to recognize each specific kind of single-strand damage of which there are probably many. This would be especially adaptive if naturally occurring damages are a mixture of types with many types represented in low frequency. Premeiotic replication produces sister chromosomes which have no apparent role under a hypothesis in which the sole function of meiosis is to produce variation. In general, by considering the molecular mechanisms of recombination and the details of meiosis it is possible to test the variation and repair hypotheses (*39*).

In summary, there are two intrinsic problems in replicating genetic information: DNA damage and mutation. We have argued that the two principal features of sex, recombination and outcrossing, are maintained, respectively, by the advantage of repairing damage and masking mutations. Variation is produced as a by-product. In focusing on the role of genetic damage and mutation in the maintenance of sex, we do not mean to suggest that the production of variation is an unimportant consequence of sex. Infrequent beneficial variants generated by recombination undoubtedly promote long-term evolutionary success, just as infrequent beneficial mutations do (*40*). We believe that the tendency toward randomization of genetic information that occurs with recombination would, under general conditions, have a negative effect on fitness in the short run, just as do mutations. This variation, or recombinational load, probably generally contributes to the immediate cost of sex, and any immediate advantage of creating variation is probably constrained to special cases.

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Benzodiazepine Receptor–Mediated Chemotaxis of Human Monocytes

Abstract. *Benzodiazepines, which are widely prescribed for their antianxiety effects, are shown to be potent stimulators of human monocyte chemotaxis. The chemotactic effects of benzodiazepine receptor agonists were blocked by the peripheral benzodiazepine receptor antagonist PK-11195, suggesting that these effects are mediated by the peripheral-type benzodiazepine receptor. Diazepam was also active in inducing chemotaxis. Binding studies on purified monocytes revealed high-affinity peripheral benzodiazepine receptors, and the displacement potencies of various benzodiazepines correlated with their relative potencies in mediating chemotaxis. The demonstration of functional benzodiazepine receptors on human monocytes, together with recent evidence of receptor-mediated monocyte chemotaxis by other psychoactive peptides (such as opiate peptides), suggests a biochemical substrate for psychosomatic communication.*

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The benzodiazepines are among the most widely used of all drugs (*1*) and are commonly prescribed for their anxiolytic, hypnotic, and anticonvulsant properties (*2*). These behavioral and neurological effects of benzodiazepines are mediated through high-affinity, stereoselective receptors that are almost exclusively localized to the central nervous system (CNS) (*2, 3*). In addition to identifying CNS benzodiazepine receptors, radioreceptor assays have revealed another class of benzodiazepine recognition sites broadly distributed in many non-neuronal tissues, including kidney, heart, platelets, mast cells, adrenals, as well as several cultured cell lines (*3, 4*). These binding sites have provisionally been defined as "peripheral" benzodiazepine receptors since structure-activity studies have demonstrated marked differences between them and CNS or "central" benzodiazepine receptors. For example, Ro5-4864 (4-chlorodiazepam) is one of the most potent ligands for the peripheral benzodiazepine receptor, yet it is virtually inactive at the central benzodiazepine receptor. In contrast, clonazepam, one of the most potent behaviorally active benzodiazepines, binds with high affinity to the central benzodiazepine receptor but is essentially inactive at the peripheral receptor. Diazepam (Valium), the most commonly prescribed benzodiazepine, binds with relatively high affinity to both the peripheral and central receptors.

Despite the many reports characterizing benzodiazepine receptors on various tissues, the physiological function or functions of the peripheral receptor are largely unknown, although several effects have been observed in vitro. Benzodiazepines have been reported to inhibit mitogenesis in Swiss mouse 3T3 fibroblasts (*5*), to promote differentiation of Friend erythroleukemia cells (*5, 6*), to induce melanogenesis in B16/C3 melanoma cells (*7*), to inhibit the growth of thymoma cells in vitro (*8*), and to decrease cardiac muscle contractility (*9*). These effects, in some cases (*7–9*), have correlated with the presence of peripheral benzodiazepine receptors on these tissues.

We examined the effects of benzodiazepines on the chemotaxis of human monocytes. Monocytes or their noncirculating counterparts, macrophages, are a heterogeneous population of cells that subserve key roles in immune system function and figure prominently in many aspects of tissue repair and restructuring, inflammation, and antineoplastic defense (*10*). Agents that are chemotactic for monocytes include bacterial products, peptide fragments derived from complement or clotting activation, factors from other immune system cells, as well as proteolytic fragments of elastin, fibronectin, and collagen, leukotrienes or other prostaglandin metabolites, and numerous small peptides of diverse origin (*11*). Recently, neuropeptides such as

Table 1. Checkerboard analysis for chemotactic response of monocytes to Ro5-4864, a benzodiazepine attractant. The data represent the number of migrating cells per field ± standard error of the mean (n = 3) as described (*13*). Various concentrations of Ro5-4864 as indicated were placed in both upper and lower chemotaxis chambers.

Concentration in lower chamber (M)	Concentration in upper chamber (M)			
	0	10^{-13}	10^{-12}	10^{-11}
0	40 ± 8	32 ± 5	39 ± 7	31 ± 5
10^{-13}	90 ± 6	56 ± 6	60 ± 8	66 ± 4
10^{-12}	126 ± 5	77 ± 6	66 ± 11	61 ± 4
10^{-11}	175 ± 5	85 ± 8	71 ± 7	53 ± 5