TARGET REVIEW

A pluralist approach to sex and recombination

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Abstract

One of the greatest challenges for evolutionary biology is explaining the widespread occurrence of sexual reproduction and the associated process of genetic recombination. A large number of theories have been developed that provide a sufficient short-term advantage for sex to offset its two-fold cost. These theories can be broadly classified into environmental (or ecological) and mutation-based models. Traditionally, the different theories have been viewed as competing, and empirical work has attempted to distinguish between them. Here we highlight the advantages that may be gained from considering that multiple mechanisms (environmental and mutational) may be at work, and that interactions between the theories may be very important.

Introduction

One of the greatest challenges for evolutionary biology is explaining the widespread occurrence of sexual reproduction, and the associated process of genetic recombination (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Stearns, 1987; Michod & Levin, 1988; Hurst & Peck, 1996). Asexual females can potentially produce twice as many daughters as sexual females, so that the ratio of asexual to sexual females should initially double each generation, resulting in a 'two-fold cost of sex'. In addition, recombination breaks up favourable gene combinations that have increased in frequency under the action of natural selection. Given these costs, we would expect natural selection to favour asexual reproduction in wild populations. However, it generally does not: sexual reproduction is widespread throughout the animal and plant kingdoms.

In order to solve this apparent paradox, a considerable number (>20) of theoretical models have been developed which purport to show conditions under which there is a sufficiently large short-term advantage for sex to offset a two-fold cost (Kondrashov, 1993). In this paper we are concerned primarily with models that provide a deterministic advantage to sex and recombination through the production of genetically variable offspring (Weismann, 1889). This can increase the efficiency of selection, and hence accelerate the increase in mean fitness (Kondrashov, 1993; Barton, 1995; Feldman *et al.*, 1997). These models can be broadly classified into two groups: (1) environmental (or ecological) models and (2) mutation-based models (Kondrashov, 1988; Maynard Smith, 1988b).

Environmental models suggest that sex accelerates adaptation to a changing environment by creating new gene combinations (Bell, 1982). The biological basis of such varying selection pressures may involve a variety of biotic or abiotic mechanisms (Haldane, 1932; Bell, 1982). Currently the most popular environmental hypothesis, the Red Queen, states that sex provides an advantage in biotic interactions (Bell, 1982; Bell & Maynard Smith, 1987). Usually, parasites are assumed to provide the antagonistic driving force in this coevolutionary dance (Jaenike, 1978; Bremermann, 1980; Hamilton, 1980, 1993; Seger & Hamilton, 1988; Hamilton *et al.*, 1990), though host immune responses may also do so (Gemmill *et al.*, 1997). The 'dance' results from time-lagged selection by coevolving parasites against common host genotypes, leading to sustained oscillations in host and parasite gene frequencies (Hutson & Law, 1981; Bell, 1982).

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The population-genetic basis of environmental models such as the Red Queen are still not entirely clear (Barton, 1995; Otto & Michalakis, 1998). Two general types of deterministic population genetic model have been developed, both of which rely on epistasis (nonadditive genetic interactions) between beneficial alleles. These environmental models assume that either: (1) selection (epistasis) fluctuates, so as to sometimes favour one gene combination, and sometimes another (Sturtevant & Mather, 1938; Barton, 1995), or (2) there is directional selection (or stabilizing selection with a moving optimum) on a quantitative trait, and weak diminishing returns epistasis between favourable alleles (the fitness increase due to two favourable alleles is less than the product of their individual effects) (Maynard Smith, 1988a; Crow, 1992; Charlesworth, 1993; Barton, 1995; Kondrashov & Yampolsky, 1996). Importantly, theory suggests that fluctuating epistasis can only provide an advantage to sex and recombination when the fluctuations occur over just the right time-scale, making it unlikely to apply in response to physical variation in the environment (Charlesworth, 1976; Maynard Smith, 1978; Sasaki & Iwasa, 1987; Barton, 1995). However, it has been argued that biotic interactions such as host–parasite coevolution might tend to produce fluctuations on the right time-scale (Nee, 1989). It is not clear to what extent the simulation models of host–parasite coevolution (e.g. Hamilton, 1980, 1993; Bell & Maynard Smith, 1987; Hamilton *et al*., 1990; Howard & Lively, 1994) provide an advantage to sex and recombination because of fluctuating epistasis, directional selection, or another process such as allowing allele frequencies to change more rapidly or preventing the stochastic loss of temporarily bad alleles (Barton, 1995; Otto & Michalakis, 1998; but see Peters & Lively, in press).

Mutation-based models (the mutational deterministic hypothesis) suggest that sexual reproduction is advantageous because it allows individuals to eliminate deleterious mutations more efficiently (Kondrashov, 1988). The mutational deterministic hypothesis requires that each deleterious mutation leads to a greater decrease in log fitness than the previous mutation (synergistic epistasis between deleterious mutations) (Kondrashov, 1982). When this is the case sexual reproduction increases the variance in the number of deleterious mutations that will be carried by offspring. The low fitness of individuals carrying above average numbers of deleterious mutations will then lead to a larger number of deleterious mutations being eliminated (Kimura & Maruyama, 1966; Crow, 1970). If the mutation rate per genome per generation is sufficiently high, then this process can fully compensate for the two-fold cost of sex (Kondrashov, 1982, 1984; Charlesworth, 1990). In contrast to environmental models, the mutation-based models are able to work in an unchanging environment.

Unfortunately, data capable of discriminating between these models are almost nonexistent (Bell, 1985; Kondrashov, 1993, 1994b; Hurst & Peck, 1996). The majority of empirical work in this area has been correlational and focused on the ecological hypotheses. In these studies the occurrence of sex or rate of recombination is examined, either across or within species, with respect to key ecological variables (e.g. Glesener & Tilman, 1978; Bell, 1982; Burt & Bell, 1987; Lively, 1987, 1992; Koella, 1993; Schrag *et al*., 1994; Jokela & Lively, 1995). As such, these studies have taken a strong inference approach (Platt, 1964), with an emphasis on finding predictions that discriminate among different hypotheses. In our view these studies have played an important role in the rejection of some of the environmental models (e.g. the lottery model of Williams, 1975; Young, 1981). However, because these studies are correlational, they are open to multiple explanations, and *post hoc* scenarios can be developed which allow the results to be explained by environmental or mutation-based models (Charlesworth, 1987, 1990; Hamilton *et al*., 1990; Kondrashov, 1993; Hurst & Peck, 1996). With some ingenuity almost any hypothesis can explain any ecological correlation, and in a later section we shall discuss a particular example. It would at present seem that ecological correlations may be incapable of convincingly discriminating between the currently favoured models. These correlations are nonetheless important because any realistic model of sex should be able to account for the well-known patterns of sex and recombination.

In this commentary, we consider the advantages that may be gained from using a pluralistic framework to consider and test models of sexual reproduction. By this we mean that the different mechanisms may act simultaneously, that their relative importance may differ between species, and that they may interact synergistically in a number of ways. In particular, we aim to emphasize how environmental and mutational theories can complement each other, and cover each other's weaknesses. This is not the approach that has been taken in the majority of previous papers, and we believe there are a number of reasons for this: (1) the different hypotheses reflect different approaches (ecology vs. population genetics) and have been viewed as competing; (2) previous empirical work has concentrated on finding predictions that discriminate among the theories; and (3) at a first glance, pluralism seems to be a cop out – indeed, it has been specifically criticised for impeding useful empirical work (Kondrashov, 1993). Our purpose here is to argue that a pluralistic approach offers a useful framework with which to consider the maintenance of sexual reproduction, because it emphasizes the most useful empirical work and the importance of interactions between the theories.

The advantages of a pluralistic approach

There are at least four advantages to be gained from taking a pluralistic approach. First, it is entirely plausible

that multiple mechanisms may be providing an advantage to sex, and/or that the different mechanisms may be important in different species or environments. Indeed, many (most?) traits that apparently arose once have been put to several different uses, or lost (e.g. gill arches, mammalian forelimbs). The factors maintaining sexual reproduction may be different from those which led to its evolution. Multiple selection pressures are the norm in evolutionary biology: for instance, we do not expect, nor do we find, that the same selection pressure is responsible for female-biased sex ratios in widely divergent taxa (Charnov, 1982). Widespread traits are often not adaptations with just a single function. In the discussion we consider the possibility that different mechanisms may be working even at different levels within the same species (e.g. the maintenance of sex and clonal diversity).

Second, more than one mechanism may be required to fully balance the two-fold cost of sex. Acting alone, each of the various theories requires extreme, and possibly unreasonable, assumptions in order to be able to fully explain the maintenance of sex. The mutation-accumulation theory requires that mutation rates are high (at least >1 per genome per generation; Kondrashov, 1988; Charlesworth, 1990; Redfield, 1994), that each deleterious mutation leads to a greater decrease in log fitness than the previous mutation (synergistic epistasis; Kondrashov, 1982), that the variation between loci in the extent of epistasis is not too great (Otto & Feldman, 1997) and that population sizes are large (Kondrashov, 1982; Charlesworth, 1990; Howard, 1994). The most popular environmental model, the Red Queen, requires that parasites have severe fitness effects on their hosts (May & Anderson, 1983; Howard & Lively, 1994) or that only the most healthy hosts are able to reproduce (termed rank-order truncation selection; Hamilton *et al.*, 1990). However, even if a model is not fully able to explain the two-fold cost of sex, it may play an important role. The pluralist approach emphasizes that it is just as important to determine the magnitude of the advantage of sex due to a particular mechanism, even if it does not balance the two-fold cost. Indeed, in some cases, such as when there is a cost to finding mates, the cost of sex may be substantially greater (or less) than two-fold (Bierzychudek, 1987; Jokela *et al.*, 1997). In a later section we discuss a specific example, showing how current data from plants are consistent with the importance of more than one mechanism.

Third, a pluralist approach shifts the emphasis of empirical work from the search for a discriminating prediction to parameter estimation. Given the pluralist assumption, the major task is to estimate the relative importance of the various mechanisms. This can only be done by testing assumptions of the different models, and estimating relevant parameters. Crucially, this approach does not exclude the possible conclusion that only one mechanism is responsible for the maintenance of sex.

Finally, different mechanisms may interact not only simultaneously, but synergistically (Manning & Thompson, 1984; Howard & Lively, 1994, 1998; Lively & Howard, 1994; Peck, 1994). This possibility can only be considered from a pluralistic framework. Given such interactions, the maintenance of sexual reproduction can be explained with much more reasonable assumptions than each of the theories acting alone (Howard & Lively, 1994). This possibility has only recently been seriously considered and we believe that it may be very important. In the next section we discuss some possibilities in detail.

An important point to note here is that we are not suggesting that all possible mechanisms and their interactions should be considered equally – over 20 theories have been proposed to explain sexual reproduction (Kondrashov, 1993). Many mechanisms require restrictive assumptions such as small populations or certain population structures, and so are likely to be of limited applicability (Maynard Smith, 1988b; Kondrashov, 1993). Others, such as the genetic repair hypothesis (reviewed by Bernstein *et al.*, 1987), have been argued against on both theoretical and empirical grounds (Maynard Smith, 1988b; Charlesworth, 1989; Szathmary & Kover, 1991; Mongold, 1992; Kondrashov, 1993). Our emphasis is on the types of deterministic mechanisms (environmental and mutation-based) that are able to work even in large panmictic populations, are most likely to be widely applicable and are generally believed on theoretical and empirical grounds to be the most feasible. In particular, we discuss how environmental and mutation-based mechanisms are particularly suited to interacting synergistically, and how they can cover each other's weaknesses.

The importance of interactions between theories

Interactions between mechanisms may greatly increase the advantage of sexual reproduction. In this section we present some possibilities, whilst noting that this is a largely unexplored area, both theoretically and empirically. In particular, we will show how environmental and mutational mechanisms may complement each other, covering each other's weaknesses. Such interactions suggest that the combined effects of the two mechanisms are likely to be greater than the sum of their parts, which would relax greatly the conditions under which sex is favoured. When we discuss interactions between environmental and mutational mechanisms we shall do so in terms of the parasite (or Red Queen) hypothesis for sex. We do this because it is the most favoured environmental hypothesis (Hamilton *et al.*, 1990; Ladle, 1992), and because previous theoretical work looking at interactions has been based upon this (Howard & Lively, 1994, 1998). However, most of the scenarios which we consider would also apply to other environmental hypotheses, such as other forms of fluctuating selection, and there are other

possible interactions (e.g. Manning & Thompson, 1984; Peck, 1994).

Mutations aid the Red Queen

As we have pointed out above, one problem of the Red Queen hypothesis is that it requires that parasites have severe fitness effects on their hosts (May & Anderson, 1983; Howard & Lively, 1994) or that only the most healthy hosts are able to reproduce (Hamilton *et al*., 1990). Another problem is that it does not select for sex *per se*, but for diversity, however it is generated (Lively & Howard, 1994). Consequently, clonal diversity is able to erode any advantage that sexual reproduction gains through the production of variable progeny. Clonal diversity could arise from repeated mutation of sexual individuals to asexual reproduction, or through mutation in asexual individuals.

One possible solution to these two problems is that mutation accumulation also occurs. Howard & Lively (1994) constructed a simulation model which allowed both host–parasite interactions and mutation accumulation to occur. Their model assumed that the fitness consequences of deleterious mutations were multiplicative, and so the Mutational Deterministic process was not operating. Instead, mutation accumulation occurred through Muller's ratchet, the irreversible decrease in fitness that can occur through the stochastic accumulation of deleterious mutations in finite asexual populations (Muller, 1964; Lynch *et al*., 1993). Acting alone, Muller's ratchet operates too slowly to provide a significant short-term advantage to sex (Maynard Smith, 1978). Howard & Lively (1994) showed that moderate effects of parasites combined with reasonable rates of mutation could more than balance the two-fold cost of sex. In the short term, parasites prevented the fixation of clones and the elimination of sex. In the long term, mutation accumulation led to the eventual extinction of clones. The accumulation of mutations in clonal lineages is enormously aided by parasite-driven oscillations, because the rate of mutation accumulation is enhanced during periods in which the clone is driven to low numbers by the parasite.

The model has recently been extended to allow for synergistic epistasis between deleterious mutations (Howard & Lively, 1998). The results showed that adding synergistic epistasis increased the advantage of sex at relatively high mutation rates ($U = 1.5$; where U is the genomic deleterious mutation rate per generation), made negligible difference at intermediate mutation rates ($U = 1.0$) and decreased the advantage of sex at relatively low mutation rates ($U = 0.5$). These results can be explained by the two consequences of adding synergistic epistasis: (1) by increasing the fitness cost of deleterious mutations it slows down and can even halt Muller's ratchet (Kondrashov, 1994a), and (2) it provides a purely deterministic advantage to sexual reproduction (Mutational Deterministic hypothesis). The first of these consequences decreases the advantage of sexual reproduction, while the second increases it. The relative importance of these two mechanisms will be determined by the mutation rate. At relatively low mutation rates (e.g. $U = 0.5$) the deterministic advantage will be small and so the most important consequence will be the slowing down of Muller's ratchet. In contrast, at high mutation rates (e.g. $U = 1.5$) the deterministic advantage will become large and so have a much greater effect than the slowing down of Muller's ratchet.

It should, however, be noted that the results of the model are likely to depend upon the assumption of a single class of mutations. In reality the fitness consequences of deleterious mutations are likely to vary (Keightley, 1994, 1996; Elena & Lenski, 1997; Keightley & Ohnishi, 1998). If, instead, a distribution of mutation effects is used, then Muller's ratchet continues to operate even with synergistic epistasis (Butcher, 1995). In this case we might expect synergistic epistasis to increase the advantage of sex over the whole range of mutation rates.

Perhaps ironically, this reasoning suggests that mutation accumulation is best able to aid the Red Queen hypothesis, and therefore the Red Queen works best when there is synergistic epistasis between deleterious mutations. We have argued above that, given a distribution of mutation effects, Howard & Lively's (1998) model would generally provide the greatest advantage to sex when there is synergistic epistasis between deleterious mutations. However, this model assumed a finite population that was small enough (10^3) for Muller's ratchet to operate. At very large population sizes the importance of Muller's ratchet will decrease, and so mutation accumulation will only help provide an advantage deterministically. This will only occur if deleterious mutations exhibit synergistic epistasis.

The Red Queen aids the Mutational Deterministic hypothesis

The Mutational Deterministic hypothesis has been shown to require high rates of deleterious mutation, at least greater than approximately 1 per genome per generation (Kondrashov, 1988; Charlesworth, 1990). However, most of the relevant models have assumed infinite populations and considered populations at equilibrium in mutation-selection balance, ignoring the dynamics of reaching this situation. Relaxing these assumptions can cause problems for the hypothesis. In particular, the initial number of deleterious mutations in a new asexual lineage is always likely to be lower than the equilibrium number, and will occasionally be much lower (Charlesworth, 1990). This becomes important at finite population sizes because an asexual lineage may be able to replace the resident sexual population before it accumulates enough deleterious mutations to balance the cost of sex (Kondrashov, 1982; Charlesworth, 1990; Howard, 1994). This possibility is further increased if

stochasticity is introduced in the rate at which populations grow and accumulate mutations (Howard, 1994). A consequence of these factors is that greater genomic deleterious mutation rates are required to be able to balance the two-fold cost of sex. For example, with moderate synergistic epistasis between deleterious mutations, a deleterious mutation rate of 2 is required for a population of size 10^4; a greater rate is required for smaller populations (Howard, 1994).

An ecological mechanism, such as the Red Queen, provides a number of possible solutions to these problems. First, it produces frequency-dependent selection and so slows down the spread of asexual clones, allowing more time for mutation-selection balance to be reached (Howard & Lively, 1994). Second, it reduces the fitness advantage of asexuals and so reduces the number of deleterious mutations required to reduce the fitness of the asexuals below that of sexuals. Third, it may speed up the rate at which an asexual lineage accumulates mutations, leading to a faster decline in fitness, and allowing mutation-selection balance to be reached more quickly. This will happen because frequency-dependent selection will drive a clonal lineage through population cycles, and the accumulation of deleterious mutations will be increased by the stochastic process of Muller's ratchet at the low points of these cycles (Howard & Lively, 1994, 1998). Fourth, by increasing the stochastic accumulation of deleterious mutations, mutation-selection balance may be reached at a higher number of deleterious mutations, and so a lower fitness. This is analogous to the fact that the equilibrium number of deleterious mutations in an asexual lineage increases with the number of deleterious mutations in the individual in which the lineage arose (Kimura & Maruyama, 1966; Charlesworth, 1990).

Moreover, a combination of both hypotheses may resolve another important challenge to the Mutational Deterministic hypothesis: explaining the ecological correlates of sex. The Mutational Deterministic hypothesis does not readily explain why, for example, there is more sex where parasites are more common (e.g. Lively, 1987, 1992; Schrag *et al*., 1994; Jokela & Lively, 1995). With *post hoc* modifications it may be able to address the correlates, perhaps by arguing that parasites are the factor that causes truncation selection against high mutation loads, but such patterns do not flow as straightforward predictions from the model. This is, however, a straightforward prediction of Red Queen models. As far as we are aware, the parasite models predict the majority of within- and between-host patterns of sexuality.

Synergism at the empirical level

Different mechanisms may also interact in their direct fitness consequences. For example, individuals with high mutational loads might be considerably sicker when infected with coevolved parasites than individuals with low mutational loads (Lively & Howard, 1994). Although it has yet to be formally modelled, such synergistic interactions are likely to provide a substantial advantage to sexual reproduction. In addition, this point illustrates how the different underlying mechanisms may interact to increase the extent of truncation selection. This is important because both the Mutational Deterministic and the Red Queen models work best under truncation selection, against deleterious mutations and parasite infection, respectively. Parasites may increase the form of truncation selection against mutations, and mutations may increase the extent of truncation selection against individuals infected by coevolved parasites.

Empirical evidence for the pluralist approach

Do we have any empirical evidence for more than one mechanism acting in a species? It seems reasonable that more than one mechanism is likely to be acting in a species: parasites are prevalent; they can have large effects on the fitness and population dynamics of their hosts; and host–parasite coevolution undoubtedly does take place (e.g. Toft *et al*., 1991; Grenfell & Dobson, 1995; Clayton & Moore, 1997; Dybdahl & Lively, 1998). In addition, mutations are ubiquitous, and are far more likely to be deleterious than they are beneficial (Crow & Simmons, 1983; Keightley & Ohnishi, 1998; see also Gillespie, 1991). However, there are few data measuring the importance of the different mechanisms in any species, let alone quantifying the importance of the different mechanisms in the same species.

The possible importance of more than one mechanism working simultaneously can be demonstrated, and speculatively quantified, by available data on plants. Kelley *et al*. (1988) and Kelley (1994) studied the perennial grass *Anthoxanthum odouratum* and demonstrated that the fitness of sexual progeny was 1.55 times greater than that of asexual progeny. This immediate fitness difference cannot be explained by the Mutational Deterministic hypothesis (Charlesworth, 1990) and so is likely to represent an environmental mechanism. Evidence suggests a role of viral pathogens transmitted by aphids (Kelley, 1993, 1994). Importantly, Kelley (1993) also determined whether parental clones were infected at the beginning of the experiment, and so showed that asexual progeny were more likely to acquire new infections. In order to balance a two-fold cost of sex, a further $2/1.55 = 1.29$ advantage to sexually produced offspring is required.

This remaining advantage can be supplied by the Mutational Deterministic hypothesis, given existing estimates of the genomic deleterious mutation rate in plants. Considering only the effects of deleterious mutations, the equilibrium fitness of an asexual population/lineage is

independent of the form of selection and equal to e^{-U}, where U is the genomic deleterious mutation rate per generation (Kimura & Maruyama, 1966). Given intermediate levels of synergistic epistasis between deleterious mutations, the equilibrium fitness of a sexual population is approximately $e^{-U/2}$ (Charlesworth, 1990). Consequently, the advantage to sexually produced offspring supplied by the Mutational Deterministic hypothesis is equal to $e^{-U/2}/e^{-U} = e^{U/2}$ (Fig. 1), and the deleterious mutation rate required to provide a fitness advantage a is equal to $2\ln(a)$. An advantage to sexual reproduction equal to 1.29 is therefore supplied by a deleterious mutation rate (U) of 0.51. Indirect estimates of the deleterious mutation rate from highly inbred plant populations suggest values in the range of 0.5–1.0 (Charlesworth *et al*., 1990, 1994; Johnston & Schoen, 1995), and so provide the required value. Other empirical investigations of possible multiple mechanisms are provided by McVean & Hurst (1997) and Zeyl & Bell (1997).

This section illustrates how, in practice, it may be easier to accept the pluralist approach with empirical data than to reject the theoretically simpler models such as the Mutational Deterministic or Red Queen hypotheses. This is particularly true because of the large confidence limits that must be placed on estimates of parameters such as the mutation rate, a subject that we shall return to in the discussion. Given this, as well as the additional insights that the pluralist approach provides (e.g. synergistic interactions between mechanisms and different mechanisms working at different levels), we believe that it does more than just make things more complicated in defiance of Occam's razor.

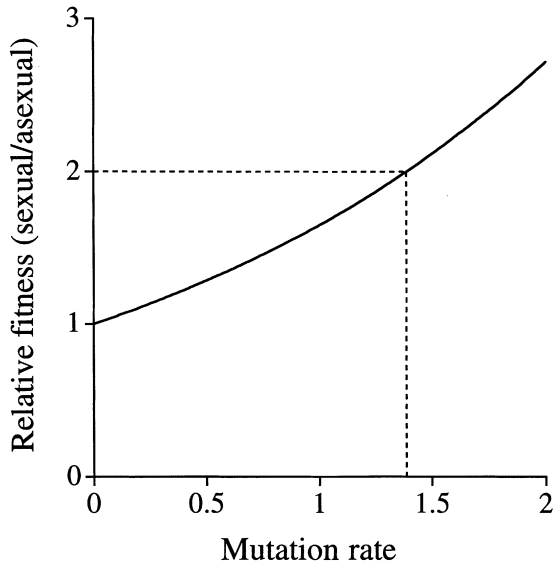

Fig. 1 The fitness advantage provided for sexual reproduction by the mutational deterministic hypothesis. Plotted is the relative fitness (at equilibrium) of a sexual population/lineage (with no cost of sex) divided by that of an asexual population/lineage against the deleterious mutation rate per genome per generation. The dashed lines show at what point this fitness advantage is able to account for a 'two-fold' cost of sex. Moderate synergistic epistasis between deleterious mutations is assumed (Charlesworth, 1990).

Discussion

Is a pluralistic approach a cop out? We believe that it is the most logically defensible approach, and have suggested a number of reasons why it is also likely to be the most useful approach. It may be required to explain the maintenance of sex, and it shifts the emphasis of empirical work away from the search for discriminating predictions to parameter estimation. Moreover, it emphasizes that the environmental and mutational mechanisms may interact synergistically in a number of ways and cover each other's potential weaknesses.

We have also suggested that, on a broader level, different mechanisms may work at different levels on related questions. For example, there are several studies that show apparent partitioning of resources among asexual clones (e.g. Vrijenhoek, 1979; Bolger & Case, 1994; Fox *et al*., 1996; Semlitsch *et al*., 1997), and it could be argued that these studies provide support for the tangled bank hypothesis, with different genotypes doing better in different environments. However, clonal coexistence and the maintenance of sex are somewhat separate issues. Consider a species such as the freshwater snail *Potamopyrgus antipodarum* that undergoes predominantly sexual reproduction in some areas, and asexual reproduction in other areas (Dybdahl & Lively, 1995; Fox *et al*., 1996). Where it occurs, sex may explained in part by the presence of coevolving parasites (e.g. Lively, 1987; Dybdahl & Lively, 1998). In areas where parasites are not present, clonal diversity may be maintained across different habitats by resource partitioning. If this is correct, then the answer depends on the question. If the question is: how do we explain the distribution of sex and the maintenance of clonal diversity across niches, then the answer almost certainly requires multiple mechanisms.

What needs to be done? Direct quantitative estimates of relevant parameters are vital. But this is not necessarily for the reason – popular in some quarters – that parameter estimates may enable particular models to be eliminated. A pluralist perspective suggests this is an overly optimistic view, true only for extreme values. For instance, Kondrashov (1993) has argued that an advantage of the Mutational Deterministic hypothesis is that it is easily falsifiable: if the genomic deleterious mutation rate is too low, the model cannot work. But unless it is extremely low (e.g. $\ll 0.1$), the crucial question is a quantitative one: how important is mutation pressure? Several estimates have placed a lower limit on the mutation rate in the range of 0.4–1.0 (Mukai, 1964;

Mukai *et al*., 1972; Ohnishi, 1977; Charlesworth *et al*., 1990; Charlesworth *et al*., 1994; Keightley, 1994; Johnston & Schoen, 1995; Deng & Lynch, 1997; Drake *et al*., 1998). This happens to be the range where the mutation rate would not fully balance the two-fold cost of sex, but would still provide a considerable advantage (Fig. 1). Moreover, empirical estimates of the mutation rate generally provide lower limits and have large confidence intervals (Keightley, 1998). Consequently, if the mutation rate is in the range of 0.4–1.0 then current empirical methodologies would not allow the Mutational Deterministic hypothesis to be easily falsified.

Similarly, if mutational effects turn out to be independent, the Mutational Deterministic hypothesis is dead. But if there is some synergism, the issue again becomes quantitative: given observed mutation rates and levels of synergism, how big is the role played by the Mutational Deterministic hypothesis? It could be argued that, in principle, parasite models could also be falsified, for example, if selection does not fluctuate. But if there is some fluctuation in selection, quantitative estimates are needed to determine the extent to which parasite pressure could, on its own or in combination with other factors, favour sexuality. Thus, while it may be relatively easy to rule out a model as a sufficient explanation of sex, it will require substantial amount of work to eliminate it as part of the explanation.

As well as parameter estimation it is also important to test the assumptions of the different models. For example, there is considerable experimental evidence showing that the amount of recombination both influences the response to selection and increases as a correlated response to selection (McPhee & Robertson, 1970; Flexon & Rodell, 1982; Burt & Bell, 1987; Korol & Iliadi, 1994). Similar work on deleterious mutations would be extremely useful. However, it should be noted that while these studies demonstrate that a certain mechanism can work, they do not quantify its importance under natural conditions.

There are several other general points that arise naturally from the pluralist standpoint. First, the importance of measuring fitness under as realistic conditions as possible cannot be overstated. The influence of any factors affecting fitness such as deleterious mutation or parasite loads are likely to vary enormously with the conditions under which fitness is measured (Dudash, 1990; Kondrashov & Houle, 1994; West *et al*., 1996). The relative contribution of different models, and of any interaction between them, is likely to similarly vary.

Second, to be of value in considering the relative importance of particular models, and any synergism between them, parameter estimates need to be derived from the same biological system. While we accept the desirability of being able to generalize, and agree that will only be possible once many different systems have been investigated, little progress will be made if we have estimates of mutation rates in a nematode, for example, and parasite-induced frequency dependence in a plant.

We also consider it highly important to estimate relevant parameters in sexual species. The form of selection must be different in sexual species than in species which are asexual or only occasionally undergo a sexual cycle. The mutation rate, for example, is generally selected to be lower in asexual species (Leigh, 1970; Kondrashov, 1995), and asexual lineages would not persist with a rate above 1.0–2.0 (Kondrashov, 1993). It is perhaps not surprising that the lowest estimates of the deleterious mutation rate have been obtained from largely nonsexual species (Kibota & Lynch, 1996; Keightley & Caballero, 1997). Similar arguments could be made that the form of epistasis between deleterious mutations is likely to differ between sexual and asexual species (Malmberg, 1977; Falush, 1998; Hurst & Smith, 1998; see also Szathmary, 1993), especially when asexuality is coupled with polyploidy, which is common in plants and animals (Bell, 1982). Environmental mechanisms could also be argued to differ between sexual and asexual species for similar reasons. Indeed, we have already suggested that even in a single species such as *P. antipodarum*, different environmental mechanisms may be responsible for the maintenance of sex and clonal diversity.

Finally, work on testing for synergistic epistasis between deleterious mutations has demonstrated the importance of developing theory for how experiments should be carried out (West *et al*., 1998). There is enormous scope for further work in this area, particularly with regards to the environmental models. In many cases, multigenerational experiments may be vital: one round of sex is often predicted to decrease mean fitness but increase its variance (Charlesworth & Barton, 1996; West *et al*., 1998). But as well as assisting in the development of effective experimental protocols, we see an important role for theory in assessing the conditions under which synergistic interaction between existing models can be most powerful. This may go a long way towards explaining the currently embarrassing existence of biparental sex, especially where the costs may be substantially more than two-fold.

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COMMENTARY

An even broader perspective on sex and recombination

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An historical note: why we needed the paper of WLR

Understanding why sexual reproduction in eukaryotes is so prevalent is a hard problem, and it has gone through a progression of stages that are typical for work on hard biological problems. First, a pioneer suggests a plausible solution, usually very general and not rigorously defined because the theory surrounding the problem has not been fully developed. Other pioneers may add competing but similarly broad and fuzzy theories. As interest in the problem spreads and the theory in which it is embedded matures, more detailed theories about more specific mechanisms are proposed; these are often presented as alternatives because of scientists' desire to be the one who solved the problem … the only one. The new theories become more and more detailed as the available theory space is used up. Occasionally, someone sits back and looks for a more general solution that includes all the detailed models as special cases. And often someone else comes forward and points out that many, if not most, of the models may be operating in nature, in different species or even in the same one.

So it has been with the question of why sexual reproduction is so prevalent among eukaryotes (Mooney, 1992). Early hypotheses, such as Weismann's (1891) that sex facilitates evolution by increasing genetic diversity, were necessarily vague and difficult to evaluate because they were devised in the absence of any real understanding of transmission genetics or population and evolutionary genetics. More sophisticated hypotheses appeared after the development of Mendelian genetics and population genetics. An important example is the hypothesis of Fisher (1930) and Muller (1932) that sex facilitates natural selection for advantageous mutations, extended to selection against detrimental mutations by Muller in 1964. This was followed in the 1970s and 1980s by a proliferation of models with increasing sophistication and detail, but of decreasing generality. The books of Williams (1975), Maynard Smith (1978) and Bell (1982) contributed to the proliferation of models directly and also indirectly by making the field more popular. We now have models for organisms with many different permutations of finite or infinite population size, advantageous or detrimental mutations, positive or negative epistasis or no epistasis, and a variety of different reproductive patterns and ecological niches. Unfortunately, the numerous models are often presented as mutually exclusive and individually sufficient to explain the prevalence of sex in most or all organisms.

Few authors have asked if there might be a more generally applicable model that subsumes most or all of the detailed models as special cases (for two exceptions, see Felsenstein (1974) and the review by Barton & Charlesworth (1998)). Even fewer have combined the detailed models to see what happens when two or more are operating simultaneously. West, Lively and Read (1999) (WLR hereafter) have done that. They are to be applauded for emphasizing that at least some of these competing hypotheses are not mutually exclusive, and for showing that they may be more powerful, as well as more realistic, when combined.

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The contribution of MR

WLR focus on population genetic models that give sexually reproducing individuals a selective advantage over asexual individuals that is sufficient to overcome the two-fold cost of sex. They initially say that they will focus on deterministic models, because they believe that the inclusion of stochastic processes restricts the generality of a model. They divide deterministic models into two classes, mutational and environmental, and say they will look at interactions between the mutational deterministic and parasite-driven Red Queen hypotheses as representatives of the mutational and environmental classes. I am dubious about their reasons for choosing these models, but it does not matter because the models they actually consider (Howard & Lively, 1994, 1998) combine host–parasite interactions with stochastic processes such as Muller's ratchet in finite populations. This is unfortunate from a truth-in-advertising standpoint, but it is probably wise scientifically. Many organisms with very large populations lead very uncertain lives and have a high variance in offspring number; moreover, the ratio of effective population size to the actual size (N_e/N) decreases as N increases (Pray *et al.*, 1996). I doubt that any approach to the evolution of sex that ignores stochastic effects of population size can be very general.

WLR's combined models give a larger advantage to sex than either model alone in many conditions. The advantage can be greater than two-fold, sufficient to overcome the cost of sex. WLR argue that the analysis of multiple models is important because it may be necessary to explain the maintenance of sex. I agree; it seems extremely unlikely that any of the existing detailed models can explain the maintenance of sex in all eukaryotes. Suppose, for example, that we had enough information about the rates, fitnesses and epistatic interactions of mutations in many different organisms to convince ourselves that the mutational deterministic model could in principle explain the maintenance of sex everywhere. This would not prove that it is the only factor involved; it would not prove that Red Queen interactions with parasites made no significant contribution to the maintenance of sex, or that Muller's ratchet did not routinely extinguish small asexual populations. It would not even prove that these models were less important than mutation accumulation; they might contribute more to the fitness differential between sexuals and asexuals than deterministic mutation accumulation. Moreover, it ignores the fact that these models might interact so as to change the parameter space in which they are effective.

I do worry about one aspect of interacting models. WLR note that 'it may be easier to accept the pluralist approach with empirical data than to reject the theoretically simpler models' and appear to see this as an advantage. The flip side of this is that multiple interacting mechanisms may be difficult to reject. There may be no way around this; I suspect that so many biological and ecological variables affect the selective value of sex that it will require an immense amount of work to identify the important ones and show which detailed model(s) are operative for any one group of organisms.

A broader perspective

WLR's work should broaden our perspective on the evolution of sex to include interactions between the various detailed models, but I believe that we need to extend the perspective in at least two more dimensions.

Not all organisms are animals or plants

The majority of theory and observation on evolution in general, and on the evolution of sex in particular, deals with vertebrates, insects and plants. This is perhaps understandable, because these organisms have both aesthetic and economic impact on humans. Nevertheless, any general theory of the advantage of sex requires a broader phylogenetic perspective. Invertebrates, fungi and eukaryotic micro-organisms have very different and diverse life styles, and the differences may provide insights into the advantages and disadvantages of sex. Many do not have a two-fold cost of sex. Nevertheless, asexual reproduction is common among these groups, and the amount and effectiveness of sex varies greatly. Many of them alternate long periods of asexual reproduction with bouts of sex. Some are basically clonal in spite of obligate sexual reproduction, suggesting that they show extreme inbreeding (e.g. Rich *et al.*, 1997). Many appear to be strictly asexual, although it is difficult to rule out sex entirely. No theory or combination of theories can claim to be a general explanation of the prevalence of sex unless it applies to these organisms. I strongly suspect that a general theory must explain not only obligate sexual and obligate asexual reproduction, but also sexual reproduction of varying degrees of effectiveness.

Interactions between selection on individuals, groups and species

In principle, selection can act on individuals within a population or species; on partially isolated populations within a species; and on species. There is some confusion about these levels of selection in the literature on sex. It is important to keep in mind that what we are trying to explain is why so many species reproduce sexually. Sexual reproduction evolved early in the eukaryotic lineage and is the ancestral state for most eukaryotes. What we have to explain is why it has been retained in lineages where asexual mutants can occur. These mutants can potentially give rise to asexual species; to do this they must go through at least two steps involving

selection at the individual and species levels, and possibly at the group level.

1 First, the mutant must be fixed: it must increase in frequency in the species, by the operation of random drift and/or selection, until the entire species is asexual. Here, asexual mutants may automatically enjoy as much as a two-fold advantage over the sexual genotype. By itself, this advantage would guarantee the fixation of the majority of asexual mutants if the sexual genotype did not have some compensating advantage. It is important to keep in mind that the two-fold advantage of asexual reproduction, and any compensating advantage of sexual reproduction, is basically a matter of individual selection. The two-fold advantage works only because the asexual and sexual genotypes are adapted to the same niche and thus subject to the same limitations on population size (the carrying capacity of the niche). Although they are reproductively isolated from each other, this does not automatically make them different groups in the classic and customary sense of group selection, which requires that the groups evolve with a high degree of independence.

Although the fate of an asexual mutant depends at least partly on individual selection, group selection might also be important if the mutant is first fixed in a subpopulation or colony that is partially isolated from the rest of the species. (Note that stochastic effects are likely to be especially effective here because the subpopulation may be small.) It can also disperse to an unoccupied habitat and found a new colony which is completely asexual. In either case the asexual subpopulation can potentially replace the sexual subpopulations, or go extinct. This is group selection in the sense that the subpopulations or colonies still occupy the same niche and can potentially exchange migrants with the rest of the species, but do so at a low rate and so show some degree of evolutionary independence. In what follows I will ignore group selection but it may not be safe to ignore it in many organisms. *A priori* arguments that group selection is weak compared with individual selection because individuals have shorter life spans than groups are compelling but probably do not apply to all organisms, and in any event we need to find ways to actually measure the relative roles of these two kinds of selection in nature.

2 Once a sexual species has become asexual as the result of fixing a mutation, selection at the species level becomes important. The fate of a species is determined by the ratio of (or difference between) its probabilities of speciation and extinction. We ignore species selection at our peril, as indicated by the following simple argument.

First, asexual mutations quickly become irreversible. This is because sex is a complex process that depends on a number of genes for its successful completion, and after one gene is inactivated by mutation, additional mutations can inactivate other genes; after two or three are inactivated, the probability of restoring all of them to functionality is effectively zero. Second, asexual mutants can be fixed by drift, even if they have a net selective disadvantage. Given these two facts, simple mathematical treatments (Van Valen, 1975; Nunney, 1989) verify what is intuitively obvious: even if asexual mutants are rarely fixed, eventually all sexual lineages will be replaced by asexuals. This will happen unless there are no viable asexual mutants, or there is species-level selection. The first possibility is probably true in mammals and possibly in some other groups, but cannot be the case in clades that contain at least one asexual lineage. The important lesson is that selection at the level of species is required to maintain sexual reproduction in most groups of eukaryotes.

Moreover, it is absolutely necessary to consider the interaction between selection at the individual and species levels. It is possible, for example, that the two-fold advantage of asexual reproduction can be completely compensated by a disadvantage of asexual reproduction in species. The relative importance of selection at the level of individuals and species is, in the final analysis, an empirical question, to be decided by observation rather than by *a priori* arguments. The answer is probably different for different taxa. Again a reminder: group selection is ignored in this treatment but might actually be important in some cases.

What is needed?

I applaud WLR's emphasis on the need for good estimates of all of the relevant parameters, such as mutation rate and parasite-induced frequency dependence, from the same organisms. I also heartily agree that we need estimates of these parameters from sexual species. I would add that we need to know the frequency and effectiveness of sexual reproduction in species that reproduce sexually part or all of the time, as well as estimates of real and effective population sizes. Besides these population genetic parameters, we need some even more fundamental information of at least three kinds.

First, we need more work on organisms other than vertebrates, insects and plants. We cannot hope to look at all groups of organisms, but granting agencies and thesis advisers should encourage people to identify representative taxa, i.e. whose life styles differ in ways that may affect the ratio of asexual to sexual species. It is important to include groups in which the sexual species have different amounts of sexual reproduction with outcrossing. Then we need to do detailed studies of these groups. Not only do we need to measure all relevant parameters in each group, but we need to test multiple hypotheses in each group, even if we believe they are mutually exclusive.

Second, we need more data on how much sex there really is. We need to know what taxa are truly obligately asexual. This is not trivial, because it is difficult to prove that an organism in which sex has never been observed is

not having sex that is rare (and thus not yet seen), or furtive (doing it under conditions in which we have not looked for it), or cryptic (sexual reproduction by a mode which we can see but do not recognize) (Judson & Normark, 1996). In taxa that are sexual, how much sex are they having, and how effective is it? It is not clear what parameters should be used to measure the amount and effectiveness of sex, although linkage disequilibrium is almost certainly one of them.

Third, we need some way to separate and measure the roles of individual, group and species selection. Measuring species selection should have high priority, and it may be possible to do this by comparing diversification in asexual and sexual clades in phylogenetic trees (for examples, see Sanderson & Donoghue, 1996).

Finally, I think it would be useful to look again for a general explanation of the prevalence of sex that applies to all eukaryotes and subsumes the detailed models such as those discussed by WLR. It is very unlikely that any of the detailed models will suffice to explain the maintenance of sex in all organisms. We need a really general model to guide our experimentation, and of course to put in general biology or genetics textbooks and explain to the public. 'Sex facilitates selection by breaking down negative linkage disequilibria' seems like a good candidate (see also Barton & Charlesworth, 1998). It can operate at the levels of both species and individuals, and probably groups as well. It may be the most specific statement that applies to all eukaryotes, or even to most eukaryotes. Happily, it also has the virtue of being relatively easy to understand and explain.

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COMMENTARY

Explanation and prediction and the maintenance of sexual reproduction

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Evolutionary biology has often sat rather uneasily with fundamental principles of scientific explanation. Hempel (1965) has pointed out that, in science, there is an equivalence of explanation and prediction. In other words, a theory and/or a set of observations A can be said to explain a set of observations B if, and only if, B is predictable from knowledge of A. This criterion, widely accepted in epistemological philosophy, is often not satisfied in adaptive explanations of the phenotype. Frequently, an evolutionary explanation for a phenotypic trait is postulated, but one in which the trait is logically predicted by the explanatory idea only if quantitative values of unobserved parameters fall in a certain range. Measurement of the parameters can be dauntingly difficult. What, regrettably often, happens instead, is that the explanatory idea is accepted as correct on the basis of its intuitive reasonableness or appeal. Once this has happened, then the conjunction of the trait to be explained and the explanatory hypothesis are seen as jointly constituting evidence that the unobserved parameters fall in the required range.

The explanations of the continued persistence of sexual reproduction in the face of a theoretical two-fold advantage for apomictic parthenogens form a good example of an incomplete logical coupling between explanation and prediction. The fundamental problem has been expressed by Maynard Smith (1978) as being that, if the number of surviving offspring produced by a female is independent of the sex of these offspring, and whether or not they are produced sexually or asexually, then a dominant mutation generating apomictic parthenogenesis would be expected to have a two-fold fitness advantage relative to its allele in a wild population. Given that such apomictic mutations are possible, why are sexual species not replaced by their apomict descendants?

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Very large numbers of hypotheses have been suggested to try to account for the persistence of sex, in the face of this expected two-fold advantage. These hypotheses postulate mechanisms whereby sexually produced offspring have a higher Darwinian fitness than their asexual competitors. Such models create a short-term advantage to sex, such that the sexual subset of the population will be able to resist invasion from apomictic mutations. Clearly, there is another suite of explanations for sex which invoke a duration of asexual species which is short in palaeontological time, although long enough for an asexual mutation to have time to selectively replace its sexual progenitor.

West *et al.* (1999) argue that traditional ways of looking at the advantage of sex may be falsely unitary, in that they tend to contrast different models and look for data sets which will convincingly resolve amongst them. In particular, considering the two most strongly supported theories of forces giving advantages to sexual organisms, that of environmental fluctuation and the Red Queen, and the Mutational Deterministic hypothesis of synergistic deleterious mutations, they believe that the ubiquity of parasites and of deleterious mutations predicts that realistic models of sex must combine these two processes.

I believe that the set of models for sexual reproduction, taken either singly or together, do not, at present, meet the criterion for being a true explanation. They are still insufficient in their details for us to be able to predict the widespread occurrence of sexual reproduction if we did not know of this independently of our modelling efforts. Indeed, what has been motivating the search for a single unitary explanation for the persistence of sex has been the conviction that precisely one of the models will,

when the parameters relevant to the model have been accurately empirically determined, turn out to be a much more powerful explanation than it currently appears.

It is impossible to deny the realism of mixed models of sexual advantage since it would seem highly likely that a series of processes favouring sex might well be acting simultaneously. Thus, it seems appropriate to multiply their relative contributions to the fitness of sexuals in the hope that the product passes the magical threshold of two, such that the suite of processes jointly explain the phenomenon. However, for all its reasonableness, there are also dangers inherent in this approach. It should be remembered that processes which lead to an advantage for sexual reproduction have been searched for and enumerated, simply because the mystery to be explained consists of the persistence of sexual types when their two-fold competitive disadvantage should, all else being equal, preclude this. There has been no correspondingly systematic logical or empirical search for mechanisms which might favour asexual genotypes, giving them fitness benefits in excess, even, of the expected two-fold advantage. Thus, the true cocktail of selective forces operating on a competition between sexual and apomictic forms would be expected to also include various selective mechanisms favouring asexuality, such as the preservation of epistatic selectively favoured genotypes by apomixis, which have not been rigorously considered or investigated. Simply taking the apparently interesting side of the balance sheet, focusing on the forces favouring sex, may result in the two-fold advantage required, but may not be a realistic description.

The authors make a plea for the empirical testing of the models being postulated, and no scientist could object to empiricism. However, I fear that a major empirical programme to put values to the relevant parameters might well be more difficult that these authors anticipate. The authors cite the work of Kelley (1994) who showed a 1.55 fitness advantage of sexually produced progeny over apomicts in a grass, due to the impact of pathogens. Since this was done after one generation of apomixis, too soon for the Mutational Deterministic process to have its effects, they could conclude that the required fitness advantage of two could be attained if synergistic deleterious mutations were arising at a mutation rate of 0.51 per genome, rather than the 1.39 required if they alone were to produce the entire two-fold advantage required.

There are two important questions to be considered in making the decision to pursue this type of experimentation more generally.

The first question is whether we require the ubiquity of sex to be matched by a ubiquity of sexual advantage. In other words, do we postulate that all sexual populations are such that a mutation which would create an apomictic clone would be incapable of spreading in that population? One of the remarkable features of the model for the spread of apomictic parthenogens is the remarkable rapidity of the process. A new mutation with a two-fold reproductive advantage has around an 80% chance of spreading to fixation in a population, and, if it does so, will complete its spread to fixation in a few tens of generations (given population sizes of up to a few millions). For almost all types of organisms, it is impossible to imagine a fixation process occurring in this time throughout a geographically widespread species, and the rate of fixation will, realistically, be limited by restricted migration between populations. In order to prevent the apomictic mutation from spreading to fixation in the species, it only has to encounter a single sexual subpopulation in an environmental situation yielding a two-fold reproductive advantage to sexuals. When it does, it will be unable to invade this subpopulation, and the species will persist with sexual and asexual subpopulations. (Whether we expect to find this situation in a typical species will depend upon the mutation rate to viable and fertile apomictic parthenogens, and this rate might be quite low, so we should not be surprised that most species consist of entirely sexual subpopulations.) Once sexual and asexual subpopulations exist, the long-term advantages of sexuality will come to the fore, and it is likely that, ultimately, only the sexual subpopulation will leave descendants. Indeed, once population subdivision is considered, the distinction between long- and short-term mechanisms for the maintenance of sexuality may be less distinct than models normally suppose. (While, here, I have envisaged a geographical subdivision of subpopulations, analogous arguments operate on an ecological scale with niche partitioning generating frequency-dependent selection (Maynard Smith, 1998).)

The point of this discussion is that sexuality may be capable of persistence at the level of the species despite its invasibility by apomictic clones in the majority of the subpopulations. Thus, in the case of Kelley's (1994) data, there is no necessary reason to suppose that the rate of deleterious mutation in this particular population was sufficiently high that, when combined with the measured fitness advantage, it would produce an overall advantage for sexuals of over two. This is quite apart from the problem that synergism is required between the deleterious mutations, for which evidence is lacking (Barton & Charlesworth, 1998).

The second and related concern about measurement of the fitness advantage of sexual progeny relative to apomicts is the extremely low repeatability expected for this measurement from species to species, population to population and perhaps even from year to year. The advantage depends on the particular spectrum of pathogens or parasite genotypes infecting the population, and there is no reason to expect it to remotely resemble a biological constant. If one is taking the approach of identifying the genome-wide deleterious mutation rate, U, as that required to generate a two-fold advantage overall, the estimates of this quantity will oscillate wildly depending upon the particular estimate of the

environmental advantage of sexuals. Had Kelley (1994) found 1.2 as the relative fitness of sexuals, the *U* required would be over one, yet if he had found 1.9, the *U* required would be 10 times less.

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COMMENTARY

Origin, age and diversity of clones

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The origin of sexual reproduction may well have been a unique evolutionary event, or series of events, but the maintenance of taxonomically widespread sex is not: it consists of many local skirmishes between sexual lineages and their asexual offshoots. Neither sexually reproducing populations nor asexual lineages are uniform in their characteristics. They vary in many ways that might influence the outcome of their evolutionary interaction, such as their ecological role, genome size and complexity, level of genetic variation, mutation rate and, for sexual populations, freedom of recombination. Although sexual reproduction predominates in animals and plants, and asexual lineages are typically short-lived and taxonomically isolated, these general patterns should not be allowed to obscure the true diversity. There are long-lived, widespread, genetically and even taxonomically diverse asexual lineages at one extreme and sexual populations with no asexual descendants at the other. In between, there are sexual species with high levels of inbreeding and asexual lineages that hybridize with sexual relatives. A complete understanding of the evolution of reproductive modes will encompass these extremes as well as the typical pattern. It seems to us that only a pluralist approach is likely to be successful in the sense that there are complex patterns to explain, not a simple dichotomy. However, this is not equivalent to the approach advocated by West *et al.* (1999) who apparently wish to abandon the search for a single mechanism capable of explaining the predominance of sexual reproduction but at the same time ignore the variety of reproductive modes found in nature.

West *et al.* (1999) draw much needed attention to the dynamics of the interaction between sexual species and asexual lineages. Initially, a new clonal lineage is very susceptible to parasites as it becomes abundant but, as it accumulates genetic diversity, this risk declines. Although individual asexual lineages may persist for short periods of time, asexual reproduction might persist if new clones originate with sufficient frequency from the sexual population. It may be that building frequent origination of clones into the models of Howard & Lively (1994, 1998) would increase the parameter space in which asexual reproduction displaces sexual reproduction. On the other hand, West *et al.* (1999) argue (p. 19) that maintenance of clonal diversity and maintenance of sex are 'somewhat separate issues'. While we agree that clonal diversity may be explained in part by resource partitioning, this is not readily separable from the outcome of interactions between sexual populations and asexual lineages: clonal diversity maintained by resource partitioning can make the asexual lineages more resistant to displacement by the sexual population because clones are better adapted to environmental conditions, because diversity in parasite resistance is maintained incidentally through linkage disequilibrium, and because resource partitioning allows higher population size and thus retards the ratchet.

The diversity of clones, their modes of origin and their rates of turnover are empirical issues that need to be addressed. The standing diversity of clones is clearly a product of origination and extinction rates but these are very hard to separate. In nonmarine ostracods, for example, clonal diversity is highly variable, as detected by allozyme electrophoresis: from seven clones in *Darwinula stevensoni* to 211 clones in *Eucypris virens* with comparable sampling efforts across Europe (Rossi *et al.*, 1998). However, the reasons for this variation are largely unexplored. Clonal diversity may be generated in at least three ways (Butlin *et al.*, 1998): mutation within existing clones (including autopolyploidy), separate origin of clones from a sexual ancestor, or hybridization between asexual females and males of the same or related species (usually generating triploid offspring). Only the first process is available to *D. stevensoni,* which lacks sexual relatives, but multiple origins of asexual reproduction and hybridization have both been demonstrated in ostracod species with sexual populations or closely related sexual species, including *E. virens* (Turgeon & Hebert, 1995; Schön & Butlin, 1998). It has been suggested that species with sexual congeners tend to have higher clonal diversity than those that do not (Havel & Hebert, 1989). On the other hand, very little is known about rates of turnover. Griffiths & Butlin (1995) found that asexual species were less abundant, and more variable in abundance, than sexual species in Holocene fossil sequences. Note that the term 'asexual species' here refers to a set of morpholog-

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ically similar asexual lineages. The variable abundance could reflect clonal turnover on a time-scale of thousands of years. However, clones may show DNA sequence divergence equivalent to several millions of years of separation (Chaplin & Hebert, 1997; Schön & Butlin, 1998; Schön *et al*., 1998). Unfortunately, molecular phylogenetic data cannot distinguish multiple origins of lineages relatively recently from smaller numbers of older origins, and can only include extant lineages. Therefore, these data do not really answer the critical question.

The situation in ostracods is as well characterized as in most taxa (Martens, 1998). Only in some asexual vertebrates, derived from interspecific hybridization in all cases, is the origin, diversity and turnover of clones better documented (Avise *et al*., 1992). Thus, it is premature to abandon studies of 'pattern' in order to concentrate on parameter estimation and the testing of assumptions, as advocated by both Kondrashov (1993) and West *et al*. (1998). The prediction that asexual lineages persist in the face of the Red Queen through clonal turnover needs to be tested, for example by determining the distribution of parasites among clones as suggested previously by Lively (1992). An explanation is needed for the persistence of asexual lineages with low clonal diversity or without sexual relatives and therefore with limited input of new genotypes. In a species with many asexual lineages like *Eucypris virens*, we need to understand why sexual populations coexist with asexuals in only a small part of the current range. There is no evidence that this is due to parasite prevalence. Currently, the best explanation is based on climate change during the Holocene (Horne & Martens, 1999). It may be true that Red Queen models *can potentially explain* patterns of sexuality but the Red Queen hypothesis should not be restricted to parasites: in its original form (Van Valen, 1973), it encompassed all environmental change, biotic and abiotic, parasites, predators and competitors. We are not convinced by the claim, unsupported by references, that 'the parasite models predict the majority of within- and between-host patterns of sexuality' (West *et al*., 1999, p. 16).

West *et al.* (1999) do not mention the so-called 'ancient asexuals' (Judson & Normark, 1996). These lineages have apparently persisted for tens of millions of years without sex (100 million years or more for the darwinulid ostracods on the basis of their excellent fossil record, Schön *et al*., 1998), have diversified (more than 20 extant darwinulid species, all asexual; Rossetti & Martens, 1998), and in some cases are abundant and widespread (e.g. *Darwinula stevensoni*; Griffiths & Butlin, 1995). They present a real difficulty for all theories but, as arguments are made that suggest additional reasons for sexual lineages to displace asexual ones, the problem becomes ever more serious. While Howard & Lively's (1994, 1998) simulations based on the pluralist approach indicate a wider parameter space for the maintenance of sex, so they automatically imply a narrower range of conditions in which asexual lineages can persist for long periods of time. Indeed, in their 1994 simulations where Muller's ratchet operates in the absence of epistatic effects of deleterious mutations, asexual lineages go extinct rapidly (150–500 generations) even in the 'asex wins' part of parameter space! With epistatic fitness effects, the ratchet is less effective or may cease to operate (Hurst & Peck, 1996; Howard & Lively, 1998) but the pluralist approach suggests that its effects will be augmented by the Red Queen so that again, even lineages that initially displace their sexual competitors are doomed to rapid extinction. Ancient asexuals must simultaneously escape both processes. Perhaps they achieve this by virtue of a 'general purpose genotype' (Lynch, 1984) or by efficient DNA repair (Schön & Martens, 1998). In any case, they are a part of the overall picture of reproductive modes and must be accommodated by any complete theory.

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COMMENTARY

The omnipresent process of sex

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West, Lively and Read (1999) (hereinafter 'the authors') note that there are upwards of 20 hypotheses for the evolutionary advantages of sexual reproduction and argue that more than one may be correct. In particular, they suggest that environmental and mutational mechanisms may both be applicable and that interactions between them could be important. I like the suggestion of a pluralistic approach. The area of interactions between different mechanisms is, as the authors say, largely unexplored. I have no criticisms of the article, only a few comments.

The quotation from Havelock Ellis, which I chose as a title, fortuitously points up the problem. I do not find it surprising that a plethora of hypotheses have been presented. It almost appears that, with each new molecular discovery, there comes another hypothesis. Is a sufficient explanation to be found among these? I suspect that among them, singly or in combination, lies the answer. But who can be sure that the happy thought that will provide a really satisfying answer will not appear. Then everyone would immediately accept the idea and say 'How obvious, why didn't I think of it?'. Welcome as this would be, it seems unlikely, and we shall continue to have a diversity of views and an increasing number of hypotheses. Yet, I suppose there is always room for one more hypothesis. Many of the newer hypotheses strike me as, if not wrong, applicable only to such special circumstances as to lack generality. I am tempted to quote Laplace: 'Sire, I have no need of that hypothesis'.

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There indeed *is* a problem, however. The evolutionary advantage of sex cannot be marginal, it must be large. The standard two-fold cost is a proper target for a quantitative assessment. But, let me emphasize that it is not alone. Here are some of the disadvantages (Crow, 1994):

- Sexual reproduction is not a very efficient means of reproduction. Meiosis and fertilization are unnecessarily complicated if reproduction is the sole objective. Asexual spores or meiosis-bypassing apomixis would appear to be far more efficient.
- The aforementioned two-fold cost. As an alternative to separate sexes, a clone of parthenogenetic females could dispense with males, with a 50% saving.
- With separate sexes, sexual selection leads to traits that are poorly adapted, such as peacock tails and destructive competition for mates.
- Sexual species cannot perpetuate what are often fitness-improving types, such as triploids, aneuploids and translocation heterozygotes.
- Species with separate sexes have to find mates, or in planktonic populations sperms have to find eggs, which can be a severe disadvantage in sparse populations.
- Sexual species are prone to sexually transmitted diseases and provide an easier opportunity for selfish DNA elements to spread.
- Sexual reproduction opens the way for 'cheating' DNA, such as meiotic drive, and for possibly adverse gametic competition.
- Short-term selection is often slower in sexual species, in which selection acts on only the additive component rather than the total genetic variance.
- The Sewall Wright dilemma. With a complex fitness surface a sexual species may not be able to cross a valley to get to a higher fitness peak.

These and other disadvantages present a formidable challenge. I believe the variety of these supports the

authors' view that there are multiple mechanisms offering an advantage to sexual reproduction. I think that research based on the two-fold cost is the best way to proceed, the other disadvantages of sex being more difficult to quantify.

I share the authors' preference for considering hypotheses that are deterministic and do not require special circumstances, such as small or structured populations. They are searching for the most generally applicable theories, and this seems to me to be the way to go.

I have long liked the mutational deterministic hypothesis. Alexey Kondrashov makes the valid point that this can be falsified by finding that mutation rates are small, say less than one per diploid genome per generation. Certainly, falsifiability is one desideratum for a scientific hypothesis. Yet, a great deal of evolutionary research – good evolutionary research – does not fit the Popper paradigm. Considering the mutation hypothesis as one that is not sufficient by itself lowers the required mutation rate and makes it less falsifiable. But the possibility of interaction among mechanisms is appealing and, in my view, can offset any loss of possible falsifiability.

The most serious problem for the mutational hypothesis arises in species with limited reproductive capacity. Most animal and plant species have high enough reproductive rates to tolerate a high mutation load and still survive. A critical place to look is in species with low rates of reproduction. Until recently there was no information on the genomic rate of deleterious mutations in any mammal. This is no longer true (Keightley, personal communication). The estimated rate for the human species is about two per diploid genome per generation. For a number of reasons, this is a minimum estimate, so a realistic range is from 2 to 5. This is high enough to require some mechanism for mutation elimination; simple exponential survival and fertility will not work.

Epistasis is regularly invoked as a means of mutation-load reduction. Yet the level of epistasis observed for quantitative traits is usually slight. I suspect, as many have suggested, that some form of rank-order selection imposes the necessary epistasis. This epistasis is a property of the way selection works rather than a function of gene interaction. No one expects nature to truncate strictly. Yet, a crude approximation, quasi-truncation selection, has almost as great a mutation-reducing effect. This was perhaps apparent to many, but my realization came with a paper by Milkman (1978), which we worked out in more detail (Crow & Kimura, 1979). All species produce more progeny than would be required to maintain the population if all survive. It seems eminently reasonable that some density-dependent selection follows and that this may be sufficiently like rank-order selection to have very similar properties. In particular, I would argue that the human species has survived, despite what increasingly seems to be a very high deleterious mutation rate and a rather low reproductive potential.

The mutation hypothesis has several advantages. All species produce deleterious mutations and selective adjustment of mutation rates is slow and inefficient. The hypothesis involves no stochastic assumption and works in all but quite small populations. It imposes no requirement for environmental fluctuations or other specialized environmental situations (e.g. parasites).

The best test of this hypothesis would be to compare deleterious mutation rates in otherwise comparable sexual and asexual species. But 'otherwise comparable' populations may be difficult to identify. The best opportunities may be in plants or perhaps some lower vertebrates. Yet, even with good data there are conceptual difficulties, as the authors have discussed.

Environmental hypotheses are more difficult to quantify and more difficult to test in ways that have general applicability. Yet there is no question of their plausibility, and the advantages of sexual reproduction in a parasite-infested world seems entirely reasonable. There are abundant observations that offer correlational support for these hypotheses, but they are rarely, if ever, capable of discriminating among rival hypotheses.

The authors emphasize that environmental hypotheses such as the Red Queen work best with strong epistasis. It is therefore inviting to regard epistasis, particularly that brought about by approximate rank-order selection, as improving the status of both hypotheses. The intriguing suggestion that there may be interactions between the two remains to be tested rigorously. A difficulty of the pluralistic approach is that it is less testable. We are giving away testability to gain plausibility and generality. And it will surely be difficult to measure interactions when the main effects are so difficult to quantify.

I think the authors are correct in emphasizing the mechanisms currently maintaining sex; these may be different from those involved in the origin of sexual mechanisms, recombination for example. I also agree that it is important to measure the magnitude of the advantage of sex for all relevant hypotheses, even though they may not individually be sufficient to balance a two-fold cost. Finally, estimation of parameters is useful even for the study of separate mechanisms, but is of special importance if they are to be considered simultaneously.

I was particularly intrigued by the possibility discussed under 'Synergism at the empirical level' by the possibility that both the parasite model and the mutation model work best under truncation selection. Each may enhance the approach to truncation selection (or quasi-truncation selection) in the other. If this turns out to be correct when specifically modelled, it will provide an excellent reason to support the authors' pluralistic view. In any case, who can object to obtaining better estimates of the relevant parameters?

I believe that the authors have emphasized the most promising hypotheses. But is there room for others? I think there is, although I would regard them as ancillary. Whether they would interact with the ones just discussed remains to be seen.

Some stochastic hypotheses certainly deserve our continued recognition. One of these is Muller's ratchet. In particular, the trade-off between this and the deterministic mutation hypothesis as population size changes is important, especially if this is combined with a model of environmental fluctuation.

Another class of hypotheses includes those that depend on individual favourable mutations sweeping through the population. In my view, these are a less likely reason for recombination than traits that depend on multiple genes (at least in multicellular eukaryotes). Yet, we know from the study of molecular evolution that favourable mutations have been incorporated in the phylogeny of various species. The well-known Fisher–Muller idea offers the possibility of incorporating favourable mutations that arise in separate individuals. Surely, there are circumstances where this would be important, but such circumstances may be rare. I suspect that Fisher's other argument is the more important. He notes that in an asexual population, in addition to the stochastic loss in the early generations (which is essentially the same in sexual or asexual species), a slightly beneficial mutation has very little chance. In Fisher's (1958) elegant prose: 'If we consider the prospect of a beneficial mutation occurring at any instant, ultimately prevailing throughout the whole group, and so leading to evolutionary progress, it is clear that its prospect of doing so will depend upon its chance of falling, out of the whole population, upon the one individual whose descendants are destined ultimately to survive.' The mutation may help that one individual, but only if the mutational effect is large, or the population is very homogeneous. But it can be very homogeneous only if the mutation rate, and therefore the chance of a favourable mutation, is small.

Data from molecular evolution are becoming more and more abundant. It should be possible eventually, perhaps soon, to compare the rate of incorporation of favourable mutations in sexual and asexual species.

In summary, I am generally supportive of the authors' views. The most fruitful approach, I believe, lies in the study of existing hypotheses, and in various combinations. The old question, 'Is Sex Necessary' (Thurber & White, 1929), is not likely to have a single answer.

Finally, let me note that it has been a great pleasure and opportunity for one who was once very closely associated with this subject, but who has fallen badly behind, to tune in again. My long association with the mutational deterministic hypothesis has been a source of much personal satisfaction. I should like to mention two items. First, Kimura and Maruyama, both now deceased, wrote their paper at a time when both were working in my lab and we were conversing regularly. Second, the fact that Alexey Kondrashov and I had each thought of this brought us together, first by correspondence and later in person during two periods in Wisconsin. The daily discussions were great.

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COMMENTARY

Measuring the benefits of sex

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West *et al*. (1999) identify and encourage a shift in studies of the evolution of sex from hypothesis testing to parameter estimation. While this trend is beneficial, relating parameter estimates to the reasons for the maintenance of sex is surprisingly difficult. This commentary discusses two of the issues involved.

Is there a two-fold benefit of sex?

West *et al*.'s argument for their pluralist view is based on the premise that sex needs a large short-term benefit in order to offset its two-fold cost. An alternative, developed by Nunney (1989) and A. Burt (personal communication), is that sex is maintained by clade selection. Clonal lineages are generally short-lived in evolutionary time (Maynard Smith, 1992). Consequently, if the rate of origination of clones is low, sex can persist even if it is out-competed by asexuality every time it arises. Nunney showed that clade selection would also reduce the rate at which sexual species produce new clonal lineages.

This clade selection hypothesis is sufficient to explain the rarity of obligate asexuality, but does not explain the persistence of systems in which sex and asex coexist (Nunney, 1989). These systems are therefore taken by many (e.g. Williams, 1975) as evidence for a large short-term benefit of sex. The existence of systems in which obligately sexual and asexual forms compete provides a basis for West *et al*.'s argument that sex needs a plurality of benefits in order to offset its two-fold cost per generation. However, these systems are rare (Bell, 1982), possibly because most of those that arise become extinct shortly afterwards (A. Burt, personal communication). Those that we do observe may be atypical in that they posses factors which promote persistence.

Possible factors include: (1) low hatch rates of parthenogenetically produced eggs; (2) residual male function of asexual hermaphrodites; (3) differences in ploidy levels between sexuals and related asexuals; (4) hybrid origins of asexuals; (5) the requirement of asexuals to be fertilized by male sexuals or other hermaphroditic asexuals. Each factor is common amongst sexual/asexual systems, but none is universal (Bell, 1982), an observation consistent with each one being common because it increases the time a system persists.

Each of these factors alters the costs and benefits of sex. The low hatch rate of parthenogenetic eggs, a common trait among facultative asexuals, reduces the fitness cost of sex relative to asex directly. Residual male function has been shown theoretically to reduce the benefit of sex necessary for coexistence in some but not all circumstances (Joshi & Moody, 1995). Ploidy differences can have an indirect effect by ensuring that sexuals and asexuals are morphologically distinct. When sexuals and asexuals inhabit different ecological niches, a wide range of values for the benefit of sex are all likely to be consistent with coexistence. Similarly, niche separation associated with speciation may protect sexual forms in hybridogenic systems. Finally, the necessity for asexuals to be fertilized by sexuals has been shown to give sexuals a frequency-dependent advantage that, in the unisexual fish *Poeciliopsis mollachaoccidentalis*, can be large enough to offset a two-fold cost of sex (Moore, 1976).

Residual male function and the necessity for asexuals to be fertilized by sexuals may also promote persistence by ensuring the creation of new clones through 'contagious asexuality' (Hebert & Crease, 1983). There is direct evidence for contagious sexuality through fertilization of unisexual females by sexual males in salamander hybrids of the Ambystoma system (Hedges *et al*., 1992). Eighteen out of 20 unisexuals, which were otherwise genetically diverse, were shown to share a common mitochondrial genotype. Contagious sexuality through male function has also been suggested as the mechanism of clone creation in a number of systems (e.g. Enghof, 1976; Hebert & Crease, 1983; Pongratz *et al*., 1998). In these systems selection to maximize female fecundity within each clonal lineage may be balanced by the increased ability of clones with lower fecundity to create new lineages. Contagious sexuality plays a multiple role in ensuring the creation and stability of sexual/asexual systems. First, it can facilitate the evolution of asexuality (Jaenike & Selander, 1979). Second it ensures the continued creation on new clones. Third, it reduces the fecundity advantage of asexuals. Fourth, it may allow asexuality to jump between species, counterbalancing clade selection against sexual species which produce asexuals. Consequently, contagious asexuality may

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ensure persistence and a reduced cost of sex in many of the systems that field workers observe.

Should fitness be measured on a log scale?

Several experimental methods have recently been proposed which measure the fitness of individual genotypes before and after sex (Charlesworth & Barton, 1996; de Visser *et al*., 1996; 1997a). This approach has the potential to provide a direct estimate of selection on sex and recombination (Barton, 1995). Unfortunately, most of the proposed methods may prove impractical (West *et al*., 1998). One problem is that each of the methods relies on a logarithrmic fitness scale to interpret data. The scale has many theoretical advantages. The most powerful analysis of the evolution of recombination (Barton, 1995) relies on the scale, treating epistasis as a deviation from multiplicativity. It also tends to make results easier to interpret. For example, under synergistic epistasis, increasing the variance in mutation number always lowers mean log fitness, but may either raise or lower mean fitness, depending on parameters (compare Tables 2 and 3 of Charlesworth & Barton, 1996).

Conversely, use of the scale can bias results, give a misleading indication of selection pressures and, in some circumstances, prove impossible. Barton's analysis, like every other, simplifies reality in order to represent it. In practice, genotypic fitnesses will not obey the relationships the analysis suggests. This is a problem because logarithmic measurements can easily over-emphasize the importance of genotypes with very low fitness. The exact fitness of these genotypes has little effect on recombination modifier dynamics but can alter epistasis estimates substantially. Additionally, measurement error is bound to have a nonlogarithmic component. Unless this source of bias is corrected for it will also exaggerate the contribution of genotypes with low fitness.

In a recent experiment, the use of a logarithmic scale proved impossible. Elena & Lenski (1997) collected *Escherichia coli* mutants in a permissive environment in order to measure the shape of the fitness function. A number of genotypes that they obtained were found to have zero fitness in the more stringent environment in which fitness was measured. For these genotypes, log fitness is undefined. Faced with this difficulty, Elena and Lenski abandoned a true logarithmic scale, calculating the log of mean fitness rather than the mean of log fitness. Better, they should have abandoned logarithms entirely. A fitness function is a statistical construct, designed to summarize the results of a number of measurements in a few parameters. The choice of function should therefore be made on statistical grounds, taking into account both the range of measurements (which in this case disqualifies the use of logarithms) and also their error. Fortunately, in Elena and Lenski's experiment the choice of fitness function did not matter much. However interpreted, the measurements do not indicate significant synergistic epistasis (Elena & Lenski, 1997).

A similar experiment has revealed another problem with the measurement of individual fitnesses. de Visser *et al*. (1997b) attempted to isolate genotypes of *Aspergillus niger* with every possible combination of a set of marker loci before measuring their fitness. They found that those combinations that were not isolated had more low fitness alleles than those that were. This experiment illustrates that some low fitness genotypes will inevitably be lost before being isolated. On a logarithmic scale the resultant bias will be large.

What can be done? One approach is to minimize the importance of low fitness genotypes by ensuring that offspring fitnesses are intermediate to those of the parents (as in the design favoured by West *et al*., 1998). A second approach is to abandon the effort to measure the fitness of individual genotypes and instead perform multigenerational experiments in which aggregate frequencies are followed for a number of generations. In an experiment of this sort, Greig *et al*. (1998) modified the capacity for sex genetically and allowed sexuals to compete with asexuals during and after sex. This approach avoids all problems with biased sampling of genotypes and nonlogarithmic error. The best solution to the problems associated with logarithmic fitness measurement may be to design experiments that do not require it.

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COMMENTARY

Sex: a pluralist approach includes species selection. (One step beyond and it's good.)

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In their paper, West *et al*. (1999) propose the idea that instead of trying to oppose two hypothetical forces (fluctuating ecology and DNA repair), concerning the evolution of sex, it could be of interest to try to explore the idea that they can act simultaneously and that the interaction between them could provide interesting mechanisms. The aim of the present commentary is to try to demonstrate two points.

1 This is a good idea; however, it suffers a handicap: namely because it tends to decrease the level of conflict between individuals (and/or 'schools'), it might be ignored by people who think that it is more fun to compete.

2 Instead of remaining stuck to individual selection alone, one could go even further and stop opposing short-term and long-term selection but explore the possibilities offered by the simultaneous action of these two forces. This has been tried by a few of us but, perhaps because it suffers the handicap stated above, it has been forgotten in most reference lists.

G. Bachelard stated that it is not sufficient for humans to be right, they must be right against somebody. The need for such fightings has already caused great trouble in evolutionary biology. In the beginning of the 19th century, Cuvier stated that all species went extinct; Lamarck answered that no species ever went extinct. Had they accepted the idea that some species could go extinct while others would not, long and sterile fights would have been avoided. Similarly, at the beginning of the 20th century, Darwinians (Pearson) stated that natural selection was the driving force of evolution while geneticists (Bateson) were putting mutation forward (see Provine, 1971). Thirty years of conflict would have been avoided if scientists had tried to assemble these two forces (as proposed by Yule, whom nobody seems to have listened to) instead of fighting.

This Bachelard-effect has probably contributed to the complexity of the debates about the maintenance of sex. In allogamous anisogamous species, a two-fold disadvantage (or 'cost') to sexual reproduction has been demonstrated by Williams (1975) and Maynard Smith (1978). This discovery seemed to imply that a two-fold advantage to sex had to exist to compensate for this cost. Moreover, because sex is a general phenomenon throughout the living world, this advantage had to be of a general nature. Consequently, numerous authors have desperately tortured their models and/or data in order to reach the magic value of 2. A list of some of them is provided in West *et al*. who state that most models and hypotheses fall into two categories. (i) Sexual reproduction, by producing variable offspring, is advantaged through sib competition and diverse sorts of variable environments, an idea starting with Williams & Mitton (1973) and later rendered more sexy by the involvement of parasites and the Red Queen by Hamilton (1980) and colleagues. (ii) By allowing repair of damages and/or mutations, sex is advantaged when the genome is large enough (Michod & Levin, 1988). West *et al*. quite rightly show that combining these two forces can provide interesting results.

However, they do not include the fact that these forces certainly act in the short term and in the long term. The observation that most asexual species appeared recently (see Judson & Normark, 1996, for the scandalous exceptions) shows that most of those which appeared earlier have gone extinct. It constitutes therefore a good proof that species selection is active on this trait (Maynard Smith, 1986).

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Indeed, in the real world, experiments and observations show that sex is usually not maintained by short-term selection. One can actually wonder how could asexual species even exist for more than a couple of generations if asexual reproduction implied a cost larger than 2 per generation (a point raised by Stearns, 1987). In aphid *Rhopalosiphum padi*, sex is maintained by the need for producing eggs (parthenogenesis is viviparous and winter frost kills all animals but not eggs) as shown by Rispe *et al.* (1999). The same kind of mechanism could be involved in *Chlamydomonas* (G. Bell, personal communication). In the fruit fly, *Drosophila mercatorum*, sex is maintained by an unexplained low fertility of parthenogenetic females (Templeton, 1982). Most plants cannot produce dispersal or resistance structures without undergoing sexual reproduction. Most animals do not use asexual reproduction simply because they cannot (for complex developmental reasons, including genomic imprinting). While a mixture of sexual and asexual reproduction constitutes probably an optimal strategy, species are, for most of them, either sexual or asexual ('the big theoretical problem' according to Hurst & Peck, 1996). Those which are sexual seem to keep this reproductive system for a variety of reasons. Moreover, in most of them, sex seems to be mainly maintained by constraints. All these features remain incomprehensible as long as multilevel selection is not taken into account.

The idea of multilevel selection in that context is that, everything being equal, individual selection is unable to resist the two-fold cost of sex but that species selection sorts out as extant those species which, for whatever reason, are unable to become asexual (i.e. are unable to produce 'good' asexual progenies). The others become asexual and then go extinct. From this point of view, species selection has favoured diverse mechanisms acting in the short term (e.g. constraints or other short-term forces including selection). It is thus not surprising to find that different studies provide divergent results. This idea was proposed by Gouyon & Gliddon (1988) and Gliddon & Gouyon (1989), and Nunney (1989) who formalized it. In this context, it is important to realize that evolutionists are used to forget that questions asked at different levels may ask for answers at different levels. In the present case, the questions 'Why are most species reproducing specially?' and 'Why are aphids *R. padi* reproducing sexually?' do not deserve the same treatment. The answer to the latter can be 'Because they need sex to produce eggs which resist to frost' while the answer to the former will be 'Because those which could evolve asexual reproduction eventually went extinct'. As stated earlier, the generality of sexual reproduction implies that there must be a general explanation but this general explanation can be found at the interspecific level while short-term reasons need not be general. Restricting the research to a general short-term reason has thus been misleading.

The multilevel explanation is an extension of the idea proposed by West *et al*. It is in perfect agreement with their statement that 'the factors maintaining sexual reproduction may be different from those which led to its evolution'. It is probably one of the major challenges for evolutionary theory of the next century to try to put together the different bits and pieces produced by different 'schools', particularly concerning levels of selection, chance, necessity and contingency (or constraints). Only if we are able to play this game, instead of systematically opposing the different possible hypotheses, shall we make significant progresses and avoid endless and sterile debates.

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COMMENTARY

Being too nice may be not too wise

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Unfortunately, it cannot be ruled out that the main point of West *et al*. (1999) is correct. Indeed, it is entirely possible that in some populations sex exists due to rapidly changing selection, while in other populations it is present as the consequence of invariant selection against deleterious mutations. Even worse, both these mechanisms (together with, God forbid, some third force) may be essential for the maintenance of sex in every population.

I do not like this possibility because such a beautiful phenomenon as sex deserves a nice, simple explanation and messy interactions of very different processes would spoil the story. Of course, this does not mean that such interactions are not, nevertheless, essential.

However, I believe that the pluralistic explanation of sex can be admitted only if all its components are shown to be (1) important and (2) individually insufficient. Before this happens, we need to keep testing the importance of these components, hoping that one of them will provide the complete explanation. I see no other way to improve our understanding of the evolution of sex.

In particular, validation/rejection of the Mutational Deterministic hypothesis is straightforward. If the genomic deleterious mutation rate U in some population is below $\sim$0.8, selection against mutations cannot alone maintain sex, as long as asex enjoys the two-fold advantage. Moreover, if $U < \sim 0.2$, deleterious mutations cannot be very important, even in combination with the Red Queen. On the other hand, with $U > 1$–2, deleterious mutations maintain sex (and explain a lot of other things) alone, Red Queen or no Red Queen. Thus, only a rather narrow range of U values is consistent with the pluralistic approach.

I believe that the case of the Red Queen is similar: except for a relatively narrow grey area, changing selection is either irrelevant, or can maintain sex without any help from deleterious mutation or any other process. Testing the Red Queen is more difficult than testing the Mutational Deterministic hypothesis: while mutation rates can be (we hope) measured indoors, fluctuating selection must be measured in nature. Still, this is not impossible.

I believe that in 10 years U will be known with good confidence for a range of organisms through (1) measuring the per nucleotide mutation rate μ (for which several approaches are possible) and (2) estimating, using the comparative analysis of moderately different genomes, the total genomic number of selectively important nucleotides. Currently, we know that $\mu \approx 2 \times 10^{-8}$ in humans, implying a total diploid mutation rate > 100 (there are $\sim 3.5 \times 10^9$ nucleotides in the human haploid genome) and $U > 1$, because there is little doubt that more than 1% of human DNA is selectively important (see Kondrashov, 1998). However, there is no real problem with maintaining sex in mammals, where genome imprinting makes reversal to asex impossible.

Thus, we need to measure U in *Drosophila melanogaster*, and this is within reach, because μ can be estimated in locus-specific tests, and its genome will be sequenced soon. Only if this measurement produces $0.2 < U < 0.8$, will I accept, with regret, the pluralistic explanation of sex.

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COMMENT

Hybrid theories of sex

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West *et al*. (1999) propose that we should examine the situations when more than one mechanism works to protect a sexual population from invasion by asexual clones. They think this pluralistic view is better than trying to choose only one theory for the advantage of sex and drop the others. In particular, they look for the joint action of theories considering mutation accumulation (the stochastic Muller's ratchet, and the mutational deterministic hypothesis of Kondrashov) and the effect of parasites (the Red Queen). As every species must fight against both parasites and deleterious mutations, it is a realistic situation. Note that, taken separately, all the hypotheses have difficulties with assigning a two-fold advantage to sex in the short run irrespective of population size. If two mechanisms act simultaneously, one does not need to assume extremely high mutation rates or severe effects and extreme transmission probabilities of parasites.

West *et al*. argue that different mechanisms may interact *not only simultaneously but synergistically*, i.e. the combined effect of the two mechanisms is greater than the sum of their contributions. We would like to comment on this aspect of the pluralistic view.

We think there are three main reasons why this synergism can exist: (i) the action of one mechanism supports the key assumption of the other, so the latter can work better; (ii) one mechanism slows down the spread of the clone in the short run so that the other has more time to render it a disadvantageous strategy; (iii) the selective forces assumed in the two mechanisms work against each other in the clone while in the sexual population they act independently, hence the clone cannot climb onto the adaptive peak. We comment below on some (maybe not all) possibilities for these types of synergistic interaction between theories.

(i) The Red Queen helps Muller's ratchet

As all the asexual clones originate from one founder individual and undergo a period with small population size at the beginning, they are prone to accumulate deleterious mutations stochastically according to Muller's ratchet. Because of the slowness of this process, the clone can reach a reasonable size well before its fitness decreases to half the average fitness of sexuals (required to counterbalance the two-fold cost of sex), but Muller's ratchet clicks rarely in a large clone. Howard & Lively (1994) showed that the Red Queen can help because parasites depress the size of the clone cyclically, and thus Muller's ratchet can accelerate at the bottlenecks.

We are not aware of any model in which the Red Queen is aided by some other mechanism in the sense of providing better conditions for it, provided the effect of parasites is more serious for the clone. One possibility might be to consider deleterious (null) mutations in the resistance loci of the host (e.g. in Hamilton's model) and then calculate the effect of Muller's ratchet on these loci.

Neither do we know about any model where the Red Queen provides the necessary conditions for the mutational deterministic hypothesis, except the brief note of West *et al*. that parasites might be the factor causing truncation selection against highly contaminated genomes.

(ii) The Red Queen and Muller's ratchet aid the mutational deterministic hypotheses

The problem with the mutational deterministic hypothesis occurs when an asexual clone invades a sexual population of modest size (10^3–10^4), because the clone can win before it reaches its higher equilibrium contamination and lower fitness (even with $U = 1$ and truncation selection when the equilibrium fitness of sexuals is more than two-fold). The effect of parasites slows down the spread of the clone providing more time for the deterministic advantage to develop and at the same time Muller's ratchet speeds up the deterministic process of mutation accumulation stochastically. The odd thing is that the latter aid given by Muller's ratchet is aided further by the Red Queen as we described in (i). Note that while it is true that Muller's ratchet can hardly doom a clone to extinction in case of truncation selection, it does decrease the time required to reach its equilibrium where the mutational deterministic hypotheses kills the clone.

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Muller's ratchet helps the Red Queen fighting against repeatedly occurring clones

Lively & Howard (1994) called the attention to the fact that parasites do not select for sex *per se*, but for genetic (clonal) diversity. If the clones occur repeatedly then it can happen that the first one has not yet been eliminated when the second or third one emerges. Thus clonal diversity develops and the sexual population has no further hope except in the case when some mechanism speeds up the elimination of the clones. Muller's ratchet is appropriate for this task because the clones oscillate and mutation accumulation accelerates at every bottleneck causing eventual mutational meltdown. In consequence, the probability that more than one clone exists at the same time decreases. In Lively and Howard's model the size of the sexual population was only 10^3 and they did not ask if the mutational deterministic hypothesis can give a similar aid. We think that it possibly cannot give much aid, because the deterministic mutational load difference develops only slowly, and unlike Muller's ratchet it does not speed up at the bottlenecks. Thus we do not know any model which shows that the Red Queen is helped by the mutational deterministic hypothesis.

(iii) Selective forces

Selective forces eliminating deleterious mutations and spreading a favourable one work against each other in an asexual poplation because of sochastically generated linkage disequilibrium, as Manning & Thompson (1984) and later Peck (1994) described well in the 'ruby in the rubbish' type of model. It is a kind of synergism between Muller's ratchet and the old Fisher–Muller theory.

A similar disadvantage of asexuality can occur if we think further on Manning's (1982) argument that frequency-dependent selection and the spread of a favourable mutation can work again antagonistically. As the Red Queen results in frequency-dependent selection, synergism may exist between it and the Fisher–Muller theory. This possibility has not yet been formally modelled.

What is missing from the picture? For example, the shifting optimum model of Maynard Smith and the models with patchy environment (Lottery, Tangled Bank) have not yet been considered in this pluralistic sense.

Summarizing, one can identify several cases when considering two models we can give a greater advantage to sex than the sum of the separate effects, but note that all the models mentioned above assumed sexual populations of modest size and in the majority of cases at least one of the interacting mechanisms is stochastic. So the aim declared in West *et al.*'s paper to find interactions among deterministic mechanisms working in large populations has not been completely achieved.

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COMMENTARY

A distinction between the origin and maintenance of sex

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origin;
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West *et al*. (1999) present a reasonable argument for the pluralistic view that multiple adaptive mechanisms may simultaneously, and even synergistically, favour sexual reproduction. However, they ignore a fundamental and potentially important distinction between the adaptive role of sex in extant organisms and the evolutionary origin of sex. If one seeks to explain the current utility of sex, then I am inclined to agree with their view that multiple factors contribute to its maintenance and prevalence. On the other hand, I suspect that one selective factor may have been important in the emergence of sexual organisms from an asexual ancestor (although I do not have a preferred candidate for what that single factor was). Even if several factors were involved in the origin of sex, they may have acted sequentially rather than simultaneously, so that each step along the way provided a solution to one problem (e.g. Maynard Smith & Szathmáry, 1995). This distinction between multiple factors acting simultaneously to maintain sex, versus a single factor or several sequential factors accounting for its origin, is merely a hunch on my part.

It often seems to be the case that evolutionary innovations are initially driven by one primary factor; but after an innovation has been integrated into an organism's way of life, the organism becomes multiply dependent on the continuation of that trait. For example, consider the origin of insect wings during the Devonian period (Kingsolver & Koehl, 1985). Small proto-wings may have been initially beneficial for thermoregulatory capacity. As the size of these structures increased, owing to change either in relative proportions or overall body size, they subsequently acquired aerodynamic properties that were beneficial in certain settings. Despite the initially one-dimensional selection to acquire the antecedents of wings, a thorough study of natural selection acting on these structures in extant organisms would probably find evidence to support multiple adaptive roles, including not only thermoregulation and locomotion but also courtship display in certain groups. Thus, selection against loss of a derived trait may often be more complex and multifaceted than was selection to produce that trait originally, as a consequence of the subsequent integration of the trait into the whole being.

That this distinction is potentially relevant to the evolution of sex can be seen more clearly by considering the recommendations that derive from the pluralistic viewpoint of West *et al*. (1999). They suggest focusing future research on a few biological systems to obtain a detailed picture of multiple selective forces and their interactions. They then state that it is '… highly important to estimate relevant parameters in sexual species. The form of selection must be different in sexual species than in species which are asexual …' This advice is quite sensible if one seeks only to understand the selective factors that are responsible for the maintenance of sexual reproduction in extant organisms. But it can be turned on its head with respect to understanding the evolutionary origin of sex. After all, sexual organisms evolved from asexual organisms that experienced the conditions – ecological and genetical – necessary to promote the emergence of sex.

The origin of sex can be addressed not only from a historical perspective (using comparative methods and evidence from palaeontology), but it can also be studied by performing experiments with extant asexual organisms to determine whether they fulfil essential preconditions for the emergence of sexuality according to a particular hypothesis. For example, in our own work

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(Elena & Lenski, 1997), we sought to test the mutational deterministic hypothesis for the evolution of sex. According to this hypothesis, sex is advantageous because it allows deleterious mutations to be more efficiently eliminated from a population, even one that is very large and hence not affected by the random drift that advances Muller's ratchet (Kondrashov, 1988). The validity of the mutational deterministic hypothesis depends on key assumptions being met, including a tendency toward synergistic interactions between deleterious mutations. That is, two or more mutations together should be worse, on average, than would be expected from their individual effects. To test this assumption, we used the bacterium *Escherichia coli*, an organism that reproduces asexually. [In nature, *E. coli* undergoes occasional recombination via parasexual processes, but the effective recombination rate is very low (Guttman & Dykhuizen, 1994).] We chose *E. coli* for this research for two important reasons. First, *E. coli* offers exceptional opportunities for genetical precision and statistical power. We constructed some 250 genotypes with different combinations of mutations, and we measured the fitness of each genotype relative to an unmutated common competitor. Second, and more subtly, we wanted to know whether there exists 'a general tendency for genetic architectures to exhibit synergistic epistasis among deleterious mutations' (Elena & Lenski, 1997). If such a tendency were manifest even in this asexual bacterium (and in other bacteria more generally), then this would fulfil an essential precondition for the evolutionary origin of sex according to the mutational deterministic hypothesis. In our study, we found abundant evidence for epistasis among deleterious mutations, but the interactions were not primarily synergistic in form. Thus, we rejected the general proposition that genetic architectures are structured such that there exists a substantial excess of synergistic interactions.

If one were to perform the same experiment, but using a sexual organism, then one might get a misleading answer with regard to the origin of sex, for the following reason. Imagine that sex evolved originally for some reason other than the one postulated by the mutational deterministic hypothesis. Once sex became integrated into the way of life of early sexual organisms, this may have allowed the subsequent evolution of a higher genomic mutation rate. This secondary change might reflect the fact that mutator alleles, which increase the genomic mutation rate, are penalized more directly in asexual than in sexual organisms (Leigh, 1970), or the fact that the conditions under which higher mutation rates promote more rapid adaptive evolution are restrictive in asexual organisms due to clonal interference (Gerrish & Lenski, 1998). In either case, a higher genomic mutation rate would produce a higher genetic load, which in turn might favour the evolution of a genetic architecture biased toward synergistic epistasis, because such an architecture reduces the equilibrium load of deleterious mutations in sexual – but not asexual – organisms (Charlesworth, 1990). *Voila*! If this hypothetical cascade of evolutionary events occurred, then one would find evidence among extant sexual organisms to support the mutational deterministic hypothesis for the evolution of sex, even though the effect that is postulated by this hypothesis only evolved later and had nothing to do with the origin of sex. (Let me emphasize that I am not arguing that this precise sequence of events unfolded in this manner. Rather, I offer this scenario to illustrate how secondary evolutionary change could lead to some mistaken inference concerning the origin of sex, especially if that inference rested entirely on studies of extant sexual organisms.)

West *et al*. (1999) themselves point out that 'The mutation rate … is generally selected to be lower in asexual species … [and] the form of epistasis between deleterious mutations is likely to differ between asexual and sexual species.' Indeed, they use these points to bolster their recommendation that parameter estimates used for testing various hypotheses should be obtained in sexual species. But they fail to realize that this recommendation is a two-edged sword, one that may cut differently depending on whether one seeks to understand the present utility or the evolutionary origin of sex.

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COMMENTARY

Individual control over reproduction: an underestimated element in the maintenance of sex?

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Introduction

West *et al.* (1999) convincingly argue that combining traditional hypotheses on the maintenance of sex into a pluralistic framework provides a more plausible explanation for the enigmatic success of sexuality. By merging (1) more efficient elimination of deleterious mutations with (2) better tracking of environmental changes (often parasites) and allowing for synergism between both, West *et al.* (1999) show that sexuality becomes much more robust against invasion by asexuality. Their approach abandons traditional attempts to find a single and sufficient explanation for sex. However, once accepting that a mixture of ingredients may be the best recipe to explain sex, we strongly suggest adding at least one more component. Here, we argue that the pluralistic approach (West *et al.*, 1999) could be further strengthened by not concentrating solely on population-level processes, but by encompassing the important role that individuals may play.

We focus on two assumptions that population geneticists often make and that are inherent to the hypotheses within the pluralistic framework, namely that offspring are produced (1) randomly and (2) without paternal care. Under these assumptions, offspring produced by a sexual female can be represented as a quality array of randomly produced progeny in a 1:1 sex ratio (Fig. 1, bold lines). For asexuals, this distribution is compressed to a single all-female class with some small variance due to mutation (not shown). Sex is favoured when the advantage of producing few, better adapted and less mutation-loaded offspring outweighs the cost of producing males plus the cost of producing low-fitness offspring. Under random mating, high-quality sexual individuals lose most as they are likely to have relatively poorer mates, whereas low-quality individuals will benefit as they are likely to have better mates. This equalizing effect limits the benefits of sex.

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However sex is not usually random. Sexual individuals can actively influence the quality of their progeny (Fig. 1, dashed lines) and data from behavioural ecology suggest that they do this specifically in an attempt to capitalize on the benefits and reduce the costs of recombination. This results in a net advantage of

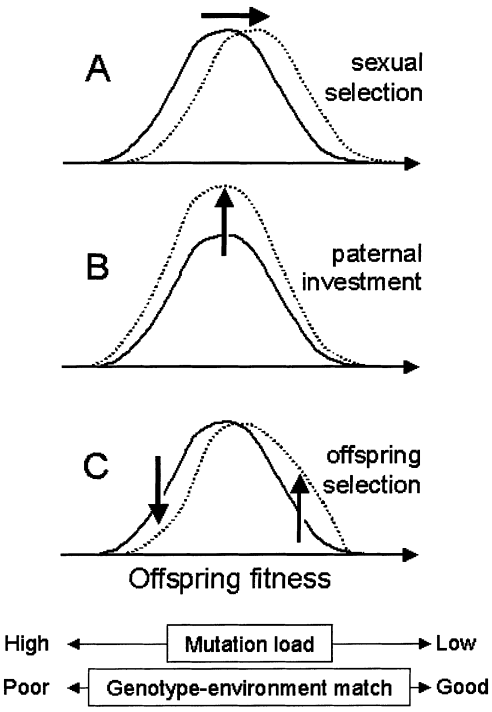

Fig. 1 Distribution of offspring quality that a sexual individual can expect to produce. Bold curve: sample distribution under random mating (shape arbitrarily chosen). (A–C) Three ways in which individuals can improve offspring fitness (dashed lines): (A) by selecting better mates, (B) by obtaining help in raising offspring and (C) by differential treatment of offspring. Option A is the only one that can move the upper range of the distribution. Options B and C can improve the shape of the distribution, but only within the set range. Options B and C are also available to asexuals, but since they start out with a very narrow distribution (not shown), the scope for improvement is accordingly limited.

sexuality at the population level. We suggest four processes sexual individuals have at their disposal to improve offspring quality and quantity. The first two (sexual selection and paternal care) are inherently linked since their strengths are inversely correlated (Trivers, 1972). The third one is specific for animals that can reduce the cost of producing males. The fourth summarizes post-zygotic effects.

Better offspring through sexual selection (Fig. 1A)

Differential selection on individuals for access to mates through mate choice and competition for mates is known to be particularly strong on traits that indicate parasite resistance and/or mutation load (often measured as developmental instability) (Hamilton *et al.*, 1990; Møller & Swaddle, 1997). Nonrandom mating in relation to these traits gives parents the possibility of actively choosing the appropriate mate to obtain offspring with fewer mutations and/or a better genome–environment match than under random mating (Fig. 1A; A. J. Pemberton, in preparation). For example, mammals actively choose mates of dissimilar MHC genotype and thereby increase the genetic variation in the immune response system of their offspring (Jordan & Bruford, 1998). Choosiness is particularly important for high-quality individuals in order to prevent their offspring from sliding back to a given population mean. The resultant assortative mating within this 'upper class' may force low-quality individuals to accept mates of a lower than average quality. At the population level, sexual selection has the potential to flatten the quality distribution of the progeny produced, thereby increasing exposure of deleterious mutations and enhancing the genotype–environment match in the next generation. If parent quality affects offspring number in addition to quality, sexual selection may also result in a larger skew towards fewer low-quality and more high-quality progeny.

Sexual selection is ubiquitous (Anderson & Iwasa, 1996) and takes place at all conceivable levels: from precopulatory interactions between individuals (Andersson, 1994) down to post-copulatory selection through sperm and pollen competition (Birkhead & Møller, 1998) or cryptic female choice (Eberhard, 1995). Parents may be able to select their own gametes. In mice, mutant spermatocytes appear to be selectively eliminated during spermatogenesis (Walter *et al.*, 1998). There is also growing evidence for nonrandom fusion of oocytes with sperm. For example, the combination of MHC alleles in eggs and sperm affects the fertilization efficiency (Wedekind *et al.*, 1996; Rulicke *et al.*, 1998). In addition to MHC-dependent mate choice (review by Jordan & Bruford, 1998) all this indicates active control over disease resistance in progeny, resulting in a better genome–environment match.

More young through paternal care (Fig. 1B)

Males are the main cost of sex, since the production of sons reduces a sexual population's intrinsic growth rate by a factor two (Maynard Smith, 1978). However, this only applies when males are mere sperm donors. When they also provide resources, they can increase the number of progeny a female produces, up to the point at which they may cancel out this two-fold cost. Although both low- and high-quality offspring may benefit equally from paternal care (Fig. 1B), it is the increased number of high-quality offspring that matters most, as it is this category that potentially enhances the spread of fitter gene combinations. In addition to support from males, a female can also receive help from her own offspring. Since helpers are not exposed to sexual selection while helping, they may be of use even if unfit for reproduction themselves, thus reducing the cost of producing low-quality offspring through sexuality.

Although asexual females may cash in on male assistance in sexual populations as well, males will be under strong selection to recognize asexuals or their (all-female) broods (Loyning & Kirkendall, 1996). Refined assessment of females may already be in place since males that invest heavily in offspring will be under selection to distinguish cheating from faithful sexual females (see below).

Reduced cost of males through skewed sex allocation (not illustrated)

Individual control over sex allocation is well developed in haplodiploid organisms (Wrensch & Ebbert, 1993). Here the sex ratio is typically skewed towards females to reduce local mate competition between brothers (Hamilton, 1967). As a consequence, the cost of males is also reduced. A female-biased sex allocation is also known from many hermaphrodites (e.g. Petersen & Fischer, 1996), and is expected when matings are rare (Greeff & Michiels, 1999) or when sexuality includes some selfing (Charlesworth & Charlesworth 1981). Alternatively, asexuals arisen from hermaphroditic ancestors may not have shut down their male function completely, and still pay the cost of male allocation (Weinzierl *et al.*, 1998). All these mechanisms will reduce the cost of reproduction in sexuals relative to that in asexuals, making asexual modes of reproduction less likely.

Progeny screening (Fig. 1C)

Parents also have post-zygotic mechanisms at their disposal to improve average offspring quality. First, by cutting investment in poor offspring, there are more resources for fitter progeny, skewing the distribution of offspring quality in favour of the fittest (Fig. 1C).

Selective abortion is a first mechanism to achieve this. There is actually evidence for higher abortion rates in humans among MHC-similar couples (Alberts & Ober, 1993), suggesting that investment in offspring with a less variable (presumably less flexible) immune system is avoided. Second, young may be fed differentially after birth. Third, parents may put an upper limit on overall investment and induce competition among their progeny, which again results in quality-dependent allocation of resources. Even when parents are completely ignorant about offspring quality within a brood, less fit young may serve as food for fitter sibs. Finally, a female that reproduces repeatedly may make maternal investment in each single brood dependent on the quality of the likely father of that brood, thus economizing resource allocation over her lifetime (Møller & Thornhill, 1998).

Discussion

There appear to be many ways in which individual behaviour can increase the benefits of sex beyond the random mating expectation. Empirical data from behavioural ecology indicate that nonrandomness is strong and widespread in sexual species. Trivers (1972) pointed out that the strength of sexual selection (our first point) is inversely related to the extent of paternal care (our second point). This is because males that do not offer paternal care, and can afford to spend more on attracting mates or fighting off rivals. It means that from our first two sets of mechanisms, at least one is likely to apply to any given system. It can therefore be no surprise that the mechanisms listed above have now been documented in many and diverse species groups, and are manifested at very basic levels, such as spermatogenesis or sperm–egg interactions. Moreover, they represent such direct advantages for the individual that employs them, that there must be strong selection in favour of them. The mere fact that individuals appear to base mating decisions on environmental adaptation and mutation load actually supports the pluralistic paradigm proposed by West *et al.* (1999). Looking at what individuals do may therefore offer an alternative approach to quantify the relative importance of mutations vs. genotype–environment matches.

'Individual quality-control' should be seen as a magnifying glass that exposes small defects in potential sexual partners. Importantly, it anticipates natural selection by parasites or mutations by stressing and unveiling unfavourable genotypes before they enter the next generation. As a result, sexual populations may actually be much fitter (and evolving faster) than expected under random mating, and the advantage asexuals need to compete with sexuals should accordingly be higher. For all these reasons, we think that 'individual quality-control' deserves a central place in a pluralistic theory of sex.

One cautionary note should be added at this point. Sexual reproduction results in a number of conflicts during reproduction because the parties, parents and offspring, are not genetically identical. These conflicts may reduce the efficiency of the mechanisms we propose. For instance, offspring may attempt to deceive their parents into believing that they are actually the best or most needy of food. But here selection for honest signals in progeny could reduce the risk. Similarly, the work on conflicts between males and females regarding certainty of paternity and paternal care (Harada & Iwasa, 1996) takes on a new light in this context. In these cases of conflict, the extent to which females can deceive males will be directly related to the degree to which sexual selection and paternal care act in unison. However, when males win, as is the case with paternally imprinted genes (Haig, 1993), the cost of sex can actually be higher.

Note that co-operative behaviour and offspring selection are two mechanisms that asexuals also have at their disposal to improve their reproductive success (Chao & Levin, 1981; Lively & Johnson, 1994). Yet, the potential advantage may be much smaller. First, in co-operative asexuals resources are primarily needed to produce own eggs and helping will be reduced to providing access to common resources or sharing common tasks such as alertness or defence. A helping male, on the other hand, does not invest in eggs, and will be able to provide his female not only with services, but also with resources that may allow her to produce more offspring. Second, genetic variance among asexual offspring is so limited that the maximum benefit of offspring screening is accordingly narrow for an asexual female.

Although most theoretical studies of the evolution of sexuality have actually acknowledged that nonrandom mating or parental care may influence the outcome of their models, the importance of these phenomena has always been minimized. We hope that pluralism at the population level will also lead to open-mindedness about the constituent individuals.

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COMMENTARY

Continued hope for a general explanation of sex

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Some natural phenomena may defy explanations in terms of single causes. The genetic basis for Haldane's Rule, for example, depends in part on whether the rule manifests itself in terms of hybrid inviability or sterility and varies among taxa (Orr, 1997). West *et al*. (1999) suggest that the widespread occurrence of sexual reproduction similarly requires multiple explanations. Currently, neither models of mutation accumulation nor those of changing environments, notably the Red Queen, can on their own fully account for the maintenance of sexual reproduction given the two-fold advantage of asexuality. The motivation for West *et al*.'s argument makes sense: creatures typically suffer from parasites as well as from deleterious mutations, and sexual reproduction may be the best way to fight their combined attack. This may be especially true, they argue, because both processes can complement each other and act synergistically. Encouraged by successful simulations of a number of specific cases (e.g. Howard & Lively, 1998), the authors invite further work in this area.

We agree with West *et al*. in that an integrated approach is required to explain why sex abounds. After decades of thorough exploration, all potentially contributing factors have probably been identified. The discovery of yet another mechanism that could instantly solve the riddle seems doubtful. However, we are sceptical that the way forward lies in the analysis of pairwise or multiple interactions of potential causes as proposed by the authors.

Imagine for the moment that specific interactions of deleterious mutation loads and parasite–host dynamics can account for the maintenance of sexual reproduction. The parameter space that covers these dynamics will not be easily defined but is extremely large. The list of factors that need to be specified includes the rate of mutation and the distribution of mutational effects, the genetic basis of the parasite–host interaction, as well as the resulting multivariate fitness functions. The Red Queen dynamics alone have been modelled in a variety of different ways (Otto & Michalakis, 1998), some or all of which may be fair representations of some natural systems. The crucial point is that any one of the resulting models is necessarily specific, and its analysis is complex. A thorough exploration of a general phenomenon via a collection of specific models seems a daunting task. If the widespread occurrence of sexual reproduction indeed required such a compartmentalized analysis, then we would be a long way from understanding the problem. This would quite simply be sad.

However, the situation may not be quite so bleak. The need for a collection of distinct models would arise if the region of the parameter space in which sex is favoured had a complicated shape and was possibly even disjunct. We find this hard to imagine. Why should the vast majority of taxa exist in exactly these specific pockets and branches of the parameter space and why should they restrict their movements to these regions as they diversify and speciate? It appears much more likely that the relevant region is large and continuous, in which case there should be a general explanation for the maintenance of sexual reproduction.

This argument does not deny the usefulness of specific models to explain the recurring yet isolated emergence of asexual lineages. West *et al*. call for detailed case studies aimed at parameter estimation. We agree that such studies would be extremely useful in order to understand just what sets the balance of forces in favour of asexuality in these cases. While the yardstick of a two-fold advantage for asexuals is a useful shorthand, it does not apply universally even in anisogamous species. The benefit of paternal care in some breeding systems is only one example. Depending on the genetic mechanisms that bring about the transition to parthenogenesis, the relative fitness of asexuals may be either smaller or larger than two. For example, a new parthenogen that arises through the duplication of one haploid genome of its sexual parent would be entirely homozygous. Aside from the obvious case of recessive lethals, there should be a fitness loss due to the large class of mutants that are slightly deleterious and partially recessive. The number of such mutations in the new parthenogen need not be large to melt away the two-fold advantage. A better understanding of the genetic mechanisms of asexuality seems therefore necessary. We believe that the explanations for particular cases of asexuality may well be varied. As a consequence, they differ from the reason why the vast majority of taxa reproduces sexually. We remain hopeful that an explanation for the maintenance of

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sexual reproduction can be found that matches the phenomenon in its generality.

In agreement with the authors, we believe that varying selection in heterogeneous environments plays a key role in this general mechanism. To take a stab at it, we would like to propose a slight modification of one of the existing theories. The most robust mechanism so far proposed and one which provides a generous and immediate advantage to sexual reproduction is the interaction between stabilizing and directional selection (Charlesworth, 1993). Stabilizing selection on a quantitative trait predictably builds up negative linkage disequilibria, which can impede the response to directional selection in asexual lines. In contrast, recombination breaks down these genetic associations, increases the genetic variance and thus facilitates adaptation to a moving phenotypic optimum.

The mechanism works well when the optimum for a trait moves steadily in one direction. The phenotypic mean then follows with some delay which is smaller in sexuals than in asexuals (Charlesworth, 1993). Reversals of the optimum and cyclically fluctuating environments make the conditions for sex more stringent, in part because large genetic loads are built up and in part because reversals imply that the optimum moves occasionally through the populations mean, thus weakening the advantage of sex at those times (Charlesworth, 1993). The steady one-directional movement of the optimum, however, appears implausible, because it predicts, for example, gradual changes in the fossil record, which are typically not observed (Charlesworth, 1993).

This problem might be alleviated if one allows for selection on more than one trait. For example, steadily moving selection might be distributed over a number of traits (Crow, 1992; Charlesworth, 1993). Under conditions that favour sexual reproduction, this would lead to a reduction in the rate of phenotypic change per trait, which should nevertheless accumulate with time. Kondrashov & Yampolsky (1996) consider a model of fluctuating selection in which the trait optima cycle with offset periods. This regime appears to generate unacceptably high lag loads. Alternatively, one could imagine that selection alternates through time among traits or sets of traits. For a given trait, intervals of selection in one direction would be interspersed with those of stabilizing selection. During the latter phases, the phenotypic mean for that trait could 'catch up' with the optimum and thus reduce the overall directional load. A persistent advantage to sexual reproduction would be assured as long as there is at all times a sufficient directional component to selection on at least one trait.

A number of conditions are critical for this explanation to apply. While the increase in genetic variance due to recombination aids adaptation to a moving optimum, it causes a segregation load for those traits currently under stabilizing selection. The net advantage of recombination across both of these processes would have to be high enough for sex to be maintained. Of course, this requirement applies similarly to the case of directional selection on a single trait, as long as one allows for some sort of organismal complexity. As in the single-character model, our scheme of intermittent directional selection per trait avoids the problems of sigmoidal reversals in the phenotypic optimum. Pleiotropy naturally sets an upper limit to the number of independent axes on which selection can act. Yet partial genetic correlations could possibly aid the proposed mechanism, in the sense that antagonistic selection could maintain the directional momentum when selection on one trait is relaxed. There should be some critical level of genetic correlation between a pair of traits above which the system reverts to the case of fluctuating selection on a single character. We realize that both analysis and critical test of this scheme would be extremely challenging and in this sense hardly preferable to the approach of West *et al*. Yet it might hold the promise of a step towards a general explanation.

The time-scale on which selection alternates between traits could be such that most of the response to selection would not manifest itself in measurable phenotypic change. This view of 'frustrated adaptation' may well be a typical feature of selection in heterogeneous environments. The target traits involved would range from morphology to metabolism and life history. Imagine a natural population that is in principle subject to abiotic stresses such as drought or cold, predators, competing species and, of course, parasites. All these selective agents operate on the existing genetic variance, but one of them may be the dominant factor at any one time. For example, predator density may be on the increase for some time, or temperatures may be cooling, or food levels may be lower in some years than others. As a consequence, the population permanently responds to selection in some direction. And yet sustained selection that leads to measurable phenotypic change in a given trait happens only rarely.

The proposed mechanism is very general in that all creatures have several to many phenotypic traits, and some of these should be sufficiently uncorrelated to serve as independent axes among which selection can alternate. Selection per trait need not be strong. Some minimal length of a given selection time interval may be required, but no further assumption seems necessary in this regard. Nor does one need to specify particular genetic interactions, as in host–parasite models. As long as there is a component of stabilizing selection, negative linkage disequilibria are built up as necessary and sufficient genetic prerequisites. The mechanism also explains recombination across all of the genome, rather than subsets of it where, for example, resistance genes might be clustered.

A basic tenet of ecological theory is the existence of limiting factors. If one of them is temporarily absent, another one will take its place. To the extent that there is

relevant genetic variance, these factors constitute selective agents. Viewed this way, populations constantly chase the Red Queen in *n* dimensions on a tortuous route and without reversals.

We believe that this idea deserves further study.

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COMMENTARY

A truly pluralistic view of sex and recombination

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Although West, Lively and Read recognize the challenge that sexual reproduction poses for evolutionary biologists, their pluralist approach is so narrow as to have little chance of meeting it. Other commentators will no doubt explore the roles of nondeterministic models and of empirical and experimental tests of these models. Here I expand the investigation beyond the traditional confines of population genetics and into phylogenetics, protistology, cell biology and molecular genetics. These fields contain much that is critical to unravelling the evolution of sex, and, because researchers in these areas are largely unaware that sex poses a problem at all, the onus is on those of us who appreciate the problem to extend our search for the answers. I also expand the approach; in addition to finding out how genes ought to be selected (in theory), or how they can be selected (in the laboratory), we must consider how they have been selected over real evolutionary time. Below I discuss three approaches: the cytology and molecular biology of meiosis, the broad context of processes that generate genetic variation, and the phylogeny of meiotic sex.

Cytological and molecular mechanisms

The control of recombination within the context of sexual reproduction has received a lot of theoretical attention (Feldman *et al*., 1996) However, when we examine the processes that control recombination rates, we find little that corresponds to the theory and much that contradicts it.

The cytological function of crossing-over

The primary control on the amount of meiotic recombination is chromosome number. Yet this character appears to vary almost randomly, affected more by accidents of chromosome breakage and fusion than by any selection on recombination. The other determinant of recombination rates, the frequency of crossing over, is highly regulated, but its regulation does not appear to reflect a need for optimum recombination of alleles. Instead crossover frequency and location appear to be constrained primarily by the mechanical role of the chiasmata formed by crossovers, which physically tie homologous chromosomes together and are required for their subsequent alignment and accurate segregation. Evidence that this segregation is the primary function of crossovers comes from the phenomenon of chiasma interference, which regulates the number of crossovers per chromosome arm, ensuring that each arm undergoes at least one and no more than a few crossovers, independent of the length of the arm or the number of genes it contains (a detailed discussion and references are given by Otto & Barton, 1997). We are left with a paradox: if recombination by reassortment is neutral, and recombination by crossing over exists mainly to permit meiosis, why bother with meiosis at all?

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The hotspot paradox

Two seemingly innocuous findings about the mechanism of crossing over combine to create an even more troublesome paradox. The first finding is that meiotic crossovers do not initiate at random positions, but at specific 'crossover hot-spots' distributed along chromosomes, with the sites used in any one meiosis randomly chosen from the existing hotspots (Smith, 1994). The second finding is that genetic information is destroyed and replaced at hotspot sites during initiation. Both molecular and genetic analyses show that a segment of DNA in the initiating homologue is degraded and replaced by copying the sequence of the other homologue, a process called gene conversion (Cao *et al*., 1990). Whenever two hotspot alleles differ in their activity, this conversion will favour the less active allele, and over many generations can cause elimination of all active alleles.

Until recently, it had been assumed that selection for the recombination and segregation benefits of crossing over would be strong enough to compensate for this loss. However, we have recently modelled the evolution of these recombination hotspots, demonstrating that gene conversion causes rapid elimination of active hotspot alleles even when opposed by the maximum possible segregation benefits of recombination (Fig. 1) (Boulton *et al*., 1997). We have developed a more sophisticated model (Israel and Redfield, manuscript in preparation) that incorporates multiple hotspots, multiple

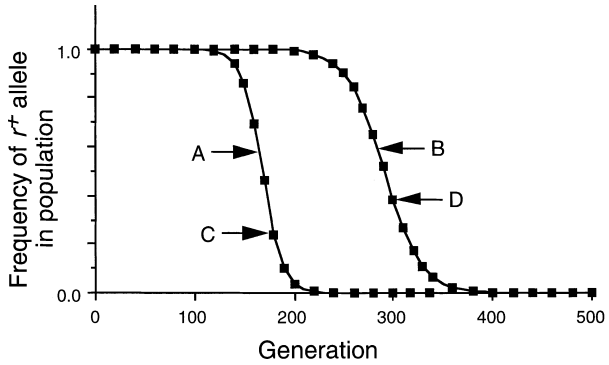

Fig. 1 Loss of active hotspot alleles in a computer simulation. The solid line A shows the loss of active hotspot (r^+) alleles due to the gene conversion associated with initiation of recombination. The solid line B shows the loss of hotspots when the conversion shown by A is opposed by the benefits of crossover-dependent chromosome segregation. The square symbols C and D show the loss of hotspots when the conversion in A and B is opposed by the benefits of genetic recombination between viability loci flanking the hotspot. See Boulton *et al*. (1997) for details.

chromosomes, and life cycles with alternation of sexual and asexual reproduction in both haploids and diploids. Our findings confirm that sites that initiate recombination unavoidably convert themselves out of existence.

We can see no simple resolution of this paradox. The experimental data that necessitate gene conversion are robust and widely accepted, but their impossible evolutionary implications appear equally irrefutable. We are forced to conclude that our theoretical understanding of recombination has no empirical foundation.

Implications for the evolution of sex

One very striking finding of the hotspot analysis is the disparity between the strength of the molecular and cytological consequences of recombination and the weakness of the genetic benefits of recombination. This is illustrated in Fig. 1, where the square symbols (analysis including recombination benefits) overlay the smooth lines (analysis excluding recombination benefits) (based on data from Boulton *et al*., 1997). Although these recombination benefits were greatly exaggerated in our analysis, they were nevertheless completely overwhelmed by the opposing molecular force.

This points to a fundamental problem with most population genetics work on the evolution of recombination. Models addressing genome-wide processes such as reduced accumulation of deleterious mutations are thought to be more realistic than those that consider only two viability loci and a modifier of their recombination. In these genome-wide models, the benefits of recombination can be large, and are often sufficient to overcome the benchmark two-fold cost of sex for females. However, at any one locus, the effects of biased molecular processes such as hotspot conversion and cytogenetic effects such as chromosome missegregation can be *much* stronger than the effects of genetic recombination. Population geneticists who ignore these effects may be constructing their models on foundations of sand.

The evolution of genetic variation

Evolution is a historically contingent process, and to understand sexual reproduction we must evaluate it in the context of other processes that generate genetic variation.

Recombination in bacteria

Bacteria have no processes comparable to sex. Not only are cell fusion and meiosis absent, they have no processes selected for producing recombinant genotypes. On the contrary, horizontal transfer of chromosomal genes in bacteria is rare, fragmentary and appears to occur only as a side-effect of processes selected for other functions, specifically transfer of parasitic plasmids and phages, uptake of DNA as a nutrient, and enzymes evolved for DNA replication and repair (Redfield, 1993; Morel *et al*., 1997).

This is not to downplay the evolutionary importance of the recombination that does occur in bacteria. Every sequenced bacterial genome contains many horizontally transferred segments, evidence of recurrent selective sweeps by recombinant ancestors (Lawrence & Ochman, 1997). Despite this, there is no evidence that such selection has had any effect on the processes that produce recombinants. Two factors probably account for this. First, most random recombination events will reduce fitness rather than increase it, so recombination may be a net cost rather than a benefit. Second, beneficial recombinants arise so rarely that they cannot influence the evolution of the genes that produce them because these genes are under constant strong selection for their immediate functions.

Mutation as a source of variation

This perspective on the evolution of genetic exchange in bacteria parallels our present understanding of the evolution of mutation rates. Although without mutation there would be no evolutionary change at all, selection on the processes that generate mutations appears to have acted entirely to *prevent* mutations rather than to facilitate them, no doubt because almost all non-neutral mutations are deleterious. The generality of mutation-prevention strategies is not contradicted by the occasional spread by hitchhiking of defective alleles of mutation-preventing genes ('mutator' alleles), which reflects only occasional decreases in the strength of selection against mutations (LeClerc *et al*., 1996; Sniegowski *et al*., 1997).

Eukaryote sexual reproduction

This perspective reveals sexual reproduction to be an oddity – the only genetic process that apparently evolved to *produce* random variation. The explanation is unlikely to be that the much higher efficiency of meiotic recombination provides benefits not available from the fragmentary bacterial processes because a small amount of recombination is sufficient to provide most of its genetic benefits (Hurst & Peck, 1996). The challenge is to understand why the genes causing this particular variation-producing mechanism, meiotic sex, have been favoured by selection. The examples of bacterial transduction and conjugation suggest we should be looking for nonrecombinational consequences of sex.

The phylogeny of sexual reproduction

Sex occurs in almost all eukaryote groups, but until we know its phylogenetic basis we cannot know what kind of an explanation it requires. If sex is polyphyletic, having become advantageous in different lineages independently, then different explanations might be appropriate. For example, perhaps sex succeeded in fungi because they have high mutation rates, and in plants because they have many parasites. But if sex is monophyletic, its persistence over more than a billion years in many diverse lineages requires a unified explanation with strong and flexible benefits. As discussed below, monophyly is supported by the available information, but only weakly.

Sex is ubiquitous and diverse

Figure 2 shows a simplified evolutionary tree, loosely based on small-subunit ribosomal RNA sequences. Sexual reproduction is typical of plants, animals, fungi and most other members of the 'crown taxa'. Within the crown taxa, sexual processes are remarkably diverse. In many groups, sex is an optional component of reproduction, induced under special and often poorly understood circumstances. Some are usually haploid with a zygotic meiosis, some diploid with a gametic meiosis. Some have clearly differentiated 'male' and 'female' gametes, others are isogamous. The ciliates have no separate gametes, instead diploid cells pair, undergo meiosis and exchange haploid nuclei. Many have multiple self-incompatible mating types (Doerder *et al.*, 1995). Commonly, one or another sexual stage is obligately linked to formation of a specialized cell type, for example an invasive stage or an environmentally resistant 'spore', selection for which confounds analysis of the benefits of sex. Some subsidiary lineages within the crown taxa lack sexual reproduction entirely – within plants and animals this is clearly due to secondary loss, as their common ancestry with many sexual groups is undisputed.

Outside of the crown taxa, the evidence for sex is sparser and not always compelling. Genetic exchange characteristic of sex has been demonstrated in trypanosomes in their insect host, but meiosis has not been observed, and their sister group, the euglenoids, are completely asexual(Gibson & Garside, 1991). Similarly, populations of the percolozoan *Naegleria* show the linkage equilibrium expected of sexual species, but sexual stages have not yet been seen. In the lineages thought to be oldest, almost all evidence for sexual reproduction comes from the painstaking microscopic observations of L. R. Cleveland on preparations of the microbial communities from the hindgut of wood-eating roaches and termites (Cleveland, 1956). The hypermastigote and oxymonad protists in these communities appear to undergo sexual reproduction in response to the hormone ecdysone which triggers moulting in their hosts.

Eukaryote phylogeny is unresolved

The ideal approach to the evolutionary history of sexual reproduction would be to map reproductive characters, such as the presence and characteristics of meiosis and the involvement of specific genes, onto a phylogenetic tree of organismal relationships, itself determined by comparing the sequences of conserved genes unrelated to mode of reproduction. Unfortunately, the dream of being handed a reliable eukaryotic phylogeny is receding, as our tree-building colleagues invoke horizontal gene transfer on a massive scale, and warn of branch-length artefacts caused by variation in rates of sequence divergence (Ribeiro & Golding, 1998). A true phylogeny will emerge only slowly and will depend on contributions from phenotypic characters as well as on sequence comparisons of multiple kinds of genes.

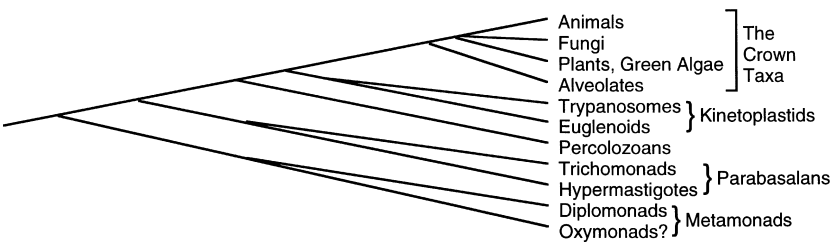

Fig. 2 Eukaryote phylogeny. Many groups have been omitted, and the relationships shown here are not yet considered to be stable. A much more detailed tree based on small-subunit rRNAs is given by Cavalier-Smith & Chao (1996).

The unreliability of deep eukaryote trees is emphasized by the recent reinterpretation of the Microsporidia. The small-subunit ribosomal RNA sequences of these parasitic protists had placed them close to the base of the eukaryote tree, where their baroque sexual practices made them objects of great interest to the cognoscenti. However, subsequent analysis of large-subunit rRNA and several protein-coding sequences has shown that they belong well within the crown, as close relatives of the fungi (Keeling & McFadden, 1998).

Sex in early eukaryotes

Sex has generally been considered to be monophyletic in all eukaryotes, both because it is so common and because of the apparent conservation of the synaptonemal complex involved in meiotic chromosome pairing (Raikov, 1995). Monophyly would imply that sexual reproduction first arose in a protist, a unicellular eukaryote whose primary mode of reproduction was asexual (mitotic). Thus sex would be originally an optional component of the reproductive cycle, presumably occurring in response to one or more signals arising intracellularly or from the environment. These issues are potentially of enormous importance in our understanding of how sex evolved and is maintained. Intervening asexual generations allow selection to act repeatedly on the products of recombination, and so can amplify its effects. Regulation of the switch to sexual reproduction can prevent sex from occurring when it is unlikely to generate a benefit. For example, sex might be induced by metabolic stress associated with high mutation loads, so that cells carrying high loads of deleterious mutations benefit from sex, and mutation-free individuals benefit from abstaining (Redfield, 1988).

On the other hand, the ancestral states of many traits will be harder to resolve. Branches thought to be early include both diploids and haploids, and both isogamous and anisogamous species (Cleveland, 1956). Some protists thought to branch deeply in the tree are reported to have 'one-step' meiosis in which homologues segregate without prior replication, others appear to use meiosis as part of an asexual ploidy cycle (Hollande & Caruette-Valentin, 1970).

One limit to phylogenetic inferences about sexual reproduction is the exploitation that sex permits. The sexual cycle provides ideal conditions for horizontal transmission of intracellular and molecular parasites such as transposable elements and meiotic-drive genes. We know that these elements are ubiquitous in modern genomes, and that they often exert strong pressures contrary to the cells' best interests (Hurst, 1995). The cumulative effects of these are certain to have repeatedly reshaped sexual systems, and, if genetic transfer in bacteria is any guide, may even have been responsible for their success.

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COMMENTARY

Genetic polarization: unifying theories for the adaptive significance of recombination

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The paper by West, Lively and Read (1999) advocates a pluralistic approach to the adaptive significance of recombination, i.e. that a combination of models may better explain the advantage of recombination compared with any single model. This certainly makes intuitive sense since there is no *a priori* reason to expect a single benefit to recombination. The paper focuses primarily on three processes: (a) deterministic accumulation of beneficial mutations in response to chronic antagonistic coevolution (Red Queen); (b) deterministic accumulation of deleterious mutations due to mutation-selection balance (mutational load) and, to a lesser extent, (c) stochastic accumulation of deleterious mutations (Muller's ratchet). Here I provide a simple genetic argument that reinforces the authors call for a pluralistic approach, i.e. I show that the advantages to recombination based on the Red Queen, mutational load and Muller's ratchet are all a direct consequence of the same underlying genetic property that is common to all nonrecombining populations – so if one process operates they should all operate, at least under the appropriate permissive conditions.

The common property of all nonrecombining populations is the movement of new deleterious mutations among individuals within the fitness distribution of a population. Substantial heritable variance in fitness among individuals is expected in all natural populations due to recurrent deleterious mutation (in addition to other factors). When recombination is present, the combination of syngamy, segregation and intrachromosomal recombination causes new deleterious mutations to move bidirectionally to better and to worse genetic backgrounds each generation. But when recombination is absent, each new deleterious mutation is trapped in its recipient genome, moving it unidirectionally toward lower fitness. This generates a continuous 'current' of new deleterious mutations flowing within the fitness distribution from greater to lower fitness classes. Recurrent mutation causes lineages from the highest-fitness class to flux unidirectionally through the population like water down a slow-motion stream.

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Eventually all genomes in the population are multiply mutated descendants from the highest-fitness class.

To accumulate in a nonrecombining population, a new mutation must make its way to the headwaters (highest-fitness class) of this stream of decaying genomes. The only way to reach the headwaters is to be introduced (fortuitously) via mutation into the highest-fitness class, or a neighbouring high-fitness class. All other new mutations (beneficial or detrimental) are trapped in inferior genetic backgrounds and thereby deterministically eliminated. Rare reverse and compensatory mutations occasionally reverse the unidirectional flow of deleterious mutations, but this effect is miniscule, analogous to turbulence occasionally moving a pebble a short distance upstream. The term 'genetic polarization' denotes the virtual unidirectional flow of new deleterious mutations (see for review, Rice, 1996). Many hundreds of mutations of very small effect are expected to accumulate in a population from a number of sources, for example: (a) nonpreferred codon mutations (selective disadvantage $\leq 10^{-5}$, Akashi *et al.*, 1998), (b) transposable element inserts (average selective disadvantage $\approx 10^{-4}$, Charlesworth *et al.*, 1992) and (c) mutations of nonessential genes (many selection coefficients $\approx 10^{-3}$, Thatcher *et al.*, 1998). The large number of accumulated mutations causes the expected number of individuals in the highest-fitness class to be quite small (one to a few individuals). This is expected even when the genome-wide mutation rate is small (e.g. 0.1) and the populations size is very large (i.e. of the order of 10^6 or higher).

Genetic polarization has two major consequences: (1) it greatly reduces the effective size of a nonrecombining population, i.e. the effective size is the number of individuals in the highest-fitness class and the neighbouring high-fitness classes (Manning & Thompson, 1984; Charlesworth, 1994; Barton, 1995), and (2) it constrains the highest-fitness class to rely solely on its own reproduction to persist, rather than being produced globally by syngamy, recombination and segregation from the population as a whole, as is the case for a recombining population (Rice, 1998).

The greatly reduced effective size of a nonrecombining population, compared with its sexual counterpart, causes

a nonrecombining population to suffer the same evolutionary maladies as small isolated sexual populations: beneficial mutations accumulate more slowly and deleterious mutations accumulate more rapidly. This occurs because selection is ineffectual unless the selection coefficient (s) is greater than the reciprocal of the effective size, i.e. $s > (1/N_e)$. From this perspective the Red-Queen advantage to a recombining population is a consequence of it being able to coevolve more effectively against enemies due to an increased efficiency in recruiting new beneficial mutations (Manning & Thompson, 1983; Charlesworth, 1994; Peck, 1994).

The Muller's ratchet disadvantage to a nonrecombining population is also a consequence of its miniscule effective population size, relative to a similar but recombining population. This small effective size causes a greater portion of the spectrum of new deleterious mutations to accumulate, i.e. deleterious mutations accumulate when $s > 1/N_e$, and since N_e is far smaller for a nonrecombining population, more new mutations can and will accumulate in the absence of recombination (Manning & Thompson, 1983; Charlesworth, 1994). It is sometimes argued that Muller's ratchet does not operate in large populations, but this conclusion is a modelling artefact that occurs when the selection coefficients (s) of all mutations are defined to have the same value (e.g. set equal to the average value of s). When variable selection coefficients are permitted, with a high density of very small selection coefficients (such as those from nonpreferred codons, transposable element inserts and lesions to nonessential genes), then the expected number mutations per genome is >100, causing the expected number of individuals in the highest extant fitness class to be one or a few individuals, and thus Muller's ratchet is expected to be ubiquitous.

The cost of the mutational load in nonrecombining vs. recombining populations also can be expressed as a direct consequence of genetic polarization. At the outset, I need to point out that I do not think that mutational load is a currency that is easily translated into the competitive ability of nonrecombining vs. recombining populations. Much of the mutational load may be expressed by selection on traits that do not directly translate into changes in the vital statistics or competitive ability of a population. For example, compensatory factors (such as increased survival of sibs when competition for parental investment is reduced by mortality within a clutch) may offset the impact of mutational load on population growth rate. As a consequence, the fact that the mutational load of one population is higher than that of another does not guarantee that the population will be competitively inferior. The major significance of mutational load occurs when it is sufficient in a nonrecombining population, but not in a recombining population, to deterministically lead to its extinction. It is this context on which I focus below.

Genetic polarization isolates the highest fitness class from the remainder of a nonrecombining population, since newly mutated individuals flow out, but not into, this class (with the exception of rare beneficial mutations in the classes neighbouring the highest-fitness class). It must therefore be maintained exclusively via its own reproduction. Assuming a Poisson distribution of new deleterious mutations, only a fraction e^{-U} (where U = the genome-wide deleterious mutation rate) do not receive new mutations, and hence the net reproductive rate of the least mutated class must be the reciprocal of this value (i.e. it must be e^{U}) to prevent deterministic mutation accumulation via recurrent extinction of the highest-fitness class (Kimura & Maruyama, 1966). When the net reproductive rate of the fittest class is less than e^{U}, then the mutation load is intolerable since the highest-fitness class is not self-sustaining and this leads to open-ended, deterministic mutation accumulation and eventual extinction. In a recombining population, the least mutated class is reconstituted each generation from the offspring produced from the population as a whole. When there is reinforcing epistasis, buffering epistasis and/or positive assortative mating for fitness (only weak levels are needed), then syngamy, segregation and recombination within the population at large builds the least mutated class faster than it would have reproduced itself via its own clonal reproduction, and recombining populations can resist open-ended deterministic mutation accumulation (i.e. tolerate a higher mutational load) at genome-wide mutations levels where their nonrecombining counterparts cannot (Rice, 1998).

In summary, the genetic polarization of nonrecombining populations guarantees the simultaneous operation of all of the processes discussed in the paper by West *et al.*: the Red Queen (unless the biotic environment is not antagonistically coevolving, which seems unlikely in any natural environment), Muller's ratchet (ubiquitous application), and mutational load (unless U is so small that the equilibrium mutational load is tolerable in both the recombining and nonrecombining populations). All populations are finite, and hence all three processes are expected to operate in all natural populations.

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Is sex in the details?

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The architect Mies van der Rohe is supposed to have said 'God is in the details'. I have always taken this to mean that life, substance and satisfaction are to be found (according to van der Rohe) in the concrete execution of a plan (that is, in the ways the particulars fit together and interact), rather than in the grand conception itself, which is necessarily abstract and therefore vague. Sex has been the grand problem of evolutionary biology for two decades. West, Lively and Read (1999) bring to full consciousness a long-standing tension in thinking about sex. This is not the familiar tension between ecological and mutational theories of sex. Instead, it is a tension between purist and integrationist approaches to the whole problem. West *et al*. propose to change the terms of the debate in ways that could have interesting and therapeutic consequences. Surely many of us have long accepted that the Red Queen and the Grim Mutator both seem likely to play significant roles in the maintenance of sex, yet we have also looked forward to a Decisive Answer in which one actor would prevail over the other. West *et al*. call attention to the inconsistency in this view.

Those who obsess about sex tend to be zoologists. We easily forget that plants defined the problem. Many angiosperms are self-compatible hermaphrodites that can self-fertilize a little, or a lot, or any level in between. In addition, many perennials can reproduce vegetatively. For such species there are no qualitative developmental or genetic barriers to incremental (and in the end, profound) retreats from sex (see Bell, 1982). Thus, many species that remain fairly sexy must do so in the face of easy access to greater asexuality. Their addiction to varying but significant levels of outcrossing should force even hopelessly unreconstructed zoocentrists to admit that ecology must explain some of the variance in rates of outcrossing and vegetative reproduction, and that for many species, sex isn't needed every generation (see Hurst & Peck, 1996). A smaller number of self-compatible hermaphroditic animals (West *et al*. mention the nematode *Caenorhabitis elegans*) illustrate the same point. This 'balance argument' (Williams, 1975; Maynard Smith, 1978) was advanced to show that sex must be advantageous in the short term. It also shows that ecology must be part of the explanation, because populations or closely related species that differ greatly in effective outcrossing rates, as some do, cannot plausibly do so (at least not in general) because they differ greatly in their underlying mutation rates, which must usually be similar.

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Unconditionally deleterious mutations must also be important, and West *et al*. review several lines of evidence that support this view. An additional line of evidence derives from well-established differences between the fixation probabilities of synonymous and nonsynonymous mutations (see Kondrashov & Crow, 1993; Crow, 1995; Drake *et al*., 1998; Eyre-Walker & Keightley, 1999). Synonymous nucleotide substitutions are typically about five times more likely to fix than nonsynonymous substitutions, on average, as estimated from comparisons between hundreds of orthologous genes in various taxa, especially rats and mice (e.g. Makalowski & Boguski, 1998). This implies that at least 4/5 of all mutations that change an amino acid must be deleterious. If mammals have about 50 000 genes averaging 2000 bp in length, then a typical mammal has around 10^8 functional base pairs. If even half of these nucleotides (5×10^7) were capable of mutating to deleterious states, and if the average mutation rate were around 4×10^{-9} per nucleotide per generation, then there would be $20 \times 10^{-2} = 0.2$ deleterious substitutions per haploid genome per generation. This number may substantially underestimate the overall deleterious mutation rate in most mammals because the per *generation* nucleotide substitution rate is undoubtedly larger than 4×10^{-9} in many species (Drake *et al*., 1998), especially those with long lifespans (e.g. Eyre-Walker & Keightley, 1999), and there are other classes of mutations (e.g. indels, including transposon hops). So deleterious mutations must go at least some distance toward supporting sex in many taxa, even if (perhaps) they do not do so, by themselves, in very many cases.

Given these well-known facts, how can anyone *not* be a pluralist? Why should West *et al*. feel compelled to argue the case? For one thing, it is necessary to establish that mutational and ecological factors may interact cooperatively to favour sex, and West *et al*. discuss this issue at length. At another level, it may also be necessary to relieve some physics envy. Simple, general, cleanly testable theories are beautiful. A universal process that could explain a pervasive pattern and that could be tested by a single decisive experiment would be a kind of

dream. Sex has seemed such a dream. But like many initially sweet dreams, this one could turn complicated, even ominous, and then wake us up. The real world may be messy, but fortunately, it may ultimately be more informative than what we would have been left with had the dream come true.

In adopting a pluralist stance we are encouraged to see more than just a qualitative contrast between sex and asex (distributed taxonomically in patterns influenced, perhaps, by several factors including ecology and mutations, and shaded, perhaps, by partial retreats such as selfing, cloning, and cyclical parthenogenesis). We are also encouraged to see (and to need to explain) continuously varying degrees of sexiness *within* species that practice sex in every generation. For example, rates of mutation and rates of recombination both appear to vary by an order of magnitude on individual mammalian chromosomes (Wolfe *et al.*, 1989; Nachman & Churchill, 1996; McVean & Hurst, 1997; Makalowski & Boguski, 1998; Nachman *et al.*, 1998). Interestingly, genes that seem to experience relatively high mutation rates (high-K_S genes) fix *disproportionately* more amino-acid substitutions, on average, than those with relatively low mutation rates (Fig. 1). This pattern would seem to suggest that many high-K_A genes may be located in chromosomal regions where high rates of mutation and low rates of recombination lead to greater than average numbers of slightly deleterious fixations for genes throughout the region, owing to background selection or to hitchhiking with linked adaptive mutations (see Barton & Charlesworth, 1998; Charlesworth & Charlesworth, 1998a,b). Alternatively, if the variation in K_S is *not* caused largely by regional variation of the mutation rate, then it must be caused by variation in the coalescence times of rat and mouse orthologs. But this would seem to require that there be strongly protected polymorphisms at an implausibly large proportion of all loci (or that most 'orthologs' are really paralogs). The various possibilities could be investigated by comparing levels of polymorphism, divergence and local recombination for genes from different parts of the joint distribution of K_S and K_A, and for chromosomal neighbours of those genes.

Why does such variation occur? If mutation is bad and sex is good, then why are they not equally bad and good for all genes in a genome? As always, the answer must be 'tradeoffs' (see McVean & Hurst, 1997; Drake *et al.*, 1998). But tradeoffs between what, balanced by what mechanisms, and in whose interests? These and other, more general questions might be illuminated by studies of quantitative variation in sexiness within genomes, where the differences occur on backgrounds well controlled for population and phylogenetic history. If such approaches prove successful, then the goddess of sex may turn out to speak less through oracles than through storytellers.

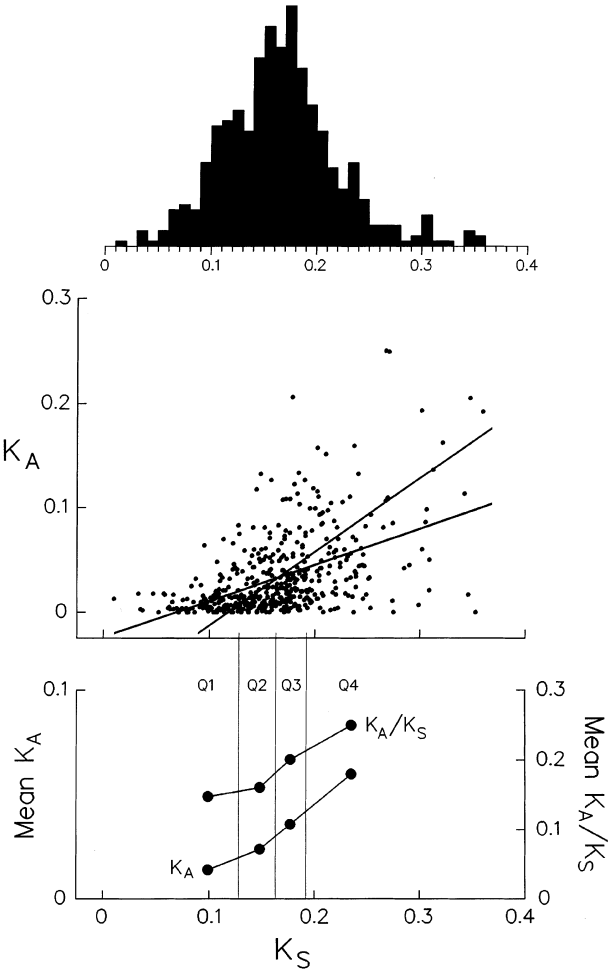

Fig. 1 The distribution of synonymous nucleotide substitutions (K_S) and the joint distribution of synonymous and nonsynonymous (K_A) substitutions for 465 orthologous gene pairs in rat and mouse. Substitutions were estimated by Makalowski & Boguski (1998) as part of a comprehensive survey of orthologous sequences from humans and rodents. The scatterplot shows K_A as a function of K_S. The linear regression (shallower slope) and reduced major axis (steeper slope) both pass below the origin, indicating that genes with high values of K_S tend to have disproportionately high K_A. This accelerating relationship between K_A and K_S is shown more clearly in the lower panel, where mean values of K_A and of the ratio K_A/K_S are shown for each of the four quartiles in K_S. On the null hypothesis, average K_A/K_S ratios would be expected to decline with increasing values of K_S because K_S and K_A are measured with error and K_S appears in the denominator of the ratio. Thus the observed positive relationship underestimates the real relationship, because it is contaminated by an artifactual negative correlation. Even so, the observed increase is formally significant by various criteria. For example, the mean $\log(K_A/K_S)$ values for nonadjacent quartiles differ significantly by two-tailed *t*-tests, as do those for Q3 vs. Q4, and for the combined upper and lower halves of the distribution (Q1 + Q2 vs. Q3 + Q4, $P < 0.00002$).

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Sex may need more than one

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The positive replies to our original paper (West *et al.*, 1999) leave us in the pleasurable position of being able to keep our final comments short.

Kondrashov (1999) demonstrates what Seger (1999) called 'physics envy'. The idea that the Mutational Deterministic hypothesis can be easily validated or rejected, once the minimum rate of deleterious mutations per genome per generation (U) is known, sounds great, especially if the magical number is $U > 1$. However, quantitative predictions (and assumptions) are almost always model-specific, so that there is unlikely to be a single, uncontroversial value, even if we ignore the large confidence limits that are placed on estimates of U (see West *et al.*, 1999). For instance, with reasonable levels of epistasis, a value of $U > 1.5$ is required (Charlesworth, 1990). If stochastic effects (which are unavoidable during the early phases of any clonal invasion) or variation in the extent of epistasis are included, then $U > 2.0$ is required (Howard, 1994; Otto & Feldman, 1997). In addition, irrespective of the value of U, the Mutational Deterministic hypothesis absolutely requires synergistic epistasis between deleterious mutations (Kondrashov, 1982). The idea that a single value of U will resolve the issue seems to us somewhat optimistic.

We agree with Kondrashov (1999) that our particular form of pluralist explanation is required for only a small fraction ($\approx 0.2 < U < \approx 2.0$) of the *entire* parameter space ($0 < U < \infty$). However, that 'small fraction' is the *relevant* parameter space: it is where the majority of estimates of the mutation rate in sexual species fall (West *et al.*, 1999). A pressing goal now is to determine the virulence of parasites in the wild, and whether they in fact evolve to infect locally common host genotypes. If the latter is not true, or if parasites are not sufficiently virulent to drive host gene frequency dynamics, then both the parasite-driven Red Queen and our particular form of pluralism are falsified.

We agree with Lenski (1999) that different factors may be responsible for the evolutionary origin and maintenance of sex. As we said, our discussion concerned the maintenance of sex. We also agree that experiments with *Escherichia coli* offer an exceptional opportunity to test for 'a general tendency for genetic structures to exhibit synergistic epistasis among deleterious mutations' (Elena & Lenski, 1997). We note, however, that such experiments cannot test whether synergistic epistasis occurs in a type of organism where sexual reproduction predominates. Do larger, more complex genomes with higher mutation rates lead to synergistic epistasis (Szathmary, 1993; Falush, 1998; Hurst & Smith, 1998)? Do the higher numbers of parasites in larger species help cause truncation selection against individuals with large numbers of mutations? Estimating relevant parameters can be much harder in more complex and sexual species (West *et al.*, 1998), but such results are crucial. We hope eventually to have a range of estimates of the mutation rate and the extent of epistasis from a number of sexual and asexual species, so that a whole slew of more subtle questions can be addressed.

Redfield's (1999) comments give us the opportunity to make the following points, orthogonal to our discussion of plurality. (1) Theoretical models suggest that the rate of crossing over is far more important than chromosome number in determining the effective amount of recombination (Burt, unpublished observations). (2) Variation in recombination rates across species are consistent with Red Queen and mutational models (Burt & Bell, 1987;

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Charlesworth, 1987). (3) Recombination 'hot-spots' could be maintained if: (a) they have some other function which helps maintain them, or (b) the use of the sequence as a recognition site is recent and temporary, with intragenomic conflict leading to a form of coevolution between recombination sites (cis) and recombination machinery (trans) (Burt, unpublished observations). (4) It is not surprising that sex occurs more often in the higher eukaryotes, where species are bigger (more parasites) and have larger genomes (more mutations). (5) We need to know whether asexuals are derived from sexual ancestors, and in many cases the phylogeny of a genus is enough, rather than a complete phylogeny of the eukaryotes. Finally, (6) Redfield appears to be advocating a basic philosophy that complex adaptations can be understood by extrapolating from mechanism. We see no precedent for this. For example, the adaptationist theory of sex allocation is perhaps the most qualitatively and quantitatively successful area in evolutionary biology; here, most insight has come from studying evolutionary ecology (population structure, male & female fitness functions) rather than sex chromosomes and eukaryote phylogeny (Charnov, 1982; Godfray & Werren, 1996).

Prompted by Butlin *et al*. (1999), we reiterate the following. First, we did not 'ignore the variety of reproductive modes found in nature.' We said that: (a) correlational studies will not be able to tell us the relative importance of mutations and environmental factors, and that (b) different mechanisms may work at different levels (see also Gouyon, 1999; Birky, 1999). Second, we did not restrict the Red Queen hypothesis to parasites. We said that: (a) the Red Queen works best through biotic interactions – abiotic changes are unlikely to lead to fluctuating epistasis on the correct time-scale (Charlesworth, 1976; Barton, 1995; Peters & Lively, in press); (b) usually parasites are assumed to be the biotic factor, but there are other possibilities such as host immune response (Gemmill *et al*., 1997); and (c) parasite models predict the majority of observed within- and between-host patterns of sexuality (the references of some of the large number of relevant correlational studies were given in the sixth paragraph of our introduction). Third, we agree wholeheartedly that there are many empirical issues that need to be addressed and parameterized, such as the diversity and turnover rates of clones and the apparent persistence of 'ancient asexuals'. Some of these need to be accounted for by any complete theory of the evolutionary maintenance of sex. We suggest that pluralism provides the most productive route to such a theory.

In fact, as we pointed out, searching for a single mechanism could be counterproductive (see also Crow, 1999; Gouyon, 1999; and for the general case, Hilborn & Stearns, 1982). We acknowledge that pluralism is not an easy approach and, like Kondrashov (1999) and physicists, we prefer simple answers. But a pluralistic approach, with explicit theory and data that at least consider the joint action of ecology and mutations, should provide the most useful advances. Crucially, such an approach does not rule out the possibility that one theory might prove sufficient.

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