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

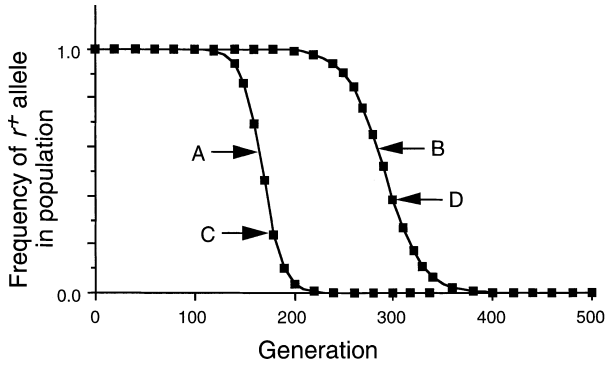

Fig. 1 Loss of active hotspot alleles in a computer simulation. The solid line A shows the loss of active hotspot (r^+) alleles due to the gene conversion associated with initiation of recombination. The solid line B shows the loss of hotspots when the conversion shown by A is opposed by the benefits 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.

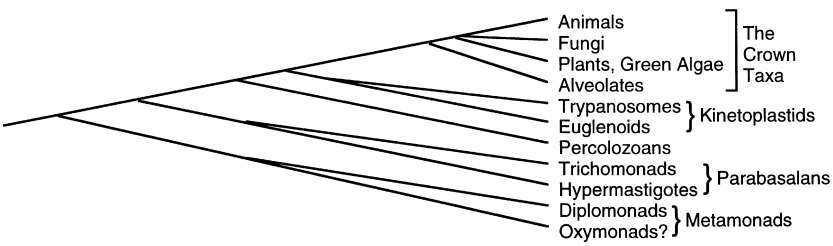

Fig. 2 Eukaryote phylogeny. Many groups have been omitted, and the relationships shown here are not yet considered to be stable. A much more detailed tree based on small-subunit rRNAs is given by Cavalier-Smith & Chao (1996).

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.

References

Boulton, A., Myers, R.S. & Redfield, R.J. 1997. The hotspot conversion paradox and the evolution of meiotic recombination. *Proc. Natl. Acad. Sci. USA* **94**: 8058–8063.

Cao, L., Alani, E. & Kleckner, N. 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisae*. *Cell* **61**: 1089–1101.

Cavalier-Smith, T. & Chao, E.E. 1996. Molecular phylogeny of the free-living archezoan *Trepomonas agilis* and the nature of the first eukaryote. *J. Mol. Evol.* **43**: 551–562.

Cleveland, L.R. 1956. Brief account of the sexual cycles of the flagellates of *Cryptocercus*. *J. Protozool.* **3**: 161–180.

Doerder, F.P., Gates, M.A., Eberhardt, F.P. & Arslanyolu, M. 1995. High frequency of sex and equal frequencies of mating types in natural populations of the ciliate *Tetrahymena thermophila*. *PNAS* **92**: 8715–8718.

Feldman, M.W., Otto, S.P. & Christiansen, F.B. 1996. Population genetic perspectives on the evolution of recombination. *Ann. Rev. Genet.* **30**: 261–295.

Gibson, W. & Garside, L. 1991. Genetic exchange in *Trypanosoma brucei brucei*: variable chromosomal location of housekeeping genes in different trypanosome stocks. *Mol. Biochem. Parasitol.* **45**: 77–89.

Hollande, A. & Caruette-Valentin, J. 1970. Appariement chromosomique et complexes synaptonematiques dans lesnoyaux en cours de depolyploidisation chez *Pyrsonympha flagelata*: le cycle evolutif des Pyrsonymphines symbiontes de *Reticulitermes lucifugus*. *Contes Rend. Acad. Sci.* **270**: 2550–2555.

Hurst, L.D. 1995. Selfish genetic elements and their role in evolution: the evolution of sex and some of what that entails. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **349**: 321–332.

Hurst, L.D. & Peck, J.R. 1996. Recent advances in understanding of the evolution and maintenance of sex. *TREE* **11**: 46–52.

Keeling, P.J. & McFadden, G.I. 1998. Origins of microsporidia. *Trends Microbiol.* **6**: 19–23.

Lawrence, J.G. & Ochman, H. 1997. Amelioration of bacterial genomes: rates of change and exchange. *J. Mol. Evol.* **44**: 383–397.

LeClerc, J.E., Li, B., Payne, W.L. & Cebula, T.A. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* **274**: 1208–1211.

Morel, P., Cherny, D., Ehrlich, S.D. & Cassuto, E. 1997. Recombination-dependent repair of DNA double-strand breaks with purified proteins from *Escherichia coli*. *J. Biol. Chem.* **272**: 17091–17096.

Otto, S.P. & Barton, N.H. 1997. The evolution of recombination: removing the limits to natural selection. *Genetics* **147**: 879–906.

Raikov, I.B. 1995. Meiosis in protists: recent adv. and persisting problems. *Eur. J. Protistol.* **31**: 1–7.

Redfield, R.J. 1988. Evolution of bacterial transformation: is sex with dead cells ever better than no sex at all? *Genetics* **119**: 213–221.

Redfield, R.J. 1993. Genes for breakfast: the have your cake and eat it too of transformation. *J. Hered.* **84**: 400–404.

Ribeiro, S. & Golding, G.B. 1998. The mosaic nature of the eukaryotic nucleus. *Mol. Biol. Evol.* **15**: 779–788.

Smith, G.R. 1994. Hotspots of homologous recombination. *Experientia* **50**: 234–238.

Sniegowski, P.D., Gerrish, P.J. & Lenski, R.E. 1997. Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* **387**: 703–705.