Evolution of sex: Using experimental genomics to select among competing theories

*Nathaniel P. Sharp and Sarah P. Otto**

Few topics have intrigued biologists as much as the evolution of sex. Understanding why sex persists despite its costs requires not just rigorous theoretical study, but also empirical data on related fundamental issues, including the nature of genetic variance for fitness, patterns of genetic interactions, and the dynamics of adaptation. The increasing feasibility of examining genomes in an experimental context is now shedding new light on these problems. Using this approach, McDonald et al. recently demonstrated that sex uncouples beneficial and deleterious mutations, allowing selection to proceed more effectively with sex than without. Here we discuss the insights provided by this study, along with other recent empirical work, in the context of the major theoretical models for the evolution of sex.

Keywords:
clonal interference; evolution of sex; experimental genomics; genetic hitchhiking; *Saccharomyces cerevisiae*; selective interference

Introduction

Sex is widespread in eukaryotes, with the vast majority – well over 99% – thought to engage in sexual reproduction, at least occasionally [2]. Strictly asexual lineages, by contrast, tend to be few and far between [3, 4]. Even the most infamous group of asexual species (bdelloid rotifers) has recently been shown to engage in some form of genetic mixing [5]. Such a widespread phenomenon as sex requires widespread favourable conditions, and nailing down exactly what conditions are most important empirically has been elusive.

Starting with the verbal arguments of Weismann [6], Fisher [7], and Muller [8], substantial theoretical advances were made in the 20th century to account for why so many eukaryotic organisms engage in sexual reproduction. These theoretical advances led Barton and Charlesworth [9] to comment that "there is reasonable confidence that the major population genetic processes that potentially yield an advantage to sex are understood."

The sense that the theory was well explored did not, however, engender a sense that the mystery of sex was solved. What was lacking was empirical work discriminating among the possible mechanisms [9, 10]. Does sex generate immediate increases in fitness? Or does it augment genetic variance within a population, hastening evolution? Does sex primarily un-link beneficial mutations from one another? Or deleterious mutations? Or a combination?

The recent paper by McDonald et al. [1] is an example of the new breed of empirical work that aims to tease apart the mechanisms that cause sex to be advantageous. By sequencing whole-population samples of the yeast *Saccharomyces cerevisiae* every 90 generations over 1,000 generations of evolution, McDonald et al. were able to pinpoint the genetic changes and allele frequency trajectories that occurred in strictly asexual versus sexual populations. Their results support a role for selective interference among loci in the evolutionary maintenance of sex. Before considering these results and other recent empirical advances, we briefly review the theoretical models to see exactly what needed to be teased apart.

When is sex beneficial?

Sex alters the associations among alleles within the genomes of a population. The major population genetic advantages of sex can be roughly grouped into two main classes, based on

DOI 10.1002/bies.201600074

Department of Zoology, University of British Columbia, Vancouver, Canada

***Corresponding author:**
Sarah P. Otto
E-mail: otto@zoology.ubc.ca

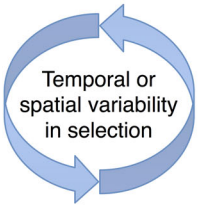
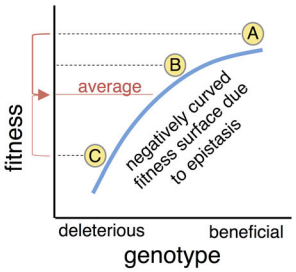
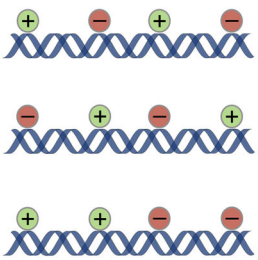
	Requirements for Advantage to Sex	Predictions	Hypothesis Names
Class I	 Abundant genotypes are currently less fit than sexually-produced genotypes.	Sex causes an immediate *increase* in offspring mean fitness relative to asex.	*Red Queen* *Spatial Heterogeneity* *Environmental Deterministic* Example references: Theory: 11, 13–17 Experiment: 53–58
Class IIa	 Intermediate genotypes (B) are more fit than extreme genotypes (average of (A) and (C)), causing genotype (B) to be more abundant than expected based on component allele frequencies (less genetic variance in fitness).	Sex causes an immediate *decrease* in offspring mean fitness relative to asex. Sex also increases genetic variance for fitness, improving response to selection and long-term fitness.	*Synergistic Epistasis* *Mutational Deterministic* Example references: Theory: 15, 19–26 Experiment: 27 (and refs. therein), 28, 29, (46)
Class IIb	 Beneficial (+) and deleterious (−) alleles are not found in all possible combinations within a finite population but are linked with one another on a limited number of genomes, reducing genetic variance for fitness.	Immediate effect of sex depends on epistasis. Sex increases genetic variance for fitness, improving response to selection and long-term fitness.	*Selective Interference* *Hill-Robertson Interference* *Clonal Interference* *Muller's Ratchet* *Mutational/Environmental Stochastic* Example references: Theory: 32–35, 38, 39 Experiment: 1, 40, 42–45, 48, 49, 59, (46)

Figure 1. Summary of theoretical models for the evolution of sex.

whether they focus on sex breaking apart genetic associations in a manner that (I) has immediate fitness benefits or (II) causes an increase in genetic variance for fitness (henceforth 'genetic variance'). We further subdivide the latter class, depending on the factors that have built these genetic associations:

Class I: Associations built by past selection are currently disadvantageous

Class IIa: Associations built by epistasis reduce genetic variance

Class IIb: Associations built by selective interference among loci reduce genetic variance

(I) Associations built by past selection are currently disadvantageous

The world is ever changing. What helped one's ancestors survive to reproduce could reduce fitness today. When this statement holds true about combinations of alleles – not just their independent effects – the genetic associations that developed ancestrally can reduce current fitness. Sex and recombination, by breaking apart these (obsolete) genetic associations, can then improve fitness. As a consequence, genes that augment the frequency of sex and recombination can spread, via this immediate fitness advantage.

Major examples of this scenario include the classic Red Queen hypothesis [11, 12] and the Spatial Heterogeneity hypothesis [13] (Fig. 1). In the Red Queen hypothesis, for example, certain combinations of host alleles (say A_1 B_1) may have helped evade parasites in the past, but co-evolutionary change in the parasites now cause different genotypes to resist infection (say A_2 B_1 or A_1 B_2). In such cases, sex and recombination can help break apart the genetic associations that are hampering the current population. While host-parasite interactions are commonly thought to underlie the Red Queen advantage, sex can have similar advantages with any temporal change in the environment that causes different combinations of alleles to be favoured at different times [14, 15].

The Spatial Heterogeneity hypothesis, by contrast, focuses on selective differences among locations, highlighting variation in selection across space rather than time. For example, selection can allow the build up of deleterious combinations (say A_2 B_2) in locations with relatively mild selection. Migration to sites experiencing harsher selection can then generate genetic associations where the most fit genotype (say

A_1 B_1) and A_2 B_2 are overabundant, even when their average fitness is less than the recombinant genotypes, again making sex immediately advantageous.

These mechanisms allow sex to be favourable (in the sense that mean fitness is increased) and to evolve (in the sense that genes promoting sex rise in frequency), but the conditions are finicky, particularly for sex to evolve: the environment needs to change often enough over time or in just the right way over space that gene combinations that have been built by past selection are commonly harmful [15–17]. While finicky, when these conditions hold, there can be strong selection for high levels of sex, because of the immediate advantages of breaking down genetic associations [18].

The open empirical question raised by this class of theoretical models is whether genetic associations present within a population are commonly harmful and whether these genetic associations were built by selection varying over space or time.

(II) Associations reduce genetic variance

The above class of hypotheses focused on the mismatch between what genetic associations are present and what associations would improve fitness. The second class of hypotheses focuses not on what would improve fitness but on what would increase genetic variance within a population.

While the ability of sex to mix genomes and generate variability is one of the most intuitive and oldest explanations, dating back to the writings of Weismann in the late 1800s, it is also a problematic explanation. Weismann's thinking was influenced greatly by considering the outcome of a mating between two individuals, in which case it is clear that the progeny will be more variable with sex ('two groups of hereditary tendencies are as it were combined ... to create those individual differences', 1889, p. 272). A population as a whole, however, may already be highly variable, in which case sex and recombination can reduce the genetic variance in fitness. Thus, a substantial amount of research has focused on the conditions that would cause genetic variance to be lacking, either as a result of (a) the shape of the fitness surface (particularly, epistatic interactions) or (b) evolution in populations with finite numbers of genotypes causing selective interference among loci.

(IIa) Associations built by epistasis reduce genetic variance

Theoretical models have helped to clarify when the shape of the fitness surface allows sex and recombination to increase variance. Specifically, when intermediate genotypes carrying both good and bad alleles are fitter than the average fitness of the extreme genotypes considered together, we say that the fitness surface is "negatively curved" and that epistasis is negative (Fig. 1). This form of selection allows intermediate genotypes to rise in abundance, relative to the frequency expected based on the component alleles. Sex and recombination can then mix these intermediate genotypes to regenerate good-with-good and bad-with-bad combinations. While this increased genetic variance can be harmful in the short term (because mean fitness is lower, on average, among the extreme genotypes), lineages that engage in more sexual reproduction can respond better to selection over the long term because good-with-good combinations become available. The spread of the good-with-good combinations can then cause genes promoting sex to hitchhike up in frequency [15].

By regenerating genetic variance that is eliminated by selection when fitness surfaces are negatively curved, sexual populations can have a greatly reduced load of deleterious mutations, a topic that received early mathematical treatment using models with infinite populations (known as the Deterministic Mutation hypothesis; [19–21]). Similarly, sexual populations can take advantage of the increased genetic variance to respond more rapidly to a changing environment [22].

The conditions under which an increase in sex can evolve are not, however, the same as the conditions where sex improves long-term fitness. The reason is that the short-term costs of breaking apart genetic combinations built by past selection can hinder the spread of genes that increase the frequency of sex. Models that track genes promoting sex and recombination have found that, in addition to negatively curved fitness surfaces, moderately high levels of sex and recombination can evolve only when the curvature is mild and does not vary greatly among pairs of genes [15, 23]. In models of deleterious mutations, relatively mild curvature can be achieved if the mutation rate is sufficiently high [15, 21, 24, 25], in part because selection becomes stronger as the mean fitness declines, causing epistasis to become relatively weaker (details in Ref. [23]).

Based on these results, Kondrashov [26] drew attention to the need for more data on mutation rates and the shape of the fitness surface, following which a great deal of empirical work aimed to estimate epistasis and the genome-wide deleterious mutation rate in order to evaluate this hypothesis (reviewed in Ref. [27]).

These empirical efforts raised concerns that the Deterministic Mutation hypothesis might fail to explain the widespread occurrence of sex. First, many organisms had mutation rates that fell below those needed to favour the evolution of high rates of sex. Second, epistasis among deleterious mutations was not generally found to be mildly negative and typically does vary greatly among pairs of genes [27].

Nevertheless, it remains an open empirical question if some classes of mutations, besides strictly deleterious mutations, do generate the right form of negatively curved fitness surfaces, depleting genetic variance and providing an advantage for sex and recombination. For example, recent work on beneficial mutations in microbes suggests that fitness tapers off over time, indicating that the fitness surface may exhibit negative curvature during adaptation [28–30]. Whether this is generally the case outside of microbial laboratory studies awaits further investigation.

(IIb) Associations built by selective interference among loci reduce genetic variance

A related class of hypotheses emphasizes that selection in finite populations depletes genetic variance. After a period of

selection, genotypes at the tails of the fitness distribution would have fixed (chromosomes with predominantly good alleles) or been eliminated (chromosomes with predominantly bad alleles). What typically remain segregating within a population are chromosomes of similar fitness: chromosomes with a mixture of good and bad alleles. In other words, genetic variance in fitness becomes limited after a period of selection in finite populations and can be regenerated from these mixtures by sex and recombination (Fig. 1).

Viewed another way, beneficial mutations that arise in the background of other beneficial alleles will rapidly rise to fixation. Beneficial mutations arising on the background of deleterious alleles will, however, remain segregating in the population for longer and contribute more to the standing genetic variance seen within a population.

This class of theories focuses, implicitly or explicitly, on the fact that the population is finite and selection acts asymmetrically on cases where, by chance, good alleles find themselves together (genetic variance is available but rapidly eliminated by selection) versus where good and bad alleles are linked (genetic variance persists for longer).

Fisher [7] was implicitly referring to this class of hypotheses when he wrote that 'Unless advantageous mutations occur so seldom that each has had time to become predominant before the next appears, they can only come to be simultaneously in the same gamete by means of recombination.' Muller [8] wrote similarly that 'Without sexual reproduction, the various favorable mutations that occur must simply compete with each other,' a phenomenon that we now call clonal interference within strictly asexual populations. When focused on beneficial mutations, this is often referred to as the Fisher-Muller hypothesis for the benefit of sex.

Yet all forms of selection run into the same fundamental problem: alleles are always selected in the context of the genomes in which they are found. This led Felsenstein [31] to emphasise that the issue really is selective interference among loci, i.e. the reduction in selective efficiency at a given locus due to selection at linked loci, regardless of the mode of reproduction. Felsenstein referred to the entire class of theories as the Hill-Robertson hypothesis, after a seminal paper quantifying selective interference [32]. Felsenstein observed that this view connected the Fisher-Muller hypothesis among adaptive mutations and also Muller's ratchet hypothesis [33] among deleterious mutations, where the stochastic loss of the fittest genotype again leads to an excess of intermediate genotypes (good-with-bad combinations).

Subsequent theoretical studies have shown that sex and recombination reduce selective interference in multiple contexts, including separating beneficial from deleterious alleles ('A Ruby in the Rubbish' [34]), separating beneficial alleles from polymorphisms maintained by selection [35], and separating beneficial alleles from sites involved in cyclic host-parasite dynamics [36, 37]. Furthermore, not only can Hill-Robertson effects give sexual populations a fitness advantage, but they can also favour the evolution of increased rates of sex and recombination because genes that promote genetic mixing hitchhike up in frequency along with the good gene combinations that they create [38, 39].

The main open empirical question raised by this theoretical work is whether selection is pervasive enough, often enough, for selective interference across the genome to account for the high levels of sex observed among most eukaryotes (not just low levels of facultative sex [18]), especially in light of the substantial costs of sex [2].

Recent experiments shed light on the benefits of sex

Experiments designed to understand the costs and benefits of sex, which were once rare, are rapidly growing in number and sophistication. Many empirical studies of laboratory model organisms have now demonstrated that the presence of sex can allow for faster adaptation to new conditions [40–47]. However, the underlying population-genetic reason behind this effect has been more difficult to establish. Although many evolution experiments focus on single species in well-mixed populations (reducing the impact of Class I hypotheses), teasing apart Classes IIa and IIb remains a challenge, which is where the experiment of McDonald et al. stands out. We next summarise the major advance of this study, before ending with a broader description of these empirical studies.

As with previous studies, McDonald et al. evolved populations of yeast in the presence and absence of sex. While the yeast were maintained primarily on standard rich lab medium, the strains were also exposed to nitrogen limitation during the sporulation phase and carried a number of genetic markers. Although the exact selective forces are unknown, fitness did improve in both asexual and sexual populations, but, as is commonly observed, sexual populations achieved higher long-term fitness over the course of the experiment than did asexual populations, consistent with the Class II hypotheses. What sets this study apart is the massive genomic sequencing effort across several points in time. This sequencing allowed the authors to determine which alleles changed substantially in frequency. By crossing evolved to ancestral lines and measuring the fitness of the resulting offspring, the authors could then estimate the fitness effects of the mutations that had spread.

McDonald et al. were able to establish that selective interference was substantial in the absence of sex: several deleterious mutations rose to high frequency in the asexual populations through hitchhiking with beneficial mutations, as seen previously [48, 49]. By contrast, in sexual populations, deleterious mutations that began to spread were uncoupled from beneficial mutations during subsequent rounds of recombination and then declined in frequency. As a consequence, less than a quarter as many mutations reached high frequency and fitness rose almost twice as much in the sexual populations.

In addition, McDonald et al. observed that sex allowed new beneficial mutations to combine with alternative alleles maintained by frequency-dependent selection, allowing for selective sweeps without disrupting a stable polymorphism [50]. This is perhaps the first empirical example of this form of selective interference, described by Strobeck et al. [35].

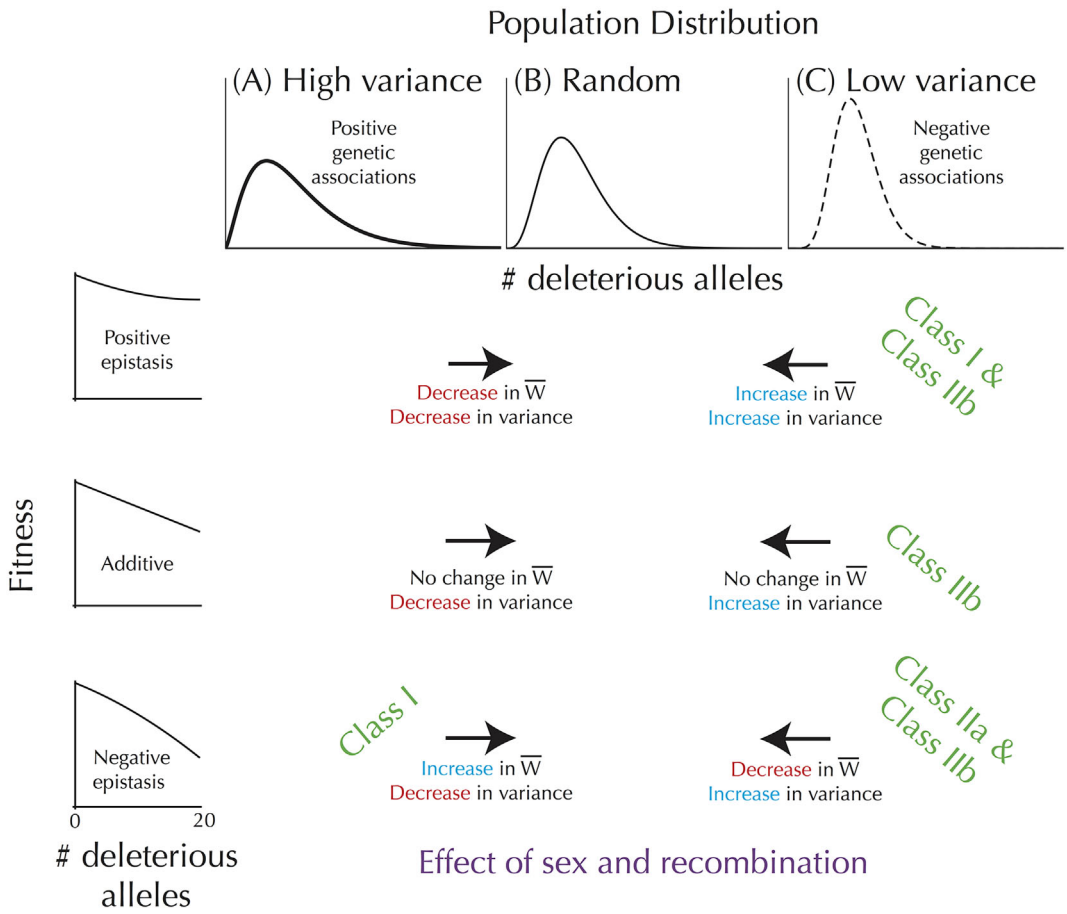


Figure 2. The short- and long-term effects of sex will depend on how alleles affecting fitness are distributed among individuals (top panels) and the shape of the fitness surface (left panels). Sex breaks down associations among alleles, moving (arrows) the distribution of genotypes towards the random expectation, given by a Poisson distribution when all mutations are equivalent (top panel B). The consequent short-term effect on the mean fitness ($\bar{W}$) of offspring depends on whether genetic associations and epistasis (measured on an additive scale) have the same sign (e.g. if genetic associations are positive and epistasis is positive, then sex decreases mean fitness) or opposite signs (e.g. if genetic associations are positive and epistasis is negative, then sex increases mean fitness). The effect on genetic variance depends on whether genetic associations are initially positive (top panel **A:** high variance; good-with-good and bad-with-bad allele combinations) or negative (top panel **C:** low variance; good-with-bad allele combinations). When the variance is increased by sex (from top panel C to B), the rate of response to selection rises, leading to greater fitness over the long-term (vice versa for reductions in variance from top panel A to B). Experimental data on the mean and variance in fitness of sexually versus asexually produced offspring thus provide information about the potential advantages of sex [10], but such experiments are rare (see [60] for a recent example and discussion of such studies). Note that this line of thinking ignores variation in epistasis among loci and assumes that the fitness surface is static, whereas recent models have explored how genetic architecture evolves in conjunction with sex [61, 62].

These results point to an important role for selective interference in the evolutionary maintenance of sex (Class IIb), with the selective spread of beneficial alleles being hampered by interference from deleterious mutations and the maintenance of balanced polymorphisms being hampered by selective sweeps in the absence of sex. Sex conferred a long-term fitness benefit by breaking down these genetic associations, moving beneficial mutations into multiple genetic backgrounds and increasing the variance in fitness.

While opening a genomic window into the evolutionary process, several questions remain. Would the advantages of sex diminish over time, if beneficial mutations were to become rare? Or would the benefits of increasing genetic variance remain, even if mutations became exclusively deleterious? The latter may be difficult to measure in yeast, given the low genome-wide mutation rate [51], making Muller's Ratchet click very slowly [52]. It would, however, be valuable to examine the strength of selective interference among deleterious mutations in organisms with higher deleterious mutation rates: do deleterious mutations tend to be under-dispersed among genomes (good-with-bad combinations)?

Although there is clear evidence for selective interference, given the rise then fall in frequency of deleterious mutations, this does not mean that other processes played no role in the experimental results of McDonald et al. For example, the experimental design included growth conditions that varied over time and space to enforce sex, leading to the possibility that Class I mechanisms could act. Furthermore, negative epistasis may have contributed to some of the observed long-term advantage of sex (Class IIa). While not measured, it would thus be interesting to know whether the fitness advantage of beneficial mutations

declined as more mutations accumulated. This possibility could be tested by measuring the fitness of strains carrying different numbers of beneficial mutations (as in Refs. [29, 30]). Interestingly, the one mutation (*met2*) assayed in multiple genetic backgrounds by McDonald et al. showed strong positive epistasis, becoming beneficial only after the spread of previous mutations. This form of epistasis is exactly the opposite of that required by Class IIa (it would contribute to higher levels of genetic variance, even in the absence of sex), but the fitness interactions among the other beneficial mutations remain unknown.

The data from *met2* raises an important caveat. Alleles cannot be strictly labeled as 'beneficial' or 'deleterious' if the sign of their fitness effects depends on the genomic background. McDonald et al. labelled mutations based largely on the fitness effects observed in crosses between evolved and ancestral strains (or, in some cases, after placement into the ancestral strain). Yet, these mutations might have had the opposite effect ('sign epistasis') in the genomes in which they arose. It would thus be worthwhile confirming that the deleterious mutations that hitchhiked up in frequency with the beneficial mutations were in fact deleterious in those genomes.

McDonald et al. have provided important data on some of the population genetic consequences of sex. However, it is important to recognise that in this study, as in many others, the presence and degree of sex was experimentally controlled, and sexual genotypes did not compete directly with asexuals. While this approach makes the effect of sex easier to measure, it does not necessarily reveal how the rate of sex would evolve over time. As mentioned above, theoretical models have found that the short-term costs of breaking apart genetic combinations built by past selection can hinder the spread of genes that increase the frequency of sex, even if sex would increase fitness in the long term when comparing completely isolated populations of sexuals and asexuals. More experiments addressing the effect of sex on the mean and variance in fitness are needed to understand the short-term and long-term costs and benefits (Fig. 2).

An alternative approach is to study populations where the rate of sex can evolve on its own, which directly sheds light on the conditions that allow sex to rise in frequency. While this approach has been attempted in yeast (e.g. Ref. [53]), the interpretation is complicated because sex in yeast must be induced by specific sporulation conditions, which causes temporal variation in selection.

Recent empirical studies have made tremendous headway working instead with the facultatively sexual monogonont rotifer *Brachionus calyciflorus*, which can produce both sexual and asexual eggs under the same culture conditions. These rotifers evolve higher rates of sex in spatially heterogeneous environments [54] and in the presence of predator-prey dynamics [55], in line with the Class I set of hypotheses. Similarly, *B. calyciflorus* evolved higher rates of sex during adaptation to a new environment [46], in line with Class II. These authors further showed that genetic associations were indeed reducing the genetic variance, but they were unable to determine whether this was due to fitness surfaces being negatively curved (Class IIa) or selective interference (Class IIb). Genomic dissections, of the kind performed by McDonald et al., would be extremely valuable in an experimental context where the rate of sex is evolving.

Conclusions and outlook

The past decade has seen major advances in our understanding on the evolution of sex. Refreshingly, many of these recent advances have been on the empirical front, both with experiments where the frequency of sex is manipulated and where it naturally evolves. Dissecting the mechanisms responsible is now possible at an even deeper level due to genomic sequencing. The recent paper by McDonald et al. used genome sequencing to pinpoint the genetic changes that occurred during experimental evolution and to track how sex affected the dynamics. For the first time, we can say definitively that sex reduced selective interference, uncoupling beneficial alleles from the deleterious alleles in their genetic background. Although this study provides experimental verification of a long-standing explanation for sex, this study does not close the book on the evolution of sex. Rather, it will encourage future use of sequencing technology to tease apart the exact dynamics of molecular evolution that have allowed sex to evolve and be maintained in the vast majority of eukaryotic organisms.

Acknowledgements

We thank the Otto lab and anonymous reviewers for comments on earlier versions of this article. This work was supported by the Natural Sciences and Engineerings Research Council of Canada (Discovery grant RGPIN-2016-03711 to SPO; Postdoctoral Fellowship to NPS).

The authors have declared no conflicts of interest.

References

1. **McDonald MJ, Rice DP, Desai MM**. 2016. Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature* **531**: 233–6.
2. **Otto SP**. 2009. The evolutionary enigma of sex. *Am Nat* **174**: S1–14.
3. **Judson OP, Normark BB**. 1996. Ancient asexual scandals. *Trends Ecol Evol* **11**: 41–6.
4. **Whitton J, Sears CJ, Baack EJ, Otto SP**. 2008. The dynamic nature of apomixis in the angiosperms. *Int J Plant Sci* **169**: 169–82.
5. **Schwander T**. 2016. Evolution: the end of an ancient asexual scandal. *Curr Biol* **26**: R233–5.
6. **Weismann A**. 1889. The significance of sexual reproduction in the theory of natural selection. In Poulton EB, Schonland S, Shipley AE, eds; *Essays upon Heredity and Kindred Biological Problems*. Oxford, UK: Clarendon Press. p. 251–332.
7. **Fisher RA**. 1930. *The Genetical Theory of National Selection*. Oxford, UK: Oxford University Press.
8. **Muller HJ**. 1932. Some genetic aspects of sex. *Am Nat* **66**: 118–38.
9. **Barton NH, Charlesworth B**. 1998. Why sex and recombination? *Science* **281**: 1986–90.
10. **Otto SP, Lenormand T**. 2002. Resolving the paradox of sex and recombination. *Nat Rev Genet* **3**: 252–61.
11. **Hamilton WD**. 1980. Sex versus non-sex versus parasite. *Oikos* **35**: 282.
12. **Lively CM**. 1996. Host-parasite coevolution and sex. *BioScience* **46**: 107–14.
13. **Lenormand T, Otto SP**. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* **156**: 423–38.

14. **Charlesworth B**. 1976. Recombination modification in a fluctuating environment. *Genetics* **83**: 181–95.
15. **Barton NH**. 1995. A general-model for the evolution of recombination. *Genet Res* **65**: 123–44.
16. **Otto SP, Nuismer SL**. 2004. Species interactions and the evolution of sex. *Science* **304**: 1018–20.
17. **Gandon S, Otto SP**. 2007. The evolution of sex and recombination in response to abiotic or coevolutionary fluctuations in epistasis. *Genetics* **175**: 1835–53.
18. **Hadany L, Comeron JM**. 2008. Why are sex and recombination so common? *Ann NY Acad Sci* **1133**: 26–43.
19. **Kimura M, Maruyama T**. 1966. The mutational load with epistatic gene interactions in fitness. *Genetics* **54**: 1337.
20. **Kondrashov AS**. 1982. Selection against harmful mutations in large sexual and asexual populations. *Genet Res* **40**: 325–32.
21. **Kondrashov AS**. 1984. Deleterious mutations as an evolutionary factor: 1. The advantage of recombination. *Genet Res* **44**: 199–217.
22. **Charlesworth B**. 1993. Directional selection and the evolution of sex and recombination. *Genet Res* **61**: 205–24.
23. **Otto SP, Feldman MW**. 1997. Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theor Popul Biol* **51**: 134–47.
24. **Kondrashov AS**. 1985. Deleterious mutations as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* **111**: 635–53.
25. **Charlesworth B**. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genet Res* **55**: 199–221.
26. **Kondrashov AS**. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**: 435–40.
27. **de Visser JAGM, Elena SF**. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat Rev Genet* **8**: 139–49.
28. **Khan AI, Dinh DM, Schneider D, Lenski RE**, et al. 2011. Negative epistasis between beneficial mutations in an evolving bacterial population. *Science* **332**: 1193–6.
29. **Chou H-H, Chiu H-C, Delaney NF, Segrè D**, et al. 2011. Diminishing returns epistasis among beneficial mutations decelerates adaptation. *Science* **332**: 1190–2.
30. **Kryazhimskiy S, Rice DP, Jerison ER, Desai MM**. 2014. Global epistasis makes adaptation predictable despite sequence-level stochasticity. *Science* **344**: 1519–22.
31. **Felsenstein J**. 1974. The evolutionary advantage of recombination. *Genetics* **78**: 737–56.
32. **Hill WG, Robertson A**. 1966. The effect of linkage on limits to artificial selection. *Genet Res* **8**: 269–94.
33. **Muller HJ**. 1964. The relation of recombination to mutational advance. *Mutation Res* **1**: 2–9.
34. **Peck JR**. 1994. A ruby in the rubbish – beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* **137**: 597–606.
35. **Strobeck C, Smith JM, Charlesworth B**. 1976. The effects of hitchhiking on a gene for recombination. *Genetics* **82**: 547–58.
36. **Peck JR**. 1993. Frequency-dependent selection, beneficial mutations, and the evolution of sex. *Proc R Soc B* **254**: 87–92.
37. **Hodgson EE, Otto SP**. 2012. The red queen coupled with directional selection favours the evolution of sex. *J Evol Biol* **25**: 797–802.
38. **Otto SP, Barton NH**. 1997. The evolution of recombination: removing the limits to natural selection. *Genetics* **147**: 879–906.
39. **Keightley PD, Otto SP**. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* **443**: 89–92.
40. **Colegrave N**. 2002. Sex releases the speed limit on evolution. *Nature* **420**: 664–6.
41. **Poon A, Chao L**. 2004. Drift increases the advantage of sex in RNA bacteriophage Phi 6. *Genetics* **166**: 19–24.
42. **Kaltz O, Bell G**. 2002. The ecology and genetics of fitness in Chlamydomonas. XII. Repeated sexual episodes increase rates of adaptation to novel environments. *Evolution* **56**: 1743–53.
43. **Goddard MR, Godfray H, Burt A**. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature* **434**: 636–40.
44. **Cooper TF**. 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. *PLoS Biol* **5**: e225–7.
45. **Gray JC, Goddard MR**. 2012. Sex enhances adaptation by unlinking beneficial from detrimental mutations in experimental yeast populations. *BMC Evol Biol* **12**: 43.
46. **Becks L, Agrawal AF**. 2012. The evolution of sex is favoured during adaptation to new environments. *PLoS Biol* **10**: e1001317.
47. **Lachapelle J, Bell G**. 2012. Evolutionary rescue of sexual and asexual populations in a deteriorating environment. *Evolution* **66**: 3508–18.
48. **Lang GI, Rice DP, Hickman MJ, Sodergren E**, et al. 2013. Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. *Nature* **500**: 571–4.
49. **Kao KC, Sherlock G**. 2008. Molecular characterization of clonal interference during adaptive evolution in asexual populations of *Saccharomyces cerevisiae*. *Nat Genet* **40**: 1499–504.
50. **Frenkel EM, McDonald MJ, Van Dyken JD, Kosheleva K**, et al. 2015. Crowded growth leads to the spontaneous evolution of semistable coexistence in laboratory yeast populations. *Proc Natl Acad Sci USA* **112**: 11306–11.
51. **Lynch M, Sung W, Morris K, Coffey N**, et al. 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. *Proc Natl Acad Sci USA* **105**: 9272–7.
52. **Gordo I, Charlesworth B**. 2000. The degeneration of asexual haploid populations and the speed of Muller's ratchet. *Genetics* **154**: 1379–87.
53. **Wolf HG, Wöhrmann K, Tomiuk J**. 1987. Experimental evidence for the adaptive value of sexual reproduction. *Genetica* **72**: 151–9.
54. **Becks L, Agrawal AF**. 2010. Higher rates of sex evolve in spatially heterogeneous environments. *Nature* **468**: 89–92.
55. **Haafke J, Abou Chakra M, Becks L**. 2016. Eco-evolutionary feedback promotes Red Queen dynamics and selects for sex in predator populations. *Evolution* **70**: 641–52.
56. **Decaestecker E, Gaba S, Raeymaekers JAM, Stoks R**, et al. 2007. Host-parasite "Red Queen" dynamics archived in pond sediment. *Nature* **450**: 870–3.
57. **Morran LT, Schmidt OG, Gelarden IA, Parrish RC**, et al. 2011. Running with the Red Queen: host-parasite coevolution selects for biparental sex. *Science* **333**: 216–8.
58. **Lively CM, Dybdahl MF**. 2000. Parasite adaptation to locally common host genotypes. *Nature* **405**: 679–81.
59. **Poon A, Chao L**. 2004. Drift increases the advantage of sex in RNA bacteriophage Φ6. *Genetics* **166**: 19–24.
60. **Becks L, Agrawal AF**. 2010. The effect of sex on the mean and variance of fitness in facultatively sexual rotifers. *J Evol Biol* **24**: 656–64.
61. **Azevedo RBR, Lohaus R, Srinivasan S, Dang KK**, et al. 2006. Sexual reproduction selects for robustness and negative epistasis in artificial gene networks. *Nature* **440**: 87–90.
62. **Whitlock AOB, Peck KM, Azevedo RBR, Burch CL**. 2016. An evolving genetic architecture interacts with Hill-Robertson interference to determine the benefit of sex. *Genetics* Early online April 20, 2016; DOI: 10.1534/genetics.116.186916.