A high price is paid for sexual reproduction compared to asexual propagation. Nevertheless, sexual reproduction apparently began to evolve as early as two billion years ago. But to what advantage?

Evolution of sexual reproduction

Why Sex?

When we sat down in July to brood over some hot topics for this issue of *Lab Times*, an inevitable theme for Darwin Year 2009 finally popped up: Why do we and other organisms have sex?

Phew, we thought, this is such a fundamental question that has been discussed and written about so many times. Should we really add another overview? Then again, why do biologists have such difficulties explaining why, despite the many disadvantages, sexual reproduction (that's to say, the reassembling of two genomes to give one new life) is so widespread in nature? Still, we had doubts there were any new observations to report. And no news = no article.

Hesitantly, we started digging through current research. To our surprise, we found lots of new and interesting reasons to explain why sex is so good and beneficial for life.

Parasite sex

Sexual reproduction is the dominant mode of propagation in eukaryotes, although in many taxa it has never been observed at all. However, recent studies indicate sex is more widespread than previously assumed. In fact, it seems meiosis and meiotic recombination can even be traced back to the earliest days of eukaryote evolution. In April, Michael Miles and colleagues at the London School of Hygiene and Tropical Medicine reported evidence for sexual recombination in *Leishmania* parasites hitherto regarded as asexual (*Science* vol. 324: 265-8). It appears they're "capable of having a sexual cycle consistent with a meiotic process, like that described for African trypanosomes." In another parasite, *Trichomonas vaginalis*, John Logsdon (University of Iowa) identified homologues to genes involved in meiotic recombination. Previously, neither sex nor meiosis had been observed in this eukaryotic microorganism or its relatives. Based on these findings, the scientists now argue that meiotic genes "arose, or were already present, early in eukaryotic evolution; thus, the eukaryotic ancestor contained most or all components of this set and was likely capable of performing meiotic recombination using near-universal meiotic machinery" (*PLoS ONE* vol. 3: e2879). Another charming asexual parasite, *Giardia intestinalis*, has also recently been shown to harbour meiosis-specific genes and to undergo recombination (*Curr. Biol.* vol. 17: 1984-8 and *Science* vol. 319: 1530-3). Taken together, these observations suggest that sexual recombination evolved some two billion years ago in a common early ancestor of all eukaryotes.

For future comparative analyses, Logsdon and colleagues have defined a "meiosis detection toolkit" – a set of meiotic genes representing markers for the presence of meiosis. When exploring the impact of meiosis on reproduction, they also dived deep into the inventory of meiosis genes and their expression patterns in obligately parthenogenetic *Daphnia duplex* strains. They found expansions in particular meiotic genes and gene families. Whether or not these are involved in the mechanism of parthenogenesis in water fleas is still an open question (*BMC Evol. Biol.* vol. 9: 78)

Really more variance?

But this still doesn't indicate *why* there's sex in the world. Sex means investing high amounts of energy and resources in courtship and copulation, to the point of sacrificing one's life, as with certain spiders where, during copulation itself, the males serve as a substantial meal for their female mates. Sex also means meiosis – a mate can only

pass on half its genome to the next generation. In 1982, Graham Bell from McGill University, Montreal, described this as *the* cost of sex. Indeed, females have to bear *two-fold* costs of sex: not only do they they transmit just half their genes but they also invest the majority of the resources for the development of their offspring – males provide little input. Yet, compared to asexual reproduction, sexual reproduction must surely have conferred some benefits to outweigh its significant costs and efforts. But just what were those benefits?

Most scientists misconceive sex's cost-benefit-equation. Sebastian Bonhoeffer (professor for theoretical biology at the ETH in Zurich) says, "When I ask biologists why there's sex, most answer it's obvious, it's because sex produces more variance, allowing natural selection to proceed more effectively." This seemingly easy and universal interpretation goes back to August Weissmann. He had already proposed in the 19th century that the breeding of more genetically variable offspring was the real purpose for sex and recombination. But doesn't that depend on whether recombination increases or decreases variance? If a certain combination of alleles at two loci is advantageous in the parents' generation then won't it tend to stay together and lead to a reduction in variance? Conversely, breaking up that combination would mean increased variance but with reduced fitness. However, if such a combination is suboptimal, its break-up will be favoured by recombination, and variance will increase. Right?

Theories over theories

Well, generations of biologists have thought about the "Why is sex in the World?"-problem and they have come up with roughly 20 theories to explain why eukaryotes and many prokaryotes exchange genetic material during reproduction. These hypotheses basically centre around two rival arguments as to why sexual propagation might offer sufficient advantages to compensate for sex's costs: environment (or ecology) or mutation. All environment-based models suggest the creation of new gene combinations will increase possibilities for dealing with selection pressure caused by changing environments. Meanwhile, advocates of mutation-based models point to the reduction of deleterious mutations as sexual reproduction's main driving force.

From these two lines of argument – environment or mutation – three main models (and many variations) have been developed:

- The Mutational Deterministic model says recombination is more efficient than asexual reproduction at breaking-up deleterious combinations of synergistically interacting alleles. In this model, negative genetic interaction (or negative epistasis), where two alleles in combination are worse than the sum of the two, is thought to have driven the evolution of sex.
- The so-called 'Red Queen' theory (see below) reckons the creation of new gene combinations increases the potential and speed necessary to deal with the challenges of selection pressure, for example, caused by parasites.
- Finally, Genetic Drift notes the random change in frequency of an allele in a population. For finite populations, it assumes that the effects of probability and selection will generate new gene combinations.

To date, it should be noted that very few experimental studies have supported any of these hypotheses or even served to discriminate between them. However, here are some of the latest interesting new studies from the "world of sex":

Susanne Paland and Michael Lynch from Indiana University used water fleas (*Science* vol. 311, 990-2) to show that sex – as they propose it – does indeed purge deleterious mutations from the genome when compared to asexual reproduction. *Daphnia pulex* can reproduce either by cyclical parthenogenesis (sexually) or by obligate parthenogenesis (asexually). Ancestrally, the fleas reproduce sexually, but parthenogenetic populations have arisen several times from sexual lines, basically due to a genetic suppression of meiosis. This feature makes *Daphnia duplex* an ideal candidate for following mutation load in different populations. They compared mutations with possibly functional effects (non-synonymous mutations) in protein-coding mitochondrial genes to mutations without such effects (synonymous mutations) in 14 sexual and asexual lines respectively. They found that more non-synonymous mutations accumulated in asexual lines (18%) than in sexual lines (4%). Paland and Lynch, therefore, concluded that roughly 90% of the non-synonymous mutations were subject to selection. This strongly supports the central claim of mutation-based models, namely that asexual reproduction leads to an accumulation of deleterious mutations. Another study revealed that deleterious mutations in the freshwater snail, *Campeloma,* were also more efficiently eliminated in sexual than asexual lines (*Evolution* vol. 61: 2728-35).

However, the "environment enforces sex"-argument has also been supported by new experimental data. Matthew Goddard and colleagues at the Centre for Population Biology, Imperial College London, showed that sexually reproducing yeast strains could better adapt to harsh conditions than asexually propagating yeast. The yeast lines were nearly isogenic, differing only by the deletion of two genes required for recombination and meiosis. Interestingly, sex had no measurable effect on fitness under conditions with little selection (*Nature* vol. 343: 636-40).

Sexual reproduction requires high energy input. But for what benefit?

Could the answer to 'Why sex?' lie in the fusion of both environment and mutation? What if there is no 'either – or' but an 'and'. In 1999, according to Stuart West, Curtis Lively and Andrew Read argued that multiple mechanisms (environmental and mutational) might be at work and that interactions between the theories might be very important (*J. Evol. Biol.* vol. 12: 1003-12).

In a highly recommendable supplement of *The American Naturalist* (July 2009), Sarah Otto from the University of British Columbia stated, "While models have identified some conditions under which sex and recombination could evolve, these conditions are fiddly and not well supported by empirical evidence. Evolutionary theory seems to be resting on a rather shaky foundation, where we cannot even explain something as commonplace as sexual reproduction." She feels that all the models proposed thus far oversimplify the real world, in which "selection varies over time and space, rates of sex vary and populations are not infinitely large." However, "the past decade has seen a strong push to clarify how including greater realism impacts the conditions under which sex can evolve and be maintained." Well then!

Results versus hypothesis

Okay, so let's go into more detail and look more closely at the Mutational Deterministic hypothesis. This states that high recombination rates evolved and are maintained, in order to break up the linkage of mutations interacting to the detriment of the fitness of an organism. This so-called negative epistasis (the negative effect of both mutations in a single genome is more pronounced than the sum of them) could be the main reason for inventing sex.

Theoretical biologists loved this hypothesis because it's so neat and plausible. But experimental studies in microorganisms and yeast came up with opposing results. One came from Bonhoeffer's lab (*Science* vol. 306:1547-50) where they looked at the diploid human immunodeficiency virus 1 (HIV-1). When two or more HIV particles infect a cell, their genomes are subsequently mixed, making the virus a very simple model for recombination. Bonhoeffer analysed sequences and fitness data for 10,000 HIV genomes, and concluded, "Our data strongly indicate that negative epistasis is not the driving force for sex and recombination." He further noted, "Negative epistasis, as the reason for sex, has been disapproved by various theoretical and empirical data." It's "the most interesting progress of the past ten years of sex and evolution in research!"

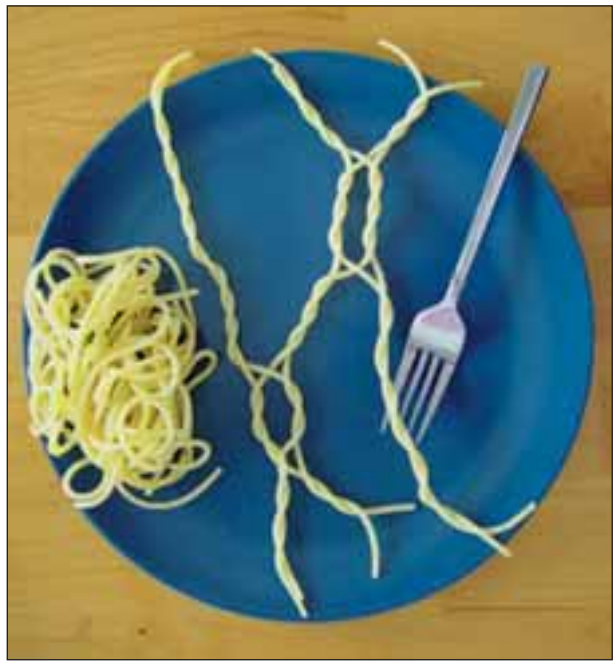
Models versus reality

Meanwhile, Otto *et al.* described how sex and recombination can theoretically evolve when epistasis is negative, absent, or positive in finite populations. This provides a strong counter-argument for genetic drift's influence on sexual evolution, especially when one realises that earlier mutation theories worked with "infinite populations" – the problem being that populations are only infinite in mathematicians' notebooks, not in nature's reality.

So, how can genetic drift influence variance? Let us assume there's a high-fitness allele at a 'locus 1', and a low-fitness allele at the linked 'locus 2'. Now, if selection ▶▶

▸▸ works more efficiently at 'locus 1' than at 'locus 2', then the less advantageous allele at 'locus 2' will also be maintained in the population. According to this pattern, selection generates stochastic (probabilistic) associations between loci. However, this is *not* epistasis because the genes *do not* interact with each other.

Finally, what about the 'Red Queen' theory? For those who are unfamiliar with the term, it is taken from Lewis Carroll's book *Through the Looking-Glass* when the Red Queen informs us that it takes "all the running you can do, to keep in the same place." This Red Queen principle was subsequently adapted by Graham Bell, who explained, "For an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the systems it is co-evolving with."



That's what it's all about: genetic recombination (here a 'spagetthi model')...

Tail wind for the 'Red Queen'

In this context, theoretical scientists calculated the benefit of recombination need not necessarily be immediately seen in the next generation. "For a long time, we thought that the next generation must profit from recombination. We looked at that issue in detail and calculated that in fact the benefits may only be seen in F2, F3, or even later generations," reports Robert Kouyos, a postdoc in Bonhoeffer's lab. "This has been overlooked for quite a while. It's important to realise that the Red Queen theory does not need to be condemned just because you don't see an effect in F1."

Researchers are now waiting for experimental tests that explore the consequences of this insight. To do this, huge data sets must be analysed – fortunately, this has now become possible thanks to new DNA sequencing technology. The first results apparently support the idea that antagonistic coevolution between hosts and parasites can involve the rapid fluctuations of genotype frequencies known as 'Red Queen dynamics'.

Alternatively, you might conduct long term ecological studies like Jukka Jokela from the Swiss Federal Institute of Aquatic Science and Technology in Switzerland and her US collaborators. For 15 years, they observed a conflict in New Zealand lakes between the snail, *Potamopyrgus antipodarum*, and its natural parasite, the trematode *Microphallus*. Here, we have a snail that can reproduce either sexually or asexually. It turns out that asexually reproducing snails do not produce males, hence their reproduction is more efficient – they can outcompete the sexual lines. But in the lakes, there are variations in the distribution of the sexual and asexual populations. In shallow water, where the parasite lives, many sexual snails also occur together with some common and rare asexual clones. Meanwhile, in the intermediate water depths, more asexual clones are found, but in the deepest water, the population is all asexually clonal.

... and fertilization.

Jokela observed that, over time, certain common asexual clones became extinct, whereas other clones were established in the lake. Such rapid fluctuations in common asexual clones support Red Queen dynamics for these snail populations: the scientists showed that the snail's parasite adapted to infect the common and previously widely resistant clones in shallow water, causing their decline. New asexual clones emerged from the sexual lines through errors in normal sexual reproduction. Some of these are resistant to parasites and subsequently become common. Finally, the decline in abundance of sexual snails in deeper water is probably due to the absence of pathogen selection.

But Jokela hasn't identified the errors that cause sexual snails to lose sex and become asexual. "We're trying to get funding for a genome project to work out such details," he says. "We can see that asexuality is derived from sex. And it's not a prehistoric issue – these lakes are less than 6,000 years old – meaning that all the asexual clones have evolved in the recent past. However, it's generally thought that asexuality is evolutionarily the oldest version of reproduction. Here we might have to flip around and look at the issue from the other side."

In this explanation for the maintenance of sex and recombination, Jokela merges environmental and mutational hypotheses. Specialisation of parasites on common genotypes works in the short run to prevent dominance of single genotypes, while mutation accumulation in asexual clones works in the long run, explaining the extinction of clonal lineages.

So what can we conclude from the new experimental and theoretical data over the past five years? The direct short-term effect of recombination is the break-up of associated genes, i.e. reduction of linkage disequilibria, releasing beneficial alleles from deleterious background and vice versa. Furthermore, effects that enhance fitness need not necessarily be seen in the next generation. Also, models indicate that indirect effects, affecting later generations, can have important influences on a sexual population.

One of these effects comes with genes that increase genetic recombi-

nation. What happens if a beneficial gene is linked to such a 'recombination modifier' gene? A modifier gene can promote its own propagation and even escape from a low-fitness background. Due to selection, it can remain in high-fitness genotypes, increasing their recombination. "This is a very important approach on the basis of one gene that selects for enhanced recombination," says Bonhoeffer.

No sex in a static world

In a static world with homogenous populations, there would almost certainly be no sex, argues Otto. But, despite being faced with its substantial disadvantages, sex will be maintained, according to new theoretical studies. "Sex is always well worth its two-fold cost," headlined Alexander Feigel and co-workers in July (*PloS One* vol. 4(7): e6012). The authors of this theoretical paper included in their calculations that individuals are able to sense the sexual state of their fellows and can consequently adapt their own sex and behaviour. The novelty of this approach is based on incorporation of information exchange in evolutionary game theory. "Sensing abilities lead to assortative interactions with the complementary sex. Such non-random interactions stabilize sexual reproduction even if overall Darwinian fitness of a population decreases (up to a two-fold reduction)," Feigel told *Lab Times*.

The world would doubtlessly be a poorer place without sexual reproduction.

Still, neither this elaboration nor other theoretical studies currently answer the *Why*-question. In the end, the answer to that question may be selfishness. Perhaps, Otto says, sex evolved "not because they [genetic elements] enhance variation, nor because they break apart unfavourable gene combinations, but rather because they are selfish and can escape bad genetic backgrounds via sex".

Ostriches wouldn't dance...

Well, since we still can't tell you *why* animals and plants have sex, you'll just have to enjoy its consequences. In a world without sex there would be no males or females, no insects, hummingbirds or butterflies, no peacock tails or birds of paradise. Ostriches wouldn't dance, deer wouldn't bell, birds wouldn't sing. And the author of this article would – perhaps – dispense with her completely uncomfortable, but 'very sexy', high heels! How lucky we are that sex is in the world!

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