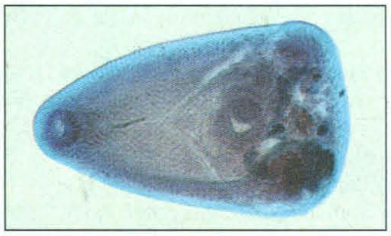
Why Sex? Putting Theory to the Test

NEWS

After decades of theorizing about the evolutionary advantages of sex, biologists are at last beginning to test their ideas in the real world

"Let copulation thrive," exhorted Shakespeare's King Lear, and it has. Today, across the tree of life, sex reigns—many unicellular and just about all multicellular organisms do it. Yet how sex began and why it thrived remain a mystery. After all, asexual organisms were here first, and new asexual species continue to arise, if only to go extinct in fairly short order. Why did sex overtake asexual reproduction some billion or more years ago, and why does it continue to upstage asexuals? What gives sex its edge?



C. LIVELY

Biologists have come up with a profusion of theories since first posing these questions a century ago. Most ideas explore some version of the notion that sex is maintained because it enhances the rate of evolution by natural selection, says evolutionary biologist Graham Bell at McGill University in Montreal, but there are dozens of variations on that general idea (see Review on p. 1986). Most of them fall into two camps: that sex brings beneficial mutations together into a single winning combination that can spread through the population, or that sex purges the genome of harmful mutations. But the devil is in the data—or lack thereof. "I emphasize experimental problems, because we have tons of theories, and some are completely crazy," says Alexey Kondrashov, an evolutionary geneticist at Cornell University.

Driven to sex? The freshwater snails (*above*) may rely on the genetic diversity created by sex to help battle their gonad-eating parasite (*top*).

Sex is a paradox in part because if nature puts a premium on genetic fidelity, asexual reproduction should come out ahead. It transmits, intact, a single parental genome that is by definition successful. Sexual reproduction, on the other hand, involves extensive makeovers of the genome. The production of gametes requires recombination, in which the two copies of each chromosome pair up and exchange DNA. Fertilization, in which genes from different parents fuse, creates yet more genetic combinations. All this shuffling is more likely to break up combinations of good genes than to create them—yet nature keeps reshuffling the deck.

This paradox is compounded by the cost of sex—which is primarily the cost of producing a male. Imagine 1 million sexually reproducing snails and a single asexual female mutant. Say that she has two daughters, who (on average) have another two daughters, and so on. Meanwhile, the sexually reproducing females would be diligently producing a female and a male—who would not directly produce any progeny. Soon, the few sexual organisms would be lost in a sea of asexuals and find it all but impossible to locate a mate. All else being equal, the asexual clone would entirely replace its sexual counterparts in only about 52 generations, says evolutionary biologist Curtis Lively of Indiana University in Bloomington. Yet this happens rarely, if ever. Despite the cost, sexual species persist, while most asexuals quickly go extinct.

In recent years, evolutionary biologists have begun to find ingenious new ways to test their explanations for the strange success of sex. They're observing populations in the wild and in the lab for evidence that rare packages of good genes really do offer an advantage. They're also counting mutation rates in organisms from water fleas to humans to see whether sex might play a role in eliminating harmful mutations. Although they haven't solved the mystery of sex yet, they are tackling what Kondrashov calls the limiting factor of "extremely lousy experimental data."

On the trail of the Red Queen

One theory put to the test in recent years is the Red Queen hypothesis, a variation of the idea that sex serves to assemble beneficial mutations and so creates a well adapted lineage in the face of a rapidly changing environment. In the case of the Red Queen, the good mutations are those that allow hosts to resist parasites. Because parasites adapt to the most common host genotype, evolution will favor hosts with rare combinations of resistant genes. Thus the Red Queen predicts that selection will favor the ability to generate diversity and rare genotypes—exactly the abilities conferred by sex and recombination.

Developed in the 1960s and '70s, the theory has been difficult to test, in part because it's hard to identify and track specific resistance alleles in sexually reproducing organisms, says Lively. Therefore, most of the evidence has been quite indirect, such as a 1987 study by Bell and Austin Burt of Imperial College in Silwood Park, U.K., showing that animals with longer generation times have more recombination. This corroborates the Red Queen, says Bell, because short-lived parasites easily adapt to long-lived animals—which therefore need extra recombination to counter the parasites.

N. ERWIN/NMNH

Sex on the fly. Mutation rates in *Drosophila* may shed light on why sex evolved.

Since 1985, Lively has been seeking more satisfying proof by scrutinizing teeming populations of a freshwater snail, *Potamopyrgus antipodarum*, which is found in both sexual and asexual variants in a cluster of 65 mountain lakes in New Zealand. *P. antipodarum* is plagued by a vicious parasite, *Microphallus*, a trematode that renders the snail sterile by eating its gonads. Lively found that lakes with few parasites tended to have mostly asexual snail populations, or clones, while lakes with more parasites tended to have mostly sexual snails. "This pattern suggests that parasites prevent the clones from eliminating sexual populations," he says.

In the August issue of *Evolution*, Lively and Indiana colleague Mark Dybdahl report more direct evidence for the Red Queen hypothesis from a 5-year study of the snails. They focused on clonal lineages, using identifiable genetic markers to finger each lineage; because each clone had a particular level of parasite resistance, they could track resistance without identifying specific genes. They couldn't use sexual organisms, because recombination would separate markers and resistance genes. But they reasoned that according to the Red Queen, selection pressure should act on these diverse clones just as it does on individuals in a sexual population, favoring unique genotypes and sparking an evolutionary race between snail and parasite. "We looked for rare advantage and a signature of coevolution," says Lively.

Specifically, they sought an oscillation in the frequency of host and parasite genotypes. As the rare host thrives and becomes more common, the parasite evolves to attack it and drive its numbers down. Then a new, resistant genotype surges ahead before the parasite evolves to hold it in check. Both species evolve as fast as they can, but neither gets far ahead, hence the theory's name, after the Red Queen's remark to Alice in Wonderland: "It takes all the running you can do, to keep in the same place."

Using markers for specific enzymes, the team identified rare clones (less than 5% of a lake's population) and common ones (more than 20%) and exposed both sets to parasite eggs. Three of four common clones were 100% infected, whereas rare clones were 50% infected. They also saw the telltale oscillation. Over 5 years, an overinfected common clone was driven down and replaced by what was initially a rare clone, which itself was driven down and replaced.

The two findings strongly indicate that parasites can grant an advantage to the rare genotypes efficiently produced by sex, and can introduce oscillatory dynamics, two key predictions of the Red Queen theory, says Lively. "Whether they maintain sex we can't say from this experiment," he acknowledges. "However, [the results] are consistent with one hypothesis and—more importantly—inconsistent with others." For example, the theory that sex purges bad mutations can't easily explain the pattern of asexual and sexual snails in the lakes, Lively says.

Other researchers also find Lively's correlations intriguing but not conclusive. "You can say [Lively's results] are consistent with the parasite hypothesis," says evolutionary geneticist Brian Charlesworth at the University of Edinburgh in Scotland, "but not that they prove it."

Purging bad genes

Kondrashov is among those skeptical of the Red Queen; he instead favors the notion that sex removes harmful mutations. Removing bad mutations is a far more common challenge for living things than is coping with the fast-changing pressure of an evolving parasite, he says. "I do not believe that everything in the world lives under rapidly changing selection—while everything suffers from bad mutations."

Underpinning this theory are two facts of life: Mutations happen, and most are bad. Meanwhile, sex produces loser and winner offspring by re-sorting the mutations. When two parents with different harmful mutations mate, sex will produce some genetic scapegoats with plenty of bad mutations, and some winners with only a few. Selection will stop the losers dead in their tracks, getting rid of several harmful mutations in one fell swoop.

When a clone reproduces, however, its offspring inherit all of its bad genes and may pick up another through a new mutation. Without sex, mutations continue to accumulate in individuals and in the population. If the mutations interact synergistically, with each new mutation triggering an ever-bigger reduction in fitness (another assumption now under experimental investigation), at some point one more mutation will spell the death of all the clonal individuals. So sexual organisms outdo their asexual competitors because sex brings together, then purges, bad mutations, while the population as a whole is maintained with organisms carrying fewer mutations. And the higher the mutation rate, the greater the advantage of sexual reproduction.

That prediction opens the way to testing this theory. To overcome the efficiency advantage of asexuals, the rate must be on the order of one harmful mutation per individual genome per generation, says Kondrashov. "Fifty percent of modern evolutionary genetic theory may depend on the deleterious mutation rate," he says. "If it's high we can explain sex, recombination, diploidy, aging, and sexual selection."

Other researchers have embraced the mutational theory of sex, in part because unlike so many others it is testable. "This theory sticks its neck out," says Laurence Hurst, an evolutionary geneticist at the University of Bath in England. "You measure the parameters and if you don't find them, the theory is wrong."

To measure those parameters, researchers raise populations of

The Asexual Life

Forty million years is a long time to go without sex, but that's apparently what the bdelloid rotifers, tiny creatures with a long fossil record, have done. Females clone themselves, and no males have ever been seen.

About 1000 asexual species of various organisms are known, but most are expected to die out soon, victims of the long-term disadvantages of asexuality. But the diversity of the freshwater bdelloids, which are their own class comprising four families and about 350 species, suggests a long and vibrant asexual history. That makes them something special: the only organism strongly suspected to have made a go of celibacy for the long haul.

If so, then the bdelloids are the perfect organism on which to test theories of sexual advantage (see main text). "They must have the answer to why asexuals go extinct, because they have figured out how not to," says molecular geneticist Matthew Meselson of Harvard University, whose team is trying to tease out their secret.

The first step is to ascertain that the animals are truly asexual—that no males sneak in occasionally. Proof may be found in the DNA, says Meselson, because in asexual species, neutral mutations will pile up independently in each member of a chromosome pair, while in sexual species, over time those mutations will be lost by genetic drift, keeping chromosomes more similar. By now the pairs of bdelloid chromosomes should be highly divergent, and so far Meselson's team has found very different copies of four genes in each of four species studied. They're now using fluorescent probes to see how similar the chromosomes are. Others say that Meselson's team is on the right track. "He has amazingly great data," says evolutionary biologist Benjamin Normark of Harvard University. –B.W.

MATTHEW MESELSON

Virginal. Like all her kind, this bdelloid rotifer is female.

organisms ranging from water fleas to worms and typically allow only one individual per generation to breed, so that if that individual has picked up a mutation, it won't be eliminated by natural selection. After every 10 generations or so, researchers test the lineages' fitness and translate any fitness decline into the deleterious mutation rate.

But the experiments are tricky, and problems can crop up if selection isn't adequately limited. Additionally, mutations of very small effect may be undetectable in the lab but important in nature, where the numbers are larger and the time is longer. Counting mutations "isn't counting beans," says geneticist James Crow of the University of Wisconsin, Madison.

So far, the results are disconcertingly mixed (see table). "The state of the whole field is very much in doubt right now," says evolutionary geneticist David Houle at the University of Toronto. Benchmark studies by Terumi Mukai and Crow in the 1970s established a deleterious mutation rate of close to one per generation in the fruit fly *Drosophila*, just enough to explain sex in Kondrashov's theory. But later reanalyses of that work put the rate considerably lower. Recent worm experiments have yielded rates as low as 0.005, and recent rates in flies have ranged from just about nil to one.

Now a few scientists are bypassing the difficulties of population genetics experiments and instead simply counting mutations in sequenced stretches of DNA. They compare DNA sequences in noncoding regions in closely related species to derive a genomewide mutation rate. Then they estimate how much of the genome is functional, or subject to selection, and apply the mutation rate to the functional DNA. Beneficial mutations are thought to be so rare that they aren't considered.

One such experiment, by Michael Nachman at the University of Arizona, Tucson, assumes that 5% of the human genome is subject to selection and concludes that each human infant is born with about six mildly deleterious mutations. If a higher proportion of the genome is functional—as some scientists suspect—then the rate would be even higher. Either way, it supports the mutational hypothesis for the maintenance of sex. But researchers agree that more work, in more organisms, is needed. Only the molecular method will vindicate or doom the theory, says geneticist Peter Keightley at the University of Edinburgh, who is now counting mutations in the worm *Caenorhabditis elegans*.

While scientists scrounge for data to support one or the other of the warring theories of sex, other researchers are considering merging the two schools of thought—that sex both collects beneficial mutations and purges bad ones. "My view is they're both going on," says McGill's Bell. "Something as complex, onerous, and laborious as sexuality is probably only going to be maintained if it's doing something very important."

DELETERIOUS MUTATION RATE

Source	Experiment type	Organism	Harmful mutations per genome per generation
T. Mukai, 1972	lab experiment	*Drosophila*, sexual fly	0.6 to 1
P. Keightley, 1996	reanalysis of Mukai	*Drosophila*, sexual fly	<<1
A. Kondrashov, 1997	lab experiment	*Drosophila*, sexual fly	1
M. Lynch, 1998	lab experiment	*Daphnia*, asexual crustacean	0.05 to 1
P. Keightley & A. Caballero, 1997	lab experiment	*C. elegans*, self-fertilizing worm	0.005
M. Nachman, 1998	genomic sequencing	Humans	6

For example, in the "ruby in the rubbish" model, the ruby—a good mutation in an asexual organism—is buried in rubbish—a glut of bad mutations that are constantly being eliminated by selection. Thus the harmful mutations drag the good ones down with them, slowing the rate of evolution relative to sexual populations that can unhitch good genes from bad ones during recombination.

But the evidence for such theories is also very indirect, and testing them is even more of a headache than testing the old theories. "We're in a world where it's easy to say such synergism is likely and harder to say how to go about falsifying it," says Bath's Hurst. For now, biologists can offer plenty of reasons why sex is good for you, but they have a ways to go before they can prove their point.

–BERNICE WUETHRICH

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A New Look at Monogamy

NEWS

Social monogamy, in which parents cooperate to raise their brood, is relatively common among animals—but true sexual fidelity is hard to find

Researchers studying the evolution of monogamy once had a straightforward task: Find those members of the animal kingdom that form lasting pair bonds—and then figure out why fidelity is in each mate's interest. But in recent years that task has grown complex. Genetic studies of organisms from birds to gibbons to rodents have revealed that some of the offspring raised by those seemingly attached parents are in fact fathered by different males. Even among those paragons of pair loyalty, the bluebirds, it turns out that the female slips away for brief liaisons with other males. Yet the two parents continue to work together to raise the young. "The first thing you have to understand is that social monogamy, where you've got a pair bond, is not the same as genetic monogamy," says Stephen Emlen, an evolutionary behavioral ecologist at Cornell University in Ithaca, New York. Indeed, genetic, or sexual, monogamy appears to be the exception rather then the rule among pairs in the animal kingdom.

Why would organisms live and work in exclusive pairs—but sometimes have sex with outsiders? Biologists have a number of theories to explain this complex behavior, as well as its extremely rare counterpart, true sexual monogamy. To test their ideas, they are examining everything from environmental factors to neural chemistry in various species that are socially—if not always genetically—monogamous. Even as they uncover the biochemical underpinnings of fidelity, they suspect that in certain circumstances, some hanky-panky has evolutionary advantages for both males and females.

For most animals, mate partnerships are thought to be somehow related to parental care. Birds, for example, were long assumed to be monogamous because two parents are needed for the prodigious labor of incubating eggs and feeding nestlings—and it was thought that males would only do this if they were certain the young were their own. But that's not the whole story. For example, although a pair of eastern bluebirds may mate, build a nest, and rear a brood together, an average of 15% to 20% of the chicks are not sired by the male in this partnership, according to ongoing research by Patricia Adair Gowaty, a behavioral ecologist at the University of Georgia, Athens. Indeed, studies in the last 10 years of the DNA of the chicks of some 180 socially monogamous species of songbirds indicate that only about 10% are sexually monogamous, says Gowaty.

Males on the prowl are simple to explain in evolutionary terms—