SCANDAL!

Some creatures have what it takes to survive long dry spells. How they do this may be revealed in their genes, reports **Erika Check Hayden**.

Sex-starved and still surviving

Bdelloid rotifers such as *Rotaria macrura* could be the only ancient asexual animals.

In the sleepy, tourist town of Woods Hole, Massachusetts, David Mark Welch is looking for a sex scandal, and he knows just where to find it.

He scrapes a small chunk of bark from a locust tree, drops it in a vial and returns to his laboratory in the imposing, red-brick Lillie building at the Marine Biology Laboratory, or MBL, as it is usually known. Once there, Mark Welch places the bark into a dish of spring water. Within hours, it is swarming with bdelloid rotifers, microscopic animals found all over the world and famous for one scandalous feature: they don't have sex. In fact, the organism may have gone without for tens of millions of years. Mark Welch wants to know how they have managed to last so long and is finding some surprising answers in the structure of their genomes and in how they repair damaged DNA.

Bdelloid rotifers reproduce entirely without males: females package a complete copy of their DNA into eggs that develop, sans fertilization, into the next generation. Asexual reproduction certainly isn't unheard of in the animal world: parasitic bacteria force some insects to reproduce without males and female sharks kept alone in captivity have surprised their keepers by giving birth to baby sharks. But an animal that always reproduces asexually is so anomalous that late biologist John Maynard Smith called the bdelloids' mere existence an "evolutionary scandal"[1]. Only bdelloids, and perhaps one or two other animals, have gone for so long without. In this time, they have thrived, adapting to harsh environments and proving extraordinarily resistant to pathogens, seeming to glean the benefits of sex without any of its drawbacks.

David Mark Welch once lived in fear of finding a hypothesis-busting male bdelloid.

Doubled up

For all its good points, sex does have complications — and not just of the morning-after sort. In the 1970s, Maynard Smith calculated what he called the "two-fold cost of sex". He reasoned that if an asexual mutant suddenly arose in a sexual species, all of its offspring — being female — would be able to reproduce. The male offspring of its sexual counterpart, however, cannot. The asexual mutants would therefore produce twice as many offspring capable of reproducing than the sexual individuals, and would soon dominate the population. Indeed, this is exactly what happens when scientists introduce asexual mutants into cultures of sexual organisms such as yeast, algae or some rotifers.

Yet outside a Petri dish the vast majority of animals are sexual. Why? Most evolutionary biologists think that asexual populations have short-term advantages, but eventually die off without new infusions of genetic variation to help them adapt to change. That's the idea behind prominent explanations for sexual reproduction, such as the Red Queen hypothesis (sex helps defeat pathogens) and Muller's ratchet (asexual organisms can't rid themselves of harmful defects).

Since the seventeenth century, when bdelloids were first spotted, legions of amateur naturalists have trained their microscopes on them. But no one has ever seen a male. Bdelloids' success flies in the face of the idea that asexuality is an evolutionary dead end. More than 400 species of them live all over the world, in all sorts of places: mosses, lichens, bird baths, puddles, sewerage outflows and streams — not to mention tree bark. But their very uniqueness has prompted doubts: surely there must be better evidence of asexuality than our inability to find males?

D. FONTANETO & G. MELONE

It doesn't take two: The bdelloid *Philodina roseola* never gets to couple with another of its species.

D. MARK WELCH

Mark Welch first started looking for such evidence in 1989, when he joined Matthew Meselson's lab at Harvard University. Meselson, a molecular biologist, proposed that bdelloid DNA might provide a definitive sexual history. In sexual organisms, such as humans, opposite sexes each package half of their genetic material into sperm or eggs through a process called meiosis. Their DNA is then mixed together in their offspring. Constant mixing over generations tends to homogenize the gene pool, keeping different individuals' genes relatively similar. But bdelloids don't undergo meiosis or exchange DNA, so each individual is free to accumulate her own mutations and pass them on. An asexual population's overall gene pool, therefore, should contain an increasingly diverse hodgepotch of genes. Testing species heterozygosity — the amount of divergence between different individuals' DNA in the same species — might thus be a test for asexuality.

Mark Welch measured heterozygosity in one gene in four bdelloid rotifers and in seven related, but sexual, monogonont species. An average sexually reproducing species is around 1% heterozygous, and so were the monogononts. But the bdelloids were anywhere from 3.5% to 54% heterozygous — a whopping difference. Mark Welch also found that individual bdelloids carried as many as four very different copies of the same gene — something he did not find in any monogonont rotifers[2]. He and Meselson had provided further evidence that bdelloids were asexual, and their methods were later used to cast doubt on other ancient asexuals (see 'Darwinulids exposed'). The small but enthusiastic worldwide community of rotifer biologists was impressed. "That paper showed they are really asexual — they are not just doing it in the dark when you're not looking," says Bill Birky, an evolutionary geneticist at the University of Arizona in Tucson.

> "Bdelloids are really asexual — they are not just doing it in the dark when you're not looking."
> — Bill Birky

Cryptic behaviour

Still, the question remained as to how the bdelloids could maintain such remarkable control. Mark Welch started to wonder whether it was related to another unusual bdelloid ability: when water is scarce, bdelloids enter into a state of suspended animation. The slightest moisture can resurrect them from this state — called anhydrobiosis — bringing them back to life after a drought.

In 2004, Mark Welch set up his own lab at the MBL. Here, he has at his disposal the power of the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, which supports more small-scale projects than other centres. Indeed, the Bay Paul centre could have burned through the sequencing required for Mark Welch's original project in a few weeks, cutting years off his decade-long toil. Mark Welch is now using that sequencing power to look more closely at rotifer genomes. He sequenced DNA in and near the four genes he and Meselson had studied previously. This sequencing expedition painted an interesting picture.

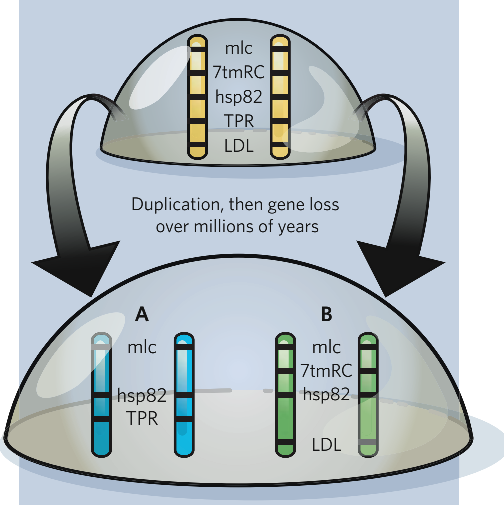
Mark Welch found that a bdelloid's genome is organized in what is called a degenerate tetraploid fashion. Organisms with this genome structure store extra copies of many genes on four separate chromosomes, each of which was originally a member of a chromosome pair that was duplicated some time in the past (see graphic)[3]. Degenerate tetraploids abound in nature — and in the lab; the standard model organism, the yeast *Saccharomyces cerevisiae*, for example, is one.

This tetraploid structure might also explain the mechanism behind another bdelloid superpower, one that has been scrutinized by Eugene Gladyshev. A few years ago, Gladyshev, a graduate student at Harvard University working with Meselson, began studying how the bdelloids could survive and revive themselves from drought. Gladyshev decided to bombard the rotifers with radiation, which affects DNA in ways similar to dehydration. He pummelled specimens of *Philodina roseola*, *Adineta vaga*, and their sexual monogonont relative, *Euchlanis dilatata*, with γ-rays from radioactive caesium. Then he measured how the radioactivity affected each species' eggs.

At 200 Grays, the sexual rotifers lost the ability to reproduce — the DNA in their eggs was so scrambled by γ-rays that most of their eggs failed to hatch. But the asexual rotifers shrugged off the dose, hatching just as many daughters as those that weren't irradiated. Gladyshev doubled, tripled and quadrupled the radiation dose — and still, the asexuals' eggs kept hatching. It wasn't until Gladyshev reached a threshold of more than 1,000 Grays that the asexuals finally failed to hatch their eggs. That dose was not only five times higher than the dose endured by the sexual rotifers; it was also a much greater dose than any tolerated by the other animals for which Gladyshev could find similar data[4].

THE MAKING of a degenerate tetraploid

At some point, the whole genome of the rotifer was duplicated. In subsequent generations, some genes were lost, but the order has stayed the same.



Gladyshev and Meselson concluded that the asexual rotifers are "extraordinarily resistant" to ionizing radiation. As the rotifers are unlikely to be forced to endure such extreme radiation in nature, the scientists speculated that the asexual rotifers have actually evolved the ability to patch up their DNA so that they can survive in "ephemeral" environments — places such as tree bark, or puddles, that might be soaking wet one day and bone-dry the next.

To Mark Welch, this idea was very exciting — and not just because it would add to the bdelloids' growing list of superpowers. It also fit nicely with his findings about the tetraploid bdelloid genome structure. When DNA is broken, by drought or by radiation, it can repair itself by using a complementary piece of DNA as a template. And Mark Welch had found two pairs of similar chromosomes, the A and B pairs, in the genome of *P. roseola*. It seemed to him that these pairs could enable the bdelloids' ephemeral lifestyle. Each drought would shatter their DNA. But one chromosome in a pair could mend the damage to its partner by serving as a template.

These super-charged self-healing powers, Mark Welch thinks, could confer certain advantages on the rotifers. Not only might they enable the bdelloids to survive in harsh places where other animals fear to tread, they could also allow the creatures to adapt to change, because their genomes are constantly remodelled during the process of DNA repair. And the self-healing might allow them to rid themselves of invasive elements, such as dreaded "retrotransposons" — genetic invaders that can insert themselves into DNA, copy themselves, and overwhelm a defenceless genome. Researchers have not found any retrotransposons in bdelloids[5]. And the organisms have so far resisted being infected by several of the pathogens that commonly infect *Caenorhabditis elegans* and *Drosophila*. The "rotifers just eat the bacteria", says Mark Welch.

Secret to success

Indeed, the combination of a tetraploid genome and an ability to tolerate drought might be enough to make up for the drawbacks of asexuality, Mark Welch says. And here at last, perhaps, is the solution to why the bdelloids are one of the world's only successful asexual animals: "You have a successful asexual because of this combination of their ecology and their unique physiology," Mark Welch says. "A lot of little things had to be in place, and perhaps nobody else came up with this particular unique set of circumstances."

Other biologists are intrigued by the idea too. "The bdelloid work is fascinating, and eventually we may be able to understand why the bdelloids have existed for so long — due to some combination of large population sizes, DNA repair and ancestral polyploidy," says evolutionary biologist Roger Butlin at the University of Sheffield, UK. "I suspect they are all contributing." Yet, he says, "I am not sure why that ends up answering the question of why sex is widespread."

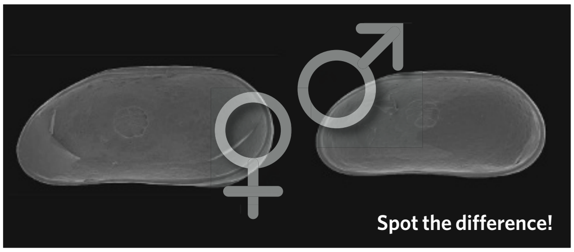
This, of course, is the big question — if the bdelloids are so unique, what are they telling us about the rest of the world? A lot, says Mark Welch. If it's true that bdelloids have survived as asexuals because they can do things like cleanse their genomes of pathogens and maintain genetic diversity, then those may be the very same things accomplished by sex. Perhaps evolution, nature's inveterate tinkerer, has come up with more than one solution for these problems.

Immunity and diversity might not sound like poetic reasons for having sex — you were expecting, perhaps, a paean to the joy of companionship and the thrill of two organisms in union? For the bdelloids, at least, it's hardly missed. ■

Erika Check Hayden writes for *Nature* from San Francisco

1. *Nature* **324,** 300–301 (1986).
2. Mark Welch, D. B. & Meselson, M. *Science* **288,** 1211–1215 (2000).
3. Mark Welch, D. B., Mark Welch, J. L. & Meselson, M. *Proc. Natl Acad. Sci. USA* **105,** 5145–5149 (2008).
4. Gladyshev, E. & Meselson, M. *Proc. Natl Acad. Sci. USA* **105,** 5139–5144 (2008).
5. Arkhipova, I. & Meselson, M. *Proc. Natl Acad. Sci. USA* **97,** 14473–14477 (2000).
6. Smith, R. J., Kamiya, T. & Horne, D. J. *Proc. Biol. Sci.* **273,** 1569–1578 (2006).

Darwinulids *EXPOSED!!*



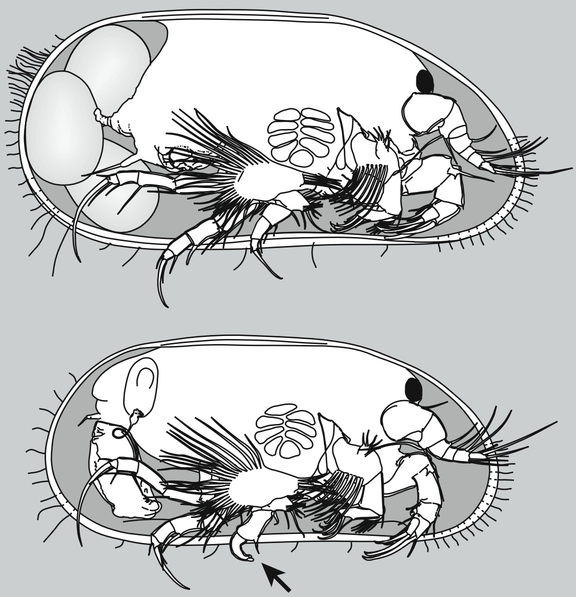
REF. 6

One day in 2003, a biologist collecting specimens stumbled across the nightmare of every scientist who studies ancient asexual organisms: a male[6].

Robin Smith from Lake Biwa Museum in Shiga, Japan, had gone hunting for darwinulid ostracods — tiny freshwater crustaceans long thought to be asexual. He was searching on a forested island called Yakushima, off the coast of southern Japan, with a colleague, Takahiro Kamiya from Kanazawa University. While looking for specimens in a stream near the coast, Smith spotted an ostracod that seemed unusually small. It wasn't until he got back to his lab and dissected the animal that he saw it wasn't just small — it was a male. It had the telltale hooks on its legs, for instance, that male ostracods use to grab their female partners while mating.

Researchers were already dubious about the asexual credentials of darwinulids. They had found low heterozygosity between individuals, suggesting that the tiny creatures could be having sex. But finding a male was a catastrophe for those who believed in the organisms' asexuality. David Mark Welch, who studies asexual rotifers called bdelloids at the Marine Biology Laboratory in Woods Hole, Massachusetts, commiserates: "Throughout my graduate career we lived in fear of reading some report of male bdelloids."

Smith and Kamiya's find raised the question of whether darwinulid males had simply been overlooked in the fossil record because they looked a lot like juvenile females. "Without this fossil evidence, the hypothesis that this group is ancient and asexual doesn't have that much other support," says Smith. He and Kamiya have gone back to Yakushima numerous times, and have only turned up two more males, among thousands more females. So it's not clear whether the males had any role in the population, or whether they were just random curiosities. Nonetheless, the finds raised a caveat about the evidence for asexuality in ostracods — and leaves the bdelloids as perhaps the last thriving asexual animals. **E.C.H.**



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Male ostracods have hooks (arrow) to grasp their partners.