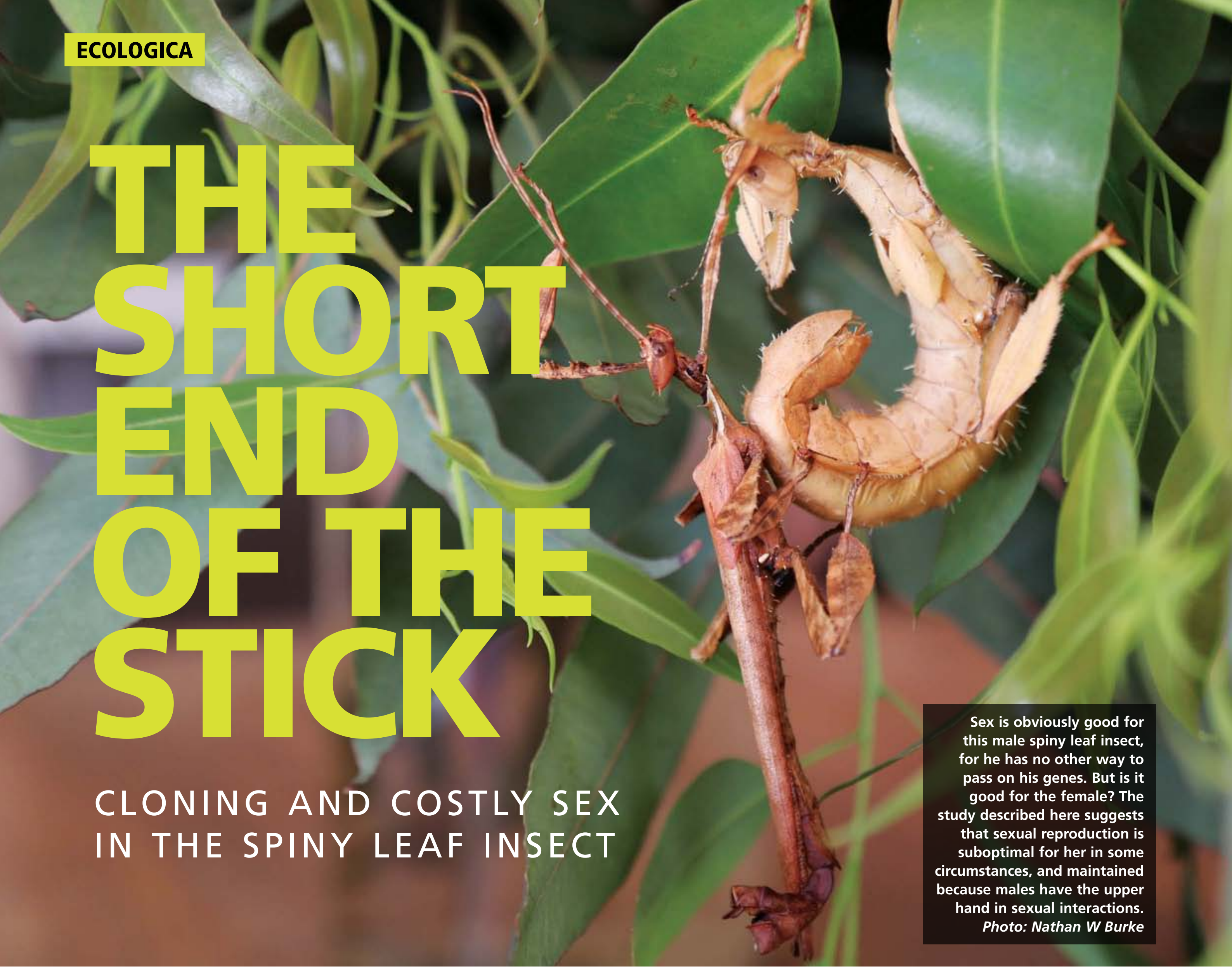
THE SHORT END OF THE STICK

CLONING AND COSTLY SEX IN THE SPINY LEAF INSECT



Sex is obviously good for this male spiny leaf insect, for he has no other way to pass on his genes. But is it good for the female? The study described here suggests that sexual reproduction is suboptimal for her in some circumstances, and maintained because males have the upper hand in sexual interactions. *Photo: Nathan W Burke*

Evolutionary ecologist **Nathan W Burke** questions conventional wisdom on why sex is so common.

More than 99% of all animal species reproduce sexually. A few species clone themselves instead, and an even smaller number reproduce both sexually and clonally. The spiny leaf insect (*Extatosoma tiaratum*) is one of those few capable of optional cloning (also called facultative parthenogenesis). When its eggs are fertilised by sperm, they develop sexually, but unfertilised eggs develop clonally.

Evolutionary biologists have long thought of optional cloning as a safety net for when males are uncommon or sex is impossible. This assumes that sex is the better option. But is sex really superior to cloning? If females that reproduce both sexually and clonally are allowed to choose, do they go for sex? I recently investigated the preferences of spiny leaf insects, and what I found suggests that sex may not be all it's cracked up to be.

The downsides of sex

The costs of sex can be high. Finding a mate can be extremely time-consuming, and attracting a dud male or a male of the wrong species can be catastrophic for females. Mating can increase the risk of predation and transmit nasty sexual diseases and parasites. For females of some species, copulation can be so traumatic that injury, infection or even death results (see Mismatched interests in sex, opposite). These are serious downsides. But by far the largest cost of sex is producing sons. Sexual females that produce both sons and daughters will generate only half as many descendants in the long run as clonal females that produce only daughters.

The benefits of sex

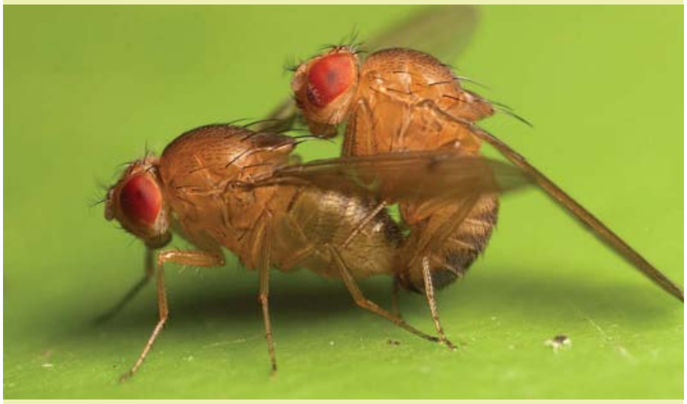
If sex is so expensive, why is it so successful? The main advantage is thought to be genetic reshuffling, which results in offspring inheriting unique combinations of genes from each parent. Genetic variation may increase the chances of some descendants adapting to environmental change or developing resistance to certain parasites and diseases.

Clonal populations, in contrast, often have very low genetic diversity and may be more likely to accumulate harmful mutations. Some evolutionary biologists consider clonal reproduction an evolutionary 'dead end' because clonal lineages seem more susceptible to extinction than sexual lineages.

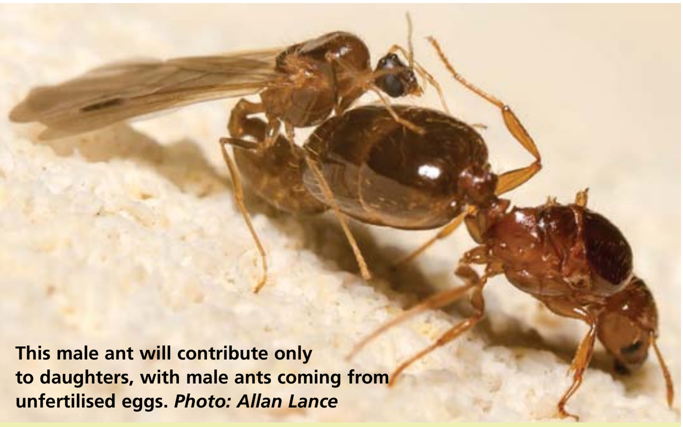
However, the advantages that come from reshuffling genes can't explain why sex is so widespread. This is because sexual populations tend to benefit from genetic variation only in the long run, whereas the benefits of cloning, such as higher population growth and escape from harmful mates, are realised immediately. The long-term benefits of sex are unlikely to beat the short-term benefits of cloning because natural selection acts on individuals in the here and now, not in the future. Natural selection favours traits that provide benefits now over traits that provide benefits later.

MISMATCHED INTERESTS IN SEX

Traumatic copulation – when males wound females during sex – evolves when there are irreconcilable differences in the evolutionary interests of females and males (for example, when females prefer few copulations and males prefer many). Because female reproduction is a 'resource' for males, sexual conflict often promotes coercive behaviours that improve their own reproductive fitness at the expense of their female mates. This is why natural selection has produced seemingly barbaric adaptations like barbed penises in seed beetles that damage the female reproductive tract, hypodermic penises in bed bugs that inject sperm directly into the female body cavity, and death-inducing compounds in fruit fly semen that immediately increase fecundity but significantly reduce female lifespan.



A compound in the semen of a male fruit fly is toxic to the female, and shortens her life. The more partners she has the sooner she will die. The purpose of the compound is to increase the chances that his sperm rather than those of a rival fertilise his mate's eggs, and to reduce her urge to mate with other males. ***Photo: Brian Valentine***



This male ant will contribute only to daughters, with male ants coming from unfertilised eggs. ***Photo: Allan Lance***

ARRHENOTOKY, AN UNUSUAL TYPE OF CLONING

Cloning doesn't always result in the production of female clones. Arrhenotoky is a special kind of cloning that produces males. Most bees, ants and wasps reproduce in this way due to their unique sex-determination system – females are produced from fertilised eggs and males from unfertilised eggs. It is also found among thrips, beetles, mites and bugs. This system offers several benefits for females. By having control over the sex ratio of their offspring, the mothers are able to bias their reproduction towards the more helpful sex. And because males obtain reproductive success only through their daughters, traits harmful to females are unlikely to spread.

Why not both?

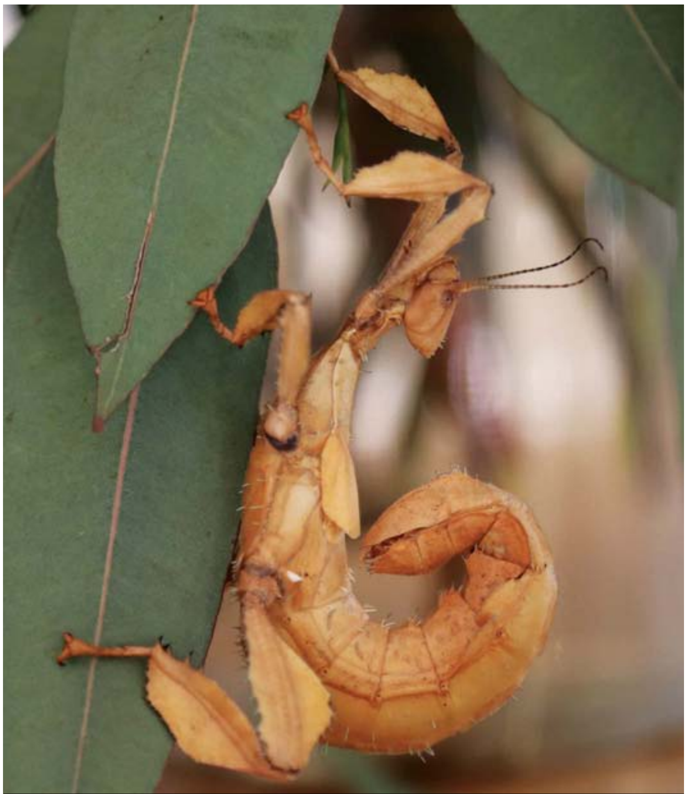
From an evolutionary standpoint, optional cloning seems to offer females the best of both worlds: the opportunity to produce clones when sex is costly or unavailable, and to have sex when cloning is the worse option. By combining the short-term benefits of cloning with the long-term benefits of sex, we would expect optional cloning to be the most successful way to reproduce. So why isn't everyone doing it?

There are three types of optional cloning, each limited to a narrow range of organisms. In the likes of water fleas and aphids, reproduction is mostly clonal until seasonal changes trigger sex. In bees and ants, daughters hatch from sexually produced eggs, while sons emerge from unfertilised eggs (see Arrhenotoky, left). And in stick insects and mayflies, cloning occurs if females don't mate or if fertilisation fails.

This last type is thought to evolve in small sexual populations when sex is difficult because males are scarce. It should be favoured by natural selection since any kind of reproduction is better than none. Lending support to this idea are several cases of spontaneous cloning in captive-bred female reptiles and sharks that have never been housed with males.

If optional cloning is merely a backup, females should strongly prefer sex when given a choice. But there is another possibility. Optional cloning could enable females to escape the many costs of sex. If this was the case, we would expect females to avoid opportunities to mate, to strongly defend themselves against male advances, and to show preferences for clonal rather than sexual reproduction.

Spiny leaf insects are an excellent animal for testing these competing ideas. Being optionally clonal, their fertilised eggs hatch into male and female offspring, while unfertilised eggs yield all-female clones. They are easy to keep in captivity and their interactions with males are easy to observe.



Female spiny leaf insects turn their anti-predator defences, including the abdomen curling shown here, against males of their own species. Their reluctance to mate is understandable – the study described here shows that more than half the females that mate after reproducing clonally die soon after mating and they lay fewer eggs than if they reproduce just clonally or just sexually. ***Photo: Nathan W Burke***

Like other phasmatids, spiny leaf insects are masters of disguise, mimicking either leaves or lichen (as shown here) to hide from predators. Adult females grow up to 20 centimetres and vary from green to dark orange. Males grow to about 11 centimetres. They eat a variety of plants in their rainforest habitat, mostly belonging to the Myrtaceae and Rosaceae families. *Photo: Peter Spradbrow*

Masters of disguise

Found in the canopy of rainforests in eastern Queensland, the spiny leaf insect is a curious creature – and not just because of its sex life. It is a popular pet around the world, and even made a cameo appearance in the Hollywood blockbuster, *Indiana Jones and the Temple of Doom*.

Like other phasmatids (commonly called leaf and stick insects), spiny leaf insects hide from predators by camouflage. Most adults mimic leaves but one subspecies looks like lichen. Eggs and hatchlings also blend with their background (see Seed-mimicking eggs, right).

When disturbed, adult spiny leaf insects secrete a chemical that smells like sour milk. Some stick insect species spray their chemicals at approaching predators, but the spiny leaf insect makes itself unappetising by secreting chemicals onto its own body. Some species also try to startle predators by unfurling their wings to reveal bright colours or fake eyespots. Female spiny leaf insects have very small non-functional wings. To repel predators, they curl their abdomen tightly over their back to reveal nasty spikes, and raise their serrated hind legs to kick at any looming threat. While keeping spiny leaf insects as pets, I often saw females behave in the same way against potential mates, as if anti-predator behaviours have a dual purpose.

Is cloning sex's understudy or its rival?

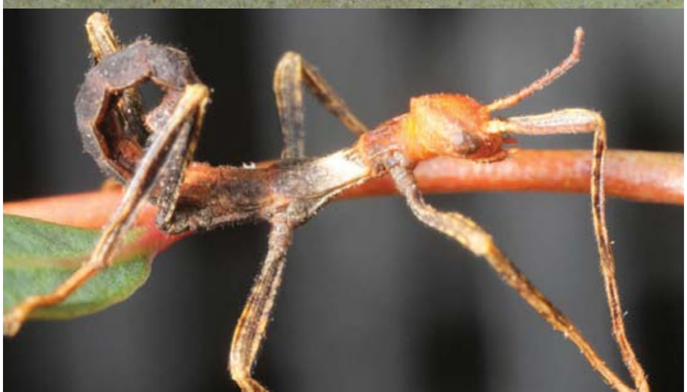
To find out what these insects can reveal about the evolution of cloning, I manipulated female access to males in a series of laboratory experiments. Females were paired with either males (for sex) or control females (for cloning), and a third group was initially paired with females and then with males (to initiate a switch from cloning to sex). I measured how long the various females lived and the number of surviving offspring they produced. I recorded their behaviours towards males and tested the responses of males to female odours.

I found that female spiny leaf insects were not keen on sex. They strongly resisted male advances – both chemically and by their behaviour.

By rubbing leaves with anti-predator secretions from different females, I found that the secretions produced by young adult females repelled males, but the secretions of other females and other males did not. This suggests that young adult females use their 'sour milk' anti-predator secretion as an anti-aphrodisiac to repel males.

I also laced leaves with pheromones (chemicals released to attract the opposite sex) produced by different females. Males were attracted to the pheromones of all females except those that had previously reproduced clonally. This suggests that females that clone themselves alter their pheromones to become invisible to males. (Whether this prevents sexual contact in the wild is not known.)

The behaviours of females suggested they did not want to mate. When females that had never encountered a male were paired with one, they kicked vigorously and curled their abdomen to prevent males grasping them.



Photos: Martin Lagerwey

SEED-MIMICKING EGGS AND ANT-MIMICKING NYMPHS

Many Australian plants, including wattles, have seeds with a fleshy knob of protein (an elaiosome). Ants collect these seeds, feed the nutritious elaiosomes to larvae in the nest, and discard the seeds in underground 'rubbish dumps', where they often germinate.

The eggs of many stick insects, including those of spiny leaf insects (top), look remarkably like these seeds. Flicked to the ground by the mother from high in the tree canopy, these seed-mimicking eggs have a fleshy knob of protein (a capitulum) which ants feed to their larvae. The discarded eggs develop and hatch inside the ants' nest free from above-ground predators and parasites. When the nymphs emerge (above), they look and smell strikingly similar to the ants whose nest they occupy. Their disguise allows them to swiftly escape the ant nest and climb to a new home at the top of the nearest tree.

The reason for these defences became clear when I looked at the fate of females and their offspring. More than half the females that started reproducing clonally but later switched to sex died soon after being paired with a male. Their survival rate was considerably less than females who reproduced just clonally. They also produced fewer eggs. This suggests that sex is particularly costly for females that switch from clonal to sexual reproduction. We don't know why. Perhaps substances in male semen have a nasty effect on females that have already reproduced clonally.

The story is more complex, however, because the offspring of clonal females that switched tended to survive better than clonal hatchlings. This could potentially compensate for some of the fitness costs of sex for these mothers, but further experiments are needed to know for sure.

Overall, the chemical secretions and resistance behaviours of spiny leaf insects imply that females have evolved to avoid males and minimise matings. This supports the idea that optional cloning evolved as a way for females to avoid the costs of sex, rather than as a suboptimal fallback for when males are scarce.

All this suggests that sex is like a high-security prison for females manned by male guards.

Do eager males keep costly sex alive?

My study offers a tantalising new perspective on sex and its evolution. Sexual reproduction might be widespread in nature not because of the long-term benefits that come from gene shuffling, but because males keep females tethered to a sexual way of life. Females of many species, not just spiny leaf insects, might benefit from giving up sex. But males cannot pass on their genes without females, so are almost always eager to mate. This sexual drive in males may lead to coercive copulations, depriving females of opportunities for cloning. So, sex might be an evolutionary trap from which females cannot escape unless, like spiny leaf insects, they can hide from males or resist mating attempts. All this suggests that sex is like a high-security prison for females manned by male guards. Although this analogy may seem extreme, it highlights the point that conflict between the sexes could shape the evolution of reproductive modes. My study suggests that females might only escape from sex if clonal reproduction and an ability to resist males evolve at the same time. Could this golden combination make females so successful at avoiding sex that males in all species eventually go extinct? It's possible. But because males are likely to counter female resistance by evolving stronger coercive abilities, widespread male extinction seems unlikely. Despite the benefits of cloning, the universal eagerness of males to mate is likely to perpetuate sex, regardless of its costs.

READING: Burke NW, Crean AJ, Bonduriansky R. 2015. The role of sexual conflict in the evolution of facultative parthenogenesis: A study on the spiny leaf stick insect. *Animal Behaviour* 101:117–27

NATHAN W BURKE is a PhD candidate in evolutionary ecology at the University of New South Wales. He is interested in understanding why most animals rely on sex to reproduce. Using stick insects, he investigates the evolutionary forces that promote and inhibit cloning to better understand why optional cloning isn't more widespread in nature.