# More Fun Than Fun: Battles of the Sexes Can Now Be Made to Order – or Vanish

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*N.G. Prasad and Bodhisatta Nandy. Right top: Flies under experimental selection. Photos: Evolutionary Biology Lab/IISER Mohali. Right bottom: A female fruit fly. Photo: R. Vijaya Anand/IISER Mohali.*

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*This article is part of the 'More Fun Than Fun' column by Prof Raghavendra Gadagkar. He will explore interesting research papers or books and, while placing them in context, make them accessible to a wide readership.*

When living organisms invented sexual reproduction over a billion years ago, they set in motion two conundrums, both for themselves and for evolutionary biologists. The first conundrum concerns the evolutionary cost of sex. In asexual species, every individual produces offspring. On the other hand, in sexually reproducing species, two parents are needed to produce offspring, so that populations of sexual species grow only at half the rate of asexual species. The evolutionary advantages of sexual reproduction that can potentially offset this two-fold cost of sex are the subject of much study and debate.

The second conundrum concerns inter-sexual conflict arising due to sexual reproduction. The conflict comes in two varieties. The first variety is because the variants of our genes that are best suited to making a female are not always the same as the variants best suited to make a male. Natural selection favours different variants in the males and females, only to be undone when genes from the male and female mix to form the next generation. It's a sobering thought that we are all not the best that natural selection can produce, but the best possible compromise between being a good female and a good male.

The second variety of conflict arises because of competition for sex partners. The greatest conflict would be in species where both sexes mate promiscuously and repeatedly. Natural selection would be expected to favour genes in one sex that help manipulate the other sex, to serve its own purpose, and favour other genes in the opposite sex that can help counteract these manipulations. This is not mere speculation. We know of some really macabre manifestations of such conflict, involving an evolutionary tug-of-war between the sexes.

**Severity of competition**

Some male insects, for example, sneak certain harmful chemicals along with their sperm into female bodies. These 'mate-harming' chemicals may make the female partner lay her lifetime supply of eggs at the present time and die. This is not in the female's best interests – but it helps the male father all her offspring. Females in these species are known to inactivate these harmful chemicals (and do much worse things to their partners, like eat them up for example).

Both sexes invest time and energy on one-upmanship at equilibrium because the two mated partners' evolutionary interests are not entirely aligned. They may well be together for the current joint offspring, but both may have future options with other partners.

The severity of competition for mates should depend on the availability of mating partners, but there is usually a curious asymmetry between the sexes in this matter. Males generally invest less time and resources per offspring than do females. Hence, males can generally have many more offspring than females can afford. Males, therefore, compete more intensely for females than the other way around. Consequently, we may predict that natural selection will favour increased conflict and competition for mates if the population is male-biased.

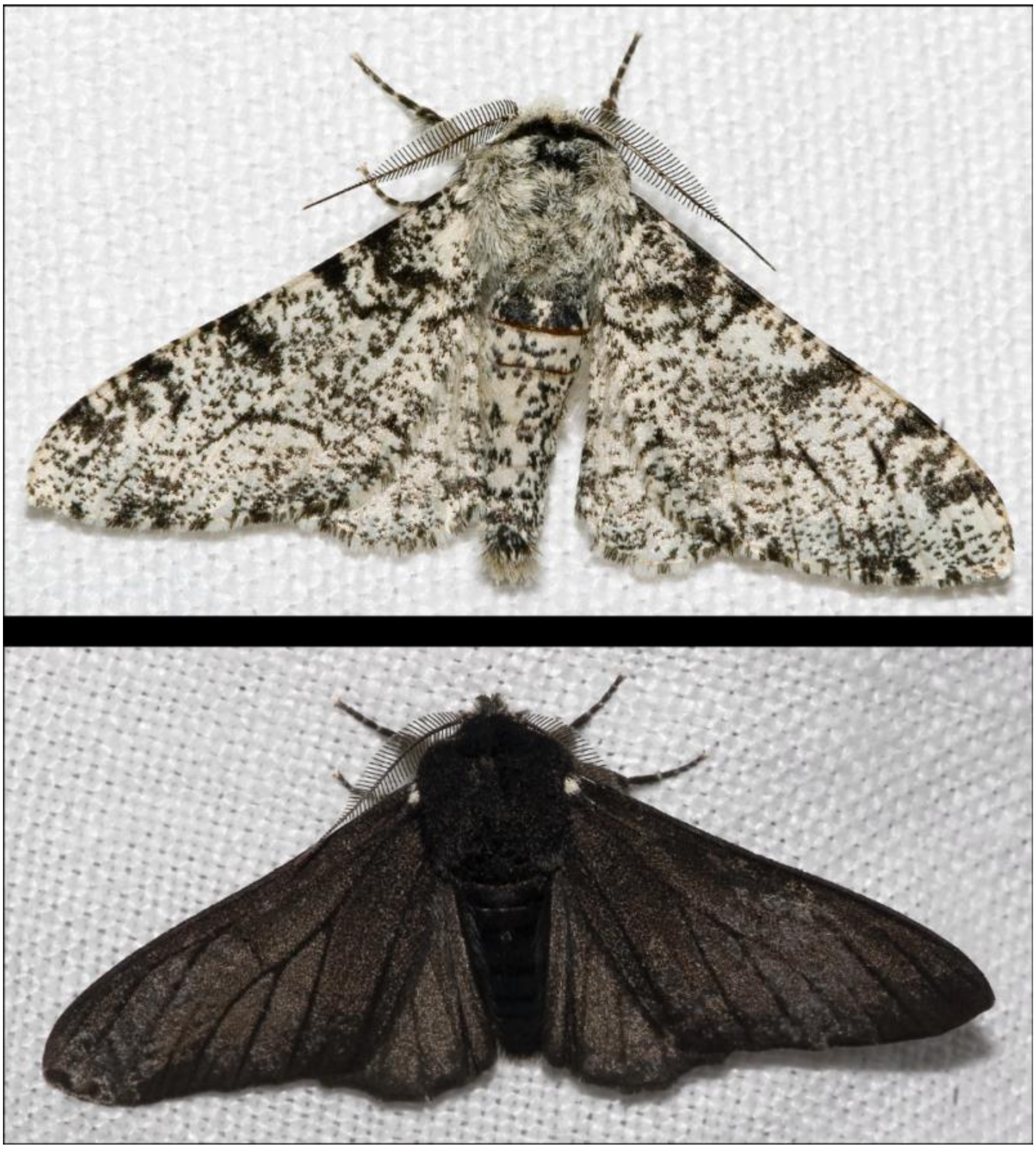
Conversely, natural selection would favour less conflict and competition for mates if the population is female-biased. There is some confirmation of these predictions in species with naturally occurring male and female-biased sex ratios. But how much more spectacular it would be if we can see increased and reduced conflicts change in real-time in response to the corresponding changes in sex ratios? Can we hope to witness this?

Evolution is generally thought to be a very slow process making experimental verifications of hypotheses impractical. To quote Charles Darwin:

> It may metaphorically be said that natural selection is daily and hourly scrutinising, throughout the world, the slightest variations; rejecting those that are bad, preserving and adding up all that are good; silently and insensibly working, whenever and wherever opportunity offers…

But having so poetically described natural selection, he lamented, "We see nothing of these slow changes in progress, until the hand of time has marked the lapse of ages, and then so imperfect is our view into long-past geological ages, that we see only that the forms of life are now different from what they formerly were."

**Of moths and finches**



The black-peppered moth. Top: peppered form; bottom: melanic form.
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There are some famous exceptions, fortunately. The peppered moth (*Biston betularia*), as its name implies, has wings flecked with black and white spots, camouflaging it well when it rests on the lichen-covered barks of trees. An occasional all-black (melanic) mutant is quickly spotted and eaten by birds.

Naturalists in England and America have recorded the changing fates of the peppered and melanic forms in response to natural selection. As industrialisation resulted in more factories emitting soot which killed the lichens and blackened the barks of trees, the peppered forms lost their protection and declined in numbers. And the melanic forms, with their new-found camouflage, rose in numbers.

Subsequently, as anti-pollution laws were implemented and the lichen grew back, naturalists recorded the peppered form's return. In the words of the English doctor and naturalist, Bernard Kettlewell, who placed these observations on a firm scientific footing, "among all living things it has fallen to the Lepidoptera [the insect group containing butterflies and moths] to provide evidence of the most striking evolutionary change in nature ever to be witnessed by man".

Another celebrated example is the remarkable 40-year study of Darwin's finches on the Galapagos islands by Peter and Rosemary Grant of Princeton University. They have shown that a single ancestor species emigrated to the islands and diversified into at least 13 different species. But more interesting for the present discussion is their detailed study of the medium ground finch (*Geospiza fortis*), on the island of Daphne Major.

Paralleling the peppered moth story, the Grants documented natural selection in the finch population in an even shorter span of time. They saw before their own eyes that the finch populations changed from small beak size to large beak size and back to small beak size again. Here, the selective pressure on beak size was the availability or lack of suitable seeds. A drought made small seeds scarce, killing off birds with small beaks, leaving mostly those with large beaks to breed and populate future generations. A few years later, torrential rains caused large seeds to become scarce, killing off birds with large beaks, leaving only birds with small beaks to breed.

There is great pleasure in store for those who endeavour to learn about this amazing research – from the Grants themselves, from a Pulitzer-Prize-winning journalistic account or from a documentary film under the guidance of Sean B. Carroll, himself a famed researcher and author.

**Custom orders**

N.G. Prasad and his fly research group. Photo: Evolutionary Biology Lab/IISER Mohali

Revealing as these stories are, there are too few examples of this kind, making observable rapid evolution in the wild, an exception rather than the rule. Besides, the researchers have no control over the strength of selection or its direction. It is not possible to have evolution made to order in nature. But that is precisely what we will need if we are to test specific evolutionary hypotheses of the kind we set out discussing.

However, there is a way to have evolution made to order and witness natural selection – not just in a researcher's lifetime but indeed, in the span of a PhD. To do so requires thinking out of the box.

In inventing the idea of natural selection and fine-tuning it to account for the observed products of evolution in the past, Darwin drew inspiration, confidence and indeed, a sense of déjà vu, from artificial selection. He devoted a great deal of his book *The Origin of Species* (1859) to illustrating how animal breeders and horticulturists had routinely obtained varieties of animals and plants with the desired characteristics by selectively breeding from ancestral populations. The trick was to selectively pick individuals with the desired characters to make up the breeding pairs in each generation, and discard (or eat) the rest.

It took over one hundred years after Darwin's book for evolutionary biologists to learn to play the role of animal and plant breeders and apply the desired selection pressure in the laboratory, under controlled conditions. But once, the trick was discovered, there was no looking back. Experimental evolution in the laboratory is now a frequently used, powerful tool, not only to test evolutionary hypotheses but to realise, often with surprise, that many more characters evolve than was intended, the so-called ‘correlated responses’. Selection experiments in the laboratory have often had the sobering effect of revealing that our imagination of how natural selection might proceed, can be entirely wrong.

Richard Lenski, at the Michigan State University, has famously subjected bacteria to selection in the laboratory for thousands of generations. Unfortunately, many interesting questions can’t be answered with the limited morphological and behavioural complexity of bacteria. It has therefore fallen upon the fruit fly (*Drosophila melanogaster*) “to provide evidence of the most striking evolutionary changes ever to be witnessed by man [and too few women so far, I am sorry to see]”.

Although not the first one to use them, much of the credit for putting *Drosophila* on the flight to unprecedented success as a model laboratory organism goes to the American biologist Thomas Hunt Morgan (1886-1945), and his students in the fly room at Columbia University, New York. In the words of the biographer Gareth Williams, “At 3 millimetres long and with roughly 2 million to the kilogram, the flies are easy and cheap to keep, especially when milk-bottles can be obtained by ‘more or less unorthodox means’ (pre-dawn raids on the doorsteps of Manhattan) … *Drosophila* need no aphrodisiac beyond decomposing bananas, and they excel at copulation: a single male put into a milk-bottle of females typically sires 1,400 offspring”.

Using little more than glass vials and cornmeal, and with the participation of a large number of bright and committed students, N.G. Prasad at the Indian Institute of Science, Education and Research (IISER), Mohali, has shown that inter-sexual conflict can indeed be made to order or made to disappear – by the action of natural selection.

In one set of experiments, Prasad and his students, led by Bodhisatta Nandy (now assistant professor, IISER Berhampur, Odisha, made the flies to evolve in the laboratory for over 45 generations with a male-biased sex ratio. They ensured that there were three times as many males in every bottle in every generation as there were females. The flies produced equal numbers of male and female progeny, but the researchers picked thrice as many males as females to start the next generation in the next bottle. Similarly, they conducted other experiments in which there were always three times as many females as males. As a basis for comparison, they also maintained bottles with equal numbers of males and females.

At the end of 45 generations or more, they compared the flies from three different sex-ratio regimes to see what natural selection might have done to the flies. Their results are striking. I will follow their nomenclature: flies from the male-biased populations are called M males and M females; flies from the female-biased populations are called F males and F females. Flies from the control populations are called C males and C females. Because males compete for females more than the other way around, the male-biased populations represent a high-conflict environment; the female-biased populations represent a low conflict environment; and the C populations represent an environment with intermediate conflict.

After 45 generation of selection, M males had become lighter, more active, showed higher courtship levels, harmed females more and died young. When mated with or exposed to M males, the ancestral females died sooner and produced fewer offspring. Clearly, M males lived life in the fast lane, competing intensely for access to the limited number of females. In contrast, F males were heavier, relatively less active, showed lower courtship levels, harmed females less and lived longer lives. When mated with F males, ancestral females lived longer and produced more offspring. F males thus lived life in the slow lane, having to compete less for access to females.

Four of Darwin's finches (clockwise from top left):
*Geospiza magnirostris, Geospiza fortis, Certhidea olivacea* and *Camarhynchus parvulus*.


**Different paths to different ends**

Male-male competition for females does not always end with copulation. If two males manage to inseminate a female, the sperm of the two males continue to compete with each other inside the body of the female. The sperm of the male who copulates first defends itself against invasion by the sperm of the second male. And sperm from the second male use offensive tactics to minimise the survival and success of the sperm of the first male. After 45 generations of evolving under high and low competition conditions, respectively, M males had evolved higher levels of both defence and offence than F males.

In contrast to some researchers in the past, Prasad and his students were not content with noting the evolution of male competitive ability and their mate harming ability. It is unthinkable that females don't fight back. Natural selection should be "daily and hourly scrutinising … the slightest variations; rejecting those that are bad, preserving and adding up all that are good" as diligently for the females as it does for the males. So, how did the females evolve in Prasad's laboratory?

F females became heavier, lived longer and produced more offspring than M females when both were paired with the ancestral males. However, F females had not evolved the ability to resist male harm nearly as well as the M females had done. At the cost of becoming lighter, producing fewer offspring and dying sooner, M females had apparently invested in defence against male harm. Thus, both M males and M females suffered due to the intense competition in their environment. This is not really surprising.

But that evolution by natural selection takes such divergent evolutionary trajectories, exactly as predicted by theory, and that we can witness this in real-time is exhilarating. By simple, clever and inexpensive experiments, of the kind that almost anyone could have done, Prasad and his students have succeeded in making the battle of the sexes to increase or decrease at their will.


The divergence in flies between the high and low conflict environments that Prasad and his students have engendered are so great that after about 100 generations of selection, the M and F flies become increasingly incapable of interbreeding – a sign of incipient speciation. Experimental evolution in the laboratory also has the potential to make new species to order. What better proof can we find for Darwin's theory of the origin of species by natural selection?

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