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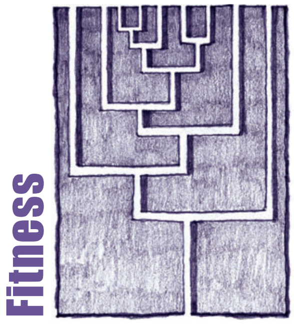
Female beetles prefer lovers, not fighters



When it comes to successfully winning a mate, there are two sides of the coin. Individuals need to both compete against members of their own sex for access to mates and convince members of the opposite sex that they are an attractive mating option. Traditionally, it is assumed that when males compete with one another for access to females, females should find the strongest and more competitive males the most attractive. However, there is no reason that this should necessarily be the case. While more competitive males might father sons that are also stronger and more competitive, strong males might also use coercive tactics against females to gain mating opportunities, which will lead females to resist these suitors. There may also be disadvantages for daughters fathered by these strong males, which could potentially balance out any benefits for sons.

Led by Kensuke Okada, a team of researchers from Okayama University and University of Tsukuba, Japan, and University of Exeter, UK, decided to investigate the two sides of the mating coin in the broad-horned flour beetle (*Gnatocerus cornutus*). In broad-horned flour beetles, males have enlarged mandibles that they use for fighting with other males. Males with the largest mandibles consistently overpower other males and have the highest mating success. First, Okada and his colleagues assessed whether females find males with larger mandibles the most attractive. As females control when mating occurs in the broad-horned flour beetles, the researchers used the time between introducing a pair and mating as a measure of male attractiveness. Surprisingly, they discovered that despite the tight link between mandible size and competitive prowess, females appeared to take no notice of this trait. Instead, they preferred males that courted more vigorously, and males that performed more courtship bouts enjoyed more rapid mating success. Thus, when given the opportunity to choose a mate, female broad-horned flour beetles prefer lovers, not fighters.

To understand why females might have this preference for lovers rather than fighters, Okada and his colleagues conducted a series of experiments to investigate potential direct and indirect benefits for females to mate with courting males versus competitive males. First, they established that although mandible size and courtship rate are both heritable traits, they are not correlated with one another. The researchers also determined that females gain no direct fitness benefits from mating with their preferred males, as females don't live longer or lay more eggs after mating with an attractive male. The offspring sired by attractive males with high courtship rates developed more quickly, although the benefits of more rapid development were unclear. However, the researchers point out that the genes associated with larger mandibles in males are associated with masculinized females that are less fecund. Further, the more competitive males are also more aggressive, and so females might suffer some injuries by mating with males with large mandibles.

Collectively, these results suggest that females suffer indirect fitness costs from mating with the stronger, more competitive males, as they have less-fertile daughters and may risk injury. On the flip side, the females gain indirect benefits from mating with males that court more vigorously, as they will have sons that also court enthusiastically and are attractive to females. Overall, it is clear from this research that in broad-horned flour beetles, attractiveness and competitive prowess are not two sides of the same coin. While males with large mandibles may win more fights, they are not the males that win female beetles' hearts.

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Bug buddy builds biotin

Bedbugs (*Cimex lectularius*) have received a lot of attention lately for their role as a human parasite. But it turns out that bedbugs are involved with another interesting ecological interaction – their mutualism with the bacterium *Wolbachia*. And now a collaborative study with researchers from multiple institutions in Japan has unravelled the mechanism for how a bacterial infection can end up being mutually beneficial.

The *Wolbachia* genus of bacteria infects a broad array of invertebrates, including nematodes, insects and spiders. Usually, it acts as a parasite, causing reproductive problems for its host. But in the bedbug, *Wolbachia* infection has positive effects on growth and reproductive output. Vertebrate blood (the bedbug's favourite meal) is low in B vitamins and bedbugs rely on their bacterial partner to synthesize them. Naruo Nikoh and his colleagues set out to investigate the origin of the relationship between these two odd bedfellows by examining the genome of the strain of *Wolbachia*, called '*w*Cle', that infects bedbugs.

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

To test whether *w*Cle was necessary for synthesis of vitamin B7 (biotin) and the development of bedbugs, the researchers raised bedbug nymphs on a diet of rabbit blood that was either supplemented with antibiotics (to produce *w*Cle-free bedbugs) or unsupplemented (to produce bedbugs with a healthy population of *w*Cle). In addition, they supplemented the diets of some of the nymphs with vitamin B7. Investigating the impact of each diet on the bedbugs and their bacterial lodgers, the team found that bedbugs that had a healthy *w*Cle population had higher concentrations of vitamin B7 while bedbugs that had been cured of *w*Cle infection and raised on rabbit blood without vitamin B7 were much less likely to survive to adulthood.

After sequencing and assembling the *w*Cle genome, the researchers examined the genes responsible for synthesizing B vitamins. They found that while most *Wolbachia* species have genes that can synthesize B2, as well as some of the genes necessary to synthesize vitamins B6 and B9, *w*Cle can also synthesize vitamin B7. Also, the genes involved in vitamin B7 synthesis were extremely similar to those involved in the synthesis in other bacteria that live as endosymbionts. The researchers concluded that *w*Cle may have received the genes through lateral gene transfer from one of these other species while infecting the same animal.

Finally, they compared the genes of proteins involved in vitamin B7 synthesis in *w*Cle with those in a *Wolbachia* species that infects the bedbug's closely related sister species *Cimex japonicus*, the bat bug. They found that the two sets of genes were almost identical, suggesting that the biotin synthesis genes were transferred to a *Wolbachia* strain and that it then infected the common ancestor of the bedbug and bat bug.

Taken together, the authors think that the lateral transfer of vitamin B7 synthesis genes is the event that permitted *w*Cle and bedbugs to strike up their symbiotic relationship. Although understanding that *w*Cle makes bedbugs stronger might not be good news for humans worried about bedbugs sharing our homes, knowing how that relationship came about could help us understand the ecology of symbiosis a little better.

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Serotonin keeps crayfish in the dark

There's nothing like a nail-biting FIFA World Cup to remind us what anxiety feels like. Properly defined, anxiety is a behavior brought on by stress that helps us cope with potentially threatening situations. Anxiety is controlled in our brains by the neurotransmitter serotonin, and differs from fear because it persists in the absence of the stressor and in a new context. While fear-like behaviour has been well documented across the animal kingdom, the more complex emotion – anxiety – has so far only been demonstrated in vertebrates. In fact, our understanding of the neurobiology behind anxiety is built almost exclusively on mammalian studies, but a recent *Science* publication from the joint efforts of Jean-Paul Delbecque and Daniel Cattaert at the Université de Bordeaux, France, offers new insight into the field using a relatively simple invertebrate, the crayfish.

The team began their study by comparing how a stressful experience impacted crayfish behavior. First, they exposed some of the animals to 30 min of mild electric pulses and then placed individuals in a plus-shaped aquatic arena with two dark arms and two light arms. Video-recording each animal's movements during a 10 min test period, the team exploited the animals' natural preference for the dark to determine whether the stress had altered their behavior by measuring how long it took stressed and unstressed animals to pluck up the courage to enter a light arm.

Compared with unstressed animals, stressed crayfish took longer to enter and spent less time in light arms, suggesting they preferred to stay in their comfort zone – the dark. This behavioral shift meets the defining terms of anxiety in that it exists in the absence of the stressor and in a new context, and is thus the first demonstration of anxiety in a non-vertebrate species. Importantly, the team also measured serotonin levels in the brains of the crayfish and discovered that stressed crayfish had higher levels of serotonin in their brains compared with unstressed controls, suggesting that the neural control of anxiety in crayfish is similar to that of mammals. Then, to confirm this evolutionary conservation, the team injected unstressed crayfish with serotonin and monitored how the crayfish behaved in the test arena. They found that unstressed crayfish given a serotonin injection behaved similarly to the stressed crayfish and stayed mainly in the dark arms, confirming that serotonin controls anxiety-like behavior in crayfish as it does in you and me.

The take-home message of this study is that the neural pathways controlling complex behavioral responses to stress are anciently wired. This study paves the way for the use of invertebrate models in studies aimed at understanding the neurobiological mechanisms driving our emotions. It also raises concerns about the broader impact of water-borne pharmaceuticals (e.g. downstream of waste-water treatment plants) on the behavior and physiology of aquatic biota. However, there could be an up-side to this study: perhaps, one day soon, your vet could prescribe Valium for your pet hermit crab who just can't seem to muster-up the courage to seek out a bigger shell.

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Buff boys and clever girls at the salt lick

Of my many vices, the one for which I am most unapologetic is my craving for salty snacks. It turns out I am not alone. Sodium is both limited and prized in nature, and animals from insects to giraffes will go far out of their way to get a taste. For some butterflies, however, human influence has made the salt search as easy as my trip to the chip isle in the supermarket. Millions of tons of salt are dumped each winter onto American roads to melt snow and ice. But where does the salt go after it has served this purpose? New research by a team of scientists lead by Emilie Snell-Rood at the University of Minnesota in the USA shows that some of it can wind up in butterflies, often to dramatic effect.

Butterflies and moths are notorious salt freaks. But because the salt they need isn't found in their preferred diet, they resort to some extreme behaviours to satisfy their cravings. Some species drink tears while others drink blood, or secretions from carrion or faeces. Less peculiar, other species congregate at the edge of water, where they engage in 'puddling', a behaviour where mainly males bioaccumulate salt by imbibing absurd quantities of water, which they eject like a lawn sprinkler. To what end? Salt, it seems, is good for these insects. It helps with flight and digestion, and can directly enhance fitness because salt from puddling males is transferred to females during mating and then from females to her eggs.

But even for these salt junkies, there can be too much of a good thing. When Snell-Rood and her colleagues reared cabbage white butterflies on high salt diets, their survival was significantly reduced. More worrying, similar reductions in survival were observed when the team reared monarch butterflies on milkweed plants isolated from the roadside versus low-sodium plants collected from a nearby meadow. In comparison to these meadow plants, roadside milkweed exposed to wintertime salting contained around 10 times the amount of sodium. And much of this excess sodium wound up in the feeding butterflies, where, for those butterflies that did survive, it caused some unexpected changes.

If my wife and I sat on the couch gorging on chips, the excess salt would potentially increase our risks of hypertension and cardiovascular disease. But what doesn't kill salty butterflies seems to make them stronger – at least for males. Surviving males of both tested butterfly species reared with higher sodium intake buffed up, investing significantly more in thoracic muscle mass than did low-sodium butterflies. Surviving females, by contrast, invested less in muscle and instead redirected their developmental energy to neural tissue. Cabbage white females reared with high salt made bigger brains, while monarch females made bigger eyes.

As yet, there is little idea of why some butterflies succumbed to the toxic effects of salt and others did not, nor of the evolutionary consequences of these salt-induced responses in butterflies. Beefcake males may be better flyers, more able to migrate or locate mates. Brainy females may be more efficient foragers or make more discerning partners, perhaps hunting down the salty males that would pass on more sodium to their eggs. And looking further, perhaps these salty eggs fare better under cold stress, the salt helping to melt the ice, just as man intended! What is clear, however, is that our footprint reaches the natural world in ways we can hardly imagine. In this case, our impact may conceivably be positive. Alternatively, because too much salt lowers survival, we may be turning our roadsides into butterfly no-fly zones.

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