Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short 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.

Outside JEB

INSECT DEFENCE

STINGLESS BEES MOUNT DEFENCE

For insects that live in colonies, social structure appears to be elevated to an art form. Numerous studies have documented that in insect societies the division of labour is typically based on age, whereby developmental maturation determines a specific job delegation, thus dividing colony members into 'temporal castes'. However, some species (ants and termites) have also been shown to use distinct morphological attributes for certain tasks, thus allocating their members into 'physical castes'. In the highest level of social organization, for example in eusocial insects, the division of labour into specialized castes is considered paramount to the success of the species.

Until now, behavioural specializations utilized by social bees and wasps were thought to be established solely based on age. However, Christoph Grüter and Francis L. W. Ratnieks, from the University of Sussex, UK, and Cristiano Menezes and Vera L. Imperatriz-Fonseca, from Preto University of São Paulo, Brazil, report for the first time in *PNAS* that the division of labour amongst eusocial neotropical stingless *Tetragonisca angustula* bees appears to be based on morphological specialization for one particular task – defence.

Stingless bees are unique in that they have a sophisticated defence system involving two groups of worker guards: hovering guards (stationed in the air near the nest entrance) and a set of soldier guards that stand inside and around the wax entrance tube of the nest. While the research team was carrying out another study on the bees, they noticed a possible morphological distinction between the various worker bees. Some workers looked larger than the forager bees and this led the team to hypothesize that a subset of workers might include a distinct physical sub-caste made up of larger-bodied guards. When they compared the size and shape of guards with that of foragers and waste-removing workers from 12 different colonies at two locations in Brazil, they found that both types of guard are 30% heavier than the foragers. The guards also display different-sized structural features – while the foragers have larger heads, the guards have larger hindlegs, and the waste-removing workers are intermediate in size. The team wondered how prevalent these larger-bodied workers are within a given population. To determine the overall size distribution of the different categories of workers in a colony, the researchers measured 300 workers (from five colonies) and found that forager-sized workers predominated, followed by waste-removing bees; specialized guard-sized workers represented only 1% of the bees in their sample.

Given that guards represent only a small subset of emerging workers, the researchers hypothesized that the colony must benefit somehow from maintaining a tightly regulated caste of larger-sized workers within the population. In fact, when they tested the fighting performance of soldier guards, they found that the larger-bodied worker bees were able to fight for longer when presented with a potential enemy, the *Lestrimelitta limao* robber bee. However, further research is needed to confirm whether the soldiers' distinct morphology, and size, correlates with a more successful defence (advantage) for the colony as a whole.

These observations also generate intriguing questions for future study, including how the colonies manage to produce morphologically distinct workers and to precisely maintain them in appropriate ratios. Towards addressing this question, the authors hope to determine whether other species of stingless bees employ similar defence tactics and to understand how a select physical sub-caste of able-bodied troops evolved in eusocial bees.

10.1242/jeb.064204

Grüter, C., Menezes, C., Imperatriz-Fonseca, V. L., and Ratnieks, F. L. W. (2012). A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proc. Natl. Acad. Sci. USA* doi: 10.1073/pnas.1113398109.

Katherine M. Parisky
Brandeis University
kparisky@brandeis.edu

ECOPHYSIOLOGY

GABAZINE HELPS CLOWNFISH COME TO THEIR SENSES

Up to a quarter of the carbon dioxide from anthropogenic sources is absorbed by the world's oceans, leading to ocean acidification. Recently, Philip Munday and his colleagues from James Cook University, Australia, have shown that clownfish (*Amphiprion percula*) and damselfish (*Neopomacentrus azysron*) larvae raised in approximate future conditions of acidified seawater lose the ability to detect homing and predatory cues. Consequently, these larvae have an increased mortality when compared with larvae raised in present day oceanic conditions. However, the physiological mechanisms behind this phenomenon remain shrouded in mystery. Göran Nilsson from the University of Oslo, Norway, in collaboration with Munday and their colleagues set out to determine whether behavioural changes in the coral reef fish larvae raised in acidified seawater were due to physiological changes in the brains of these fish. More specifically, the team looked at the effect that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) has on the behaviour of these fish; their results were published in a recent issue of *Nature Climate Change*.

After raising the clownfish in either normal or seawater acidified at levels predicted for the end of this century, the researchers measured the preference of these two groups of clownfish larvae for seawater containing an olfactory cue, from either a predator or a non-predator. Just as in previous studies, the clownfish raised in normal seawater were strongly repelled by the predatory cue, while the clownfish raised in the acidified seawater were strongly attracted to the predatory cue. However, after the team treated the fish with gabazine, a GABA receptor inhibitor, both clownfish groups were repelled by the predatory cue, showing a reversal in the behaviour of fish raised in acidified seawater. These results indicate that the GABA receptor mediates neural function and therefore olfactory behaviour in clownfish.

Next, the team wanted to know whether gabazine would reverse abnormal behaviours seen in other coral reef fish exposed to acidified seawater. Just as humans are either left or right handed, damselfish larvae show a strong preference for turning left or right when faced with this choice in a maze. However, damselfish larvae that have been exposed to acidified seawater lose this preference. Could gabazine reverse this effect too?

The team caught damselfish larvae from the wild and exposed them to normal or acidified water for 4 days. They then tested the turning preference using a T-shaped maze and repeated the test after a 30 min treatment with gabazine. Again, treatment with gabazine reversed the behaviour: damselfish larvae that had been exposed to acidified water regained their turning preference.

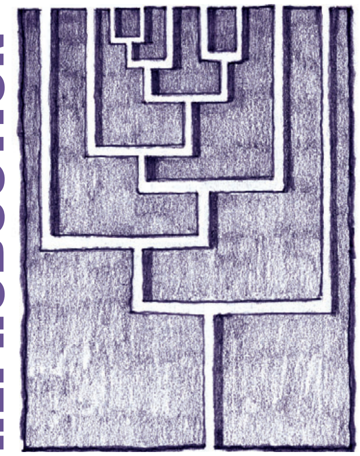
Based on their findings, the researchers hypothesized that exposure to acidified seawater alters the acid–base balance of coral reef fish and this causes inhibitory neurons that contain GABA receptors to become excitatory, which may cause shifts in the fish's olfactory and turning preferences. Therefore, inhibiting these now excitatory neurons with gabazine reverses the behaviour. Although many questions remain unanswered, these findings pave the way for future research on the impacts of ocean acidification on the physiology of fish and other aquatic organisms.

10.1242/jeb.064188

Nilsson, G. E., Dixson, D. L., Domenici, P., McCormick, M. I., Sorensen, C., Watson, S.-A. and Munday, P. L. (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nat. Clim. Chang.* **2**, 201-204.

Cosima Porteus
University of British Columbia
ciuhandu@zoology.ubc.ca

REPRODUCTION



THE KEY TO REPRODUCTIVE SUCCESS: COMING LAST

Birds do it, bees do it, even educated fleas do it: postcopulatory sexual selection. It took nearly a century after Darwin first described how competition between males forms the process of sexual selection before researchers realized that selection does not end there. Instead, males have to fight off their competitors even after copulation has started, through postcopulatory selection, either directly (through sperm competition) or indirectly (through sperm selection by the female).

Postcopulatory selection has certainly led to some interesting adaptations. Recently discovered examples include exploding male genitalia – which prevent other males from mating – and seminal compounds that enter the female's nervous system and modify her behavior, such as the *Drosophila* seminal fluid proteases (Sfps), which make her resist the amorous approaches of other males. However, no one had tried to measure the relative importance of postcopulatory selection in determining reproductive success until a study recently published in *PNAS* by Alison Pischedda and William Rice. They addressed this question by identifying the precopulatory and postcopulatory components of sexual selection in a lab-grown population of the fruit fly (*Drosophila melanogaster*).

The authors first quantified the extent of sexual selection within this population by measuring and comparing the reproductive success of male individuals. They found that there was great variability in male reproductive success: a large number of the males produced no or few offspring, while others produced large numbers of offspring. This suggested that there is indeed strong sexual selection.

The total number of offspring a male produces is determined mainly by two factors: the number of females that it mates with (mating success), which is the product of precopulatory selection, and the proportion of offspring it sires with these females (fertilization success), which is the product of postcopulatory selection. To measure the relative importance of postcopulatory selection in the process of sexual selection, the duo divided the variability in reproductive success into its precopulatory and postcopulatory components.

They found that the variability in these two parameters was roughly the same, suggesting postcopulatory selection is as important as precopulatory selection in the process of sexual selection. This is the first quantitative evidence that the number of offspring a male produces is determined in equal measure by how well it can find a willing mate and what happens during and after mating.

Knowing that *Drosophila* males rely on a complicated sequence of courtship behaviors to ensure mating success, the duo then decided to identify the factors that determine fertilization success. As the last male that copulates with a female is known to sire most of her offspring, Pischedda and Rice focused on finding how much of the postcopulatory success can be attributed to this bias by looking at the correlation between the mating order of males and their fertilization success.

They found that the vast majority of the fertilization success can be attributed to mating order. After finding a willing mate, the timing of mating is therefore the most important factor that determines the number of offspring a male produces.

The authors have established that postcopulatory selection is a crucial factor in determining the overall reproductive success of male fruit flies. While the biological mechanisms that are behind this selection are still unknown, the timing of mating is essential. The same principle could apply to other non-monogamous species, such as birds, bee, and, yes, even educated fleas.

10.1242/jeb.064196

Pischedda, A. and Rice, W. R. (2012). Partitioning sexual selection into its mating success and fertilization success components. *Proc. Natl. Acad. Sci USA* **109,** 2049-2053.

Maarten Zwart
University of Cambridge
mfz20@cam.ac.uk

VIBRATION DETECTION

CATERPILLARS KNOW WHAT'S SHAKING

The world of insects is full of vibrations, from wind-driven fluttering to deliberate drumming. Almost everything vibrates on the insect's scale. In recent years, researchers have studied the wide range of ways that insects use vibrations. Many communicate by leaf-borne vibrations. Even incidental vibrations from crawling and chewing may contain valuable information. But do insects actually respond to vibrational cues before other signals? If they do, can they distinguish useful vibrations from vibrational noise produced by wind and rain?

In a recent paper in the *Journal of Comparative Physiology A*, Raul Guedes and his colleagues in Joyce Yack's lab at Carleton University set out to answer these questions using birch caterpillars (*Drepana arcuata*). Previously, members of the Yack laboratory found that these caterpillars respond to each other with distinct vibrational signals, but they did not know how caterpillars distinguish communication signals from other environmental vibrations.

To find out whether caterpillars were listening in on others' vibrations, the researchers allowed individual caterpillars to set up shelters on leaves before allowing an intruder to approach. To see whether or not the caterpillars could distinguish between intruders, the residents were exposed to the approaches of other birch caterpillars or predatory bugs. Using a laser vibrometer, Guedes and colleagues measured leaf vibrations near the resident caterpillar over the course of each intruder's approach. As the intruder closed in, its crawling produced low amplitude vibrations. In response, the residents produced a series of deliberate vibrations, warning the intruders to back off. The speed of the response depended on the type of intruder – the residents responded much more quickly to predators than to other approaching caterpillars. Furthermore, the vibrational warnings often worked: the predators backed off about 80% of the times that the residents responded!

But Guedes and his colleagues wanted to make sure that residents were responding to vibrations from the invader, not visual or chemical cues. To check this, the researchers tried the experiments again – but this time, they made a cut in the leaf between the intruder and the resident, cutting off the resident from vibrations produced by the intruder. Without vibrational cues, resident caterpillars did not respond to the intruder until it crossed the cut in the leaf, much later than when the leaf was uncut. This suggests that leaf-borne vibrations are one of the primary signals used by caterpillars to identify the approach of others.

Next, the authors wanted to know whether birch caterpillars could recognize vibrational cues from intruders against a background of environmental noise. They simulated rain, by spraying water, and wind, using a fan, and repeated the experiments with the approaching caterpillars. In the absence of an intruder, the resident caterpillars did not respond to wind or rain. However, the residents were able to pick up and respond to an intruder's approach despite the additional environmental vibration.

Although the vibrations produced by different sources largely overlap in frequency and amplitude, caterpillars may be able to isolate and recognize specific components of vibrational signals in order to distinguish between friend and foe. In a world full of vibration, perhaps it's worth learning even more about caterpillars' sophisticated senses.

10.1242/jeb.064212

Guedes, R. N. C., Matheson, S. M., Frei, B., Smith, M. L. and Yack, J. E. (2012). Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*). *J. Comp. Physiol. A* doi: 10.1007/s00359-012-0711-8.

Kara Feilich
Harvard University
kfeilich@fas.harvard.edu