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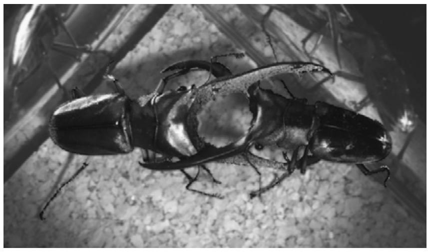
INSIDE JEB

Stag beetle males compensate for massive mandibles



Two stag beetle males fighting over mating rights. Photo credit: Jana Goyens.

Armed with a ferocious pair of mandibles, male stag beetles appear well prepared to take on the world. 'Their jaws are not just for ornamentation, they really use them to fight', says Jana Goyens from the University of Antwerp, Belgium, adding that males grapple over the choicest patches of rotten wood for their mates to lay their eggs in. Describing a stag beetle battle, Goyens explains that one beetle grabs the other one around its body and then rears up in an attempt to hurl his opponent over his head and onto its back. 'It is clear which one is the loser', says Goyens. But something puzzled her: 'It seemed unlikely that stag beetles could bite forcefully because they have these long jaws', she says. Instead of amplifying forces, the beetles' long mandibles would reduce the forces exerted at the sharp teeth halfway along the mandibles. Intrigued, Goyens and her supervisors, Joris Dirckx and Peter Aerts, decided to test how powerful stag beetle bites are (p. 1065).

Although the beetles are native to Belgium, they are endangered, so Goyens turned to Asia, where betting on insect fights is popular, to obtain the animals. However, when 10 pairs of the animals arrived from Taiwan just before Christmas, the pressure was on. 'I had been told that they only live for a couple of months but I didn't know how old they were and it was the Friday before the Christmas holidays so I worked the entire holiday to get as much data as possible while they were still alive', says Goyens, who laughs when she recalls that the beetles went on to live for another 12 months.

Testing the beetles' bite force, Goyens found that the aggressive animals were extremely cooperative, biting enthusiastically on the force transducer whenever it was held before them. And when she compared the strength of the male's and female's bites, she was impressed to see that the male's jaws gripped with an impressive 7 N force, which is six times stronger than the female's bite. Even when she scaled up the female's bite to take account of the female's smaller stature, the male's bites were still three times stronger. So, even though the male's long mandibles should reduce the strength of their nasty nips, they were still able to clamp on much harder than the females. They must have somehow compensated for their oversized mandibles; Goyens decided to take a closer look at the beetles' heads.

Comparing the males and females, Goyens realised that the males' heads were much wider than the females', suggesting the males had increased the size of some of the internal structures that move the mandibles to compensate for their size. Goyens then teamed up with Luc Van Hoorebeke and Manuel Dierick to take CT scans of the insects' heads to find out what was going on inside. She could clearly see that the muscle that pulls on the mandible is almost four times larger in male stag beetles than in females, and the lever on the side of the mandible that is pulled by the muscle is more than three times longer than the lever on female mandibles. And when Goyens checked the direction that the muscle pulls in, she saw that both the males' and females' muscles were perfectly oriented to generate the hardest bite. So, the males have successfully compensated for their ungainly mandibles by increasing the size of the internal structures that wield their mighty weapons.

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Goyens, J., Dirckx, J., Dierick, M., Van Hoorebeke, L. and Aerts, P. (2014). Biomechanical determinants of bite force dimorphism in *Cyclommatus metallifer* stag beetles. *J. Exp. Biol.* **217**, 1065-1071.

Kathryn Knight

Crabs' thermal tolerance key to invasive success

Close-up of a green crab in Maine, USA. Photo credit: Carolyn Tepolt.

The European green crab isn't too fussy about where it lives. Not content with its native range, which extends from Iceland to northern Africa, the green crab has also set up home in Japan, Australia, South Africa, Argentina and North America. These globe-trotting tendencies make the green crab an excellent model to study how animals cope with temperature changes, explains Carolyn Tepolt of Stanford University, USA. Enlisting the help of George Somero, Tepolt decided to explore the temperature tolerance – and ability to fine-tune that tolerance – of green crab populations around the world (p. 1129).

However, Tepolt realised that she wouldn't be able to bring crabs back to her lab – most countries are understandably reluctant to allow highly invasive species to cross their borders – so she would have to take the lab to them. Technician John Lee helped Tepolt transform cooler boxes into portable acclimation tanks by fitting them with aquarium heaters and chillers; but she also needed a simple method to measure the crabs' heart rate. 'Heart function is a common measure of heat tolerance in cold-blooded animals', Tepolt explains. 'As temperatures rise, the animal's heart rate increases. When you reach the animal's maximum tolerated temperature – the critical temperature – the heart rate suddenly plummets.' To let Tepolt measure crabs' heart rate in the field, Lee constructed a non-invasive portable monitor that could be attached to the crab's shell just over the heart. By measuring infrared light bouncing back from a crab's expanding or contracting heart, Tepolt was able to record heart rate.

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Taking her portable lab, Tepolt travelled to seven sites around the globe (Norway, Portugal, three sites along the east coast of North America and two sites along the west coast) to test the thermal limits of their green crab populations. At each site, she trapped green crabs and placed them in acclimation tanks for several weeks, keeping one group at 5°C and another group at 25°C. She then tested the heat tolerance of each group by attaching heart rate monitors to the crabs, ramping up the temperature in the tanks by 5°C an hour, and measuring the critical temperature at which the crabs' heart rate plummeted. To test the crabs' cold tolerance, she decreased the temperature to 0°C and measured their average heart rate. Tepolt expected that green crabs, as an invasive species, would have better heat tolerance than non-invasive species. Sure enough, she found that the average critical temperature for green crabs was 34.5–36.5°C, higher than the 30–35°C critical temperatures previously recorded for other crabs and lobsters living at the same sites. Green crabs were also remarkably cold tolerant; they all survived short-term exposure to 0°C.

But the real key to green crabs' invasive success may lie in their ability to fine-tune their response to shifting temperatures. Tepolt saw that warm-acclimated crabs coped better with higher temperatures than their cold-acclimated counterparts: the critical temperature for 25°C-acclimated crabs was 1.8–2.4°C higher than that seen for 5°C-acclimated crabs. 'This suggests that green crabs can shift their thermal limits through acclimation', says Tepolt. However, even after they had acclimated to the same temperature, crabs found in Portuguese waters were better at coping with heat and fared worse at chillier temperatures than their Norwegian cousins. This is exciting, says Tepolt, because it suggests that crab populations are locally adapted to their environments – and that perhaps not all populations are equally suited to invading new regions. In the face of global warming, such variation may mean the difference between life and death.

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Tepolt, C. K. and Somero, G. N. (2014). Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. *J. Exp. Biol.* **217**, 1129-1138.

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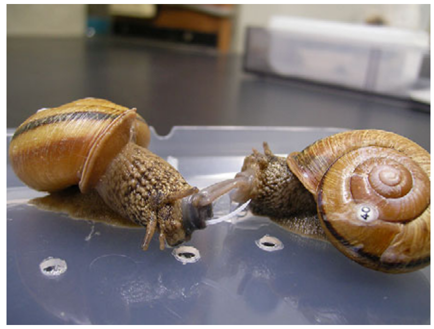
Love dart goo gives snail sperm a head start



Dart shooting in *Euhadra* snails. Photo credit: Kazuki Kimura.

For simultaneous hermaphrodites – equipped with both male and female reproductive organs – reproduction is more complex than the conventional 'boy meets girl' story. For a start, each protagonist can fertilize the eggs of the other. Common garden snails, *Cornu aspersum*, seem to improve their chances of becoming fathers – and this might not sound terribly romantic – by spearing their partner with a so-called 'love dart', smeared in mucus. Once in the partner's blood stream, the mucus improves the chances of the biological father's sperm successfully fertilising their partner's eggs by closing off the entrance to a structure that digests unused sperm and causing the sperm to be pumped into a storage organ for later use. But it wasn't clear whether this pragmatic approach to reproduction was peculiar to the common garden snail or whether other species also took advantage of the love dart's benefits. To remedy the situation, Kazuki Kimura and Satoshi Chiba from Tohoku University, Japan, working with Joris Koene from Vrije University, The Netherlands, investigated another species equipped with love darts, the distantly related Japanese *Euhadra peliomphala* snail, to find out whether the love dart mucus that they inject into their partners also provides a fertility advantage (p. 1150).

Having collected snails from a nearby local park, the team extracted the glands that produce the love dart mucus from some of the animals and produced a mucus gland extract to test on the remaining snails. They then simulated the love dart stabbing action of an ardent snail with a syringe needle coated in the extract before injecting a minute volume of coloured water that mimicked semen into the snail's genital tract to find out whether the mucus gland extract altered the fluid's fate.

The team tested whether any of the fluid made it into the organ that digests sperm and they found that it was closed in the snails that had been injected with the mucus gland extract. In a real-life situation, the mucus would have saved the semen from destruction, and Kimura and colleagues say, 'This indicates that in various dart-bearing species the mucus from the dart glands targets the same organ'.

So, having added another species to the list of simultaneous hermaphrodite snails that improve their chances of becoming fathers through the use of love dart mucus, Kimura and colleagues wonder whether these hermaphrodites benefit from sexual selection. 'Because of the relatively limited literature on sexual selection in hermaphrodites, it was unclear how important sexual selection has been for the evolution of their reproductive behaviours', they say. However, the fact that love dart mucus targets the same organ in distantly related snails suggests that simultaneous hermaphrodites do benefit from sexual selection. The team adds, 'This result supports the idea that this conspicuous reproductive trait of land snails had evolved through a conflict over sperm digestion between sperm donors and recipients'.

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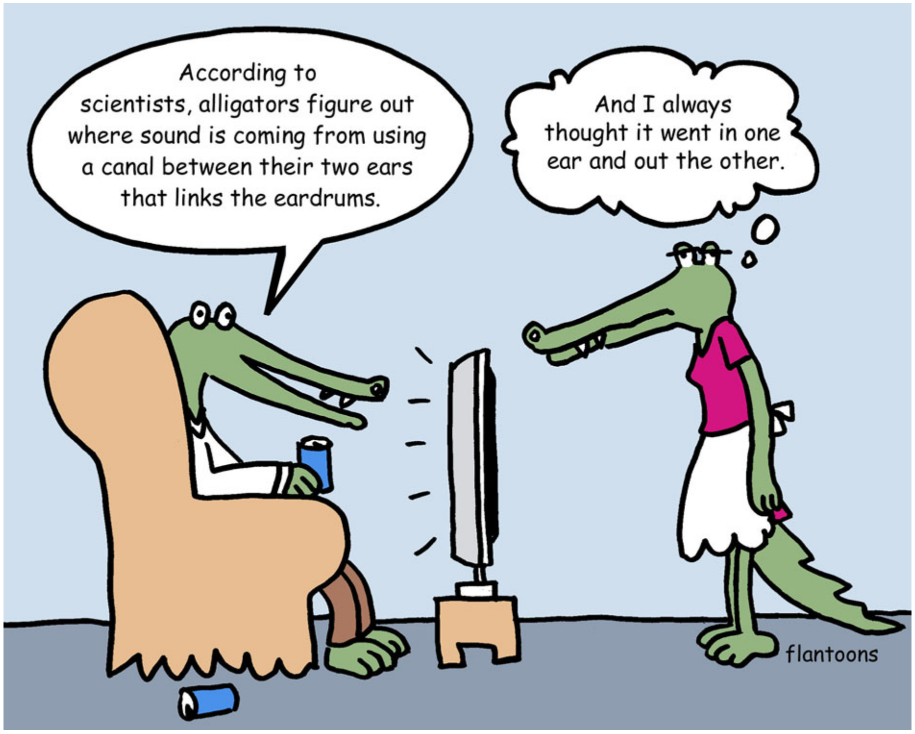
Kimura, K., Chiba, S. and Koene, J. M. (2014). Common effect of the mucus transferred during mating in two dart-shooting snail species from different families. *J. Exp. Biol.* **217**, 1150-1153.

Kathryn Knight

Canal between ears helps alligators pinpoint sound



By reptile standards, alligators are positively chatty: according to Hilary Bierman and colleagues, 'They are the most vocal of the non-avian reptiles'. The team adds that the animals are known to be able to pinpoint the source of sounds with accuracy, but it wasn't clear exactly how they did it. 'Different vertebrate lineages have evolved external and/or internal anatomical adaptations to enhance these [auditory] cues, such as pinnae and interaural canals', says Bierman, from the University of Maryland College Park, USA. However, alligators lack the external structures and it wasn't clear whether they had interaural canals linking their ears. Collaborating with a group of international scientists from the University of Massachusetts-Lowell, USA, the University of Colorado School of Medicine, USA, and the University of Southern Denmark, Bierman investigated the mechanisms that alligators use to locate sounds (p. 1094).

First, the team tested how sound travelled around the animals' heads to see whether they somehow channel sound to help them locate the origin, listening for the minute time and volume differences between the sound arriving at the two ears, but the team found no evidence that the animal's body alters sound transmission sufficiently for the animal to be able to detect the difference. And when the team measured the alligators' brainstem responses to sounds, they were too fast for the animals to be sensing the time difference between a sound arriving at the two ears.

Next, the team looked for internal structures in the alligators' heads that might propagate sound between the two eardrums. 'Acoustical coupling produces directional responses at the tympanum', says Bierman. She explains that sound reaches both sides of the eardrum – travelling externally to reach the outer side and through head structures to the internal side – to amplify the vibration at some frequencies when the head is aligned with the sound. This maximises the pressure differences on the two sides of the eardrum, magnifying the time difference between the sound arriving at the eardrum via two different paths to allow the animal to pinpoint the source. Viewing slices through the heads of young alligators, the team could clearly see two channels linking the two middle ears that could transmit sound between the two eardrums. They also measured differences in the alligator's brainstem responses to sounds depending on their location. And when the team looked at the eardrum's vibration, they could see that it was amplified at certain frequencies, as they would expect if alligators use the pressure difference at the eardrum for orientation.

Assembling all of the evidence together, Bierman and colleagues suggest that the reptiles rely on magnified time difference at the eardrum to locate noises. They also suspect that this is the mechanism that the archosaur ancestors of modern crocodilians and birds used to pinpoint sounds.

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Bierman, H. S., Thornton, J. L., Jones, H. G., Koka, K., Young, B. A., Brandt, C., Christensen-Dalsgaard, J., Carr, C. E. and Tollin, D. J. (2014). Biophysics of directional hearing in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **217**, 1094-1107.

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