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

REPRODUCTION

GUPPIES REPRODUCE FROM BEYOND THE GRAVE

Humans can be forgiven for assuming that successful reproduction requires both partners to be alive. Amazingly, this isn't the case for the Trinidadian guppy. Under laboratory conditions, female guppies are able to store sperm for up to a year, potentially allowing them to produce offspring even if the males are no longer around. However, several other studies have suggested that the last male to mate with a female ends up fertilizing the bulk of her offspring. This last-male-in advantage may mean that stored sperm is rarely used by guppies in nature. Studying this phenomenon in natural surroundings poses many logistical problems, but in a recent report, Andrés López-Sepulcre and his international team of collaborators not only proved that they were up to the challenge of tackling this problem in the wild but also discovered just how often female guppies use the sperm of deceased mates.

Undertaking a year-long mark–recapture study in Trinidad, the team introduced individually marked guppies with known genetic 'fingerprints' to a short stretch of the Guanapo River, which is isolated from existing downstream guppy populations by a swiftly flowing waterfall. Then, each month, the researchers painstakingly caught and identified almost every guppy longer than 13 mm present in the study site. These monthly assessments were used to determine when each guppy died, and also allowed the researchers to collect a few scales from each guppy born at the field site so that their mother and father could be determined using genetic fingerprinting. López-Sepulcre and colleagues then developed a complex mathematical model to estimate the reproductive contributions of dead males to the introduced guppy population.

The research team found that almost 50% of reproductively active males sired young after they had died and, amazingly, over 30% of reproductive males were successful only after they were dead. Some offspring were even fathered by males that had been dead for 8 months. Perhaps this allows relatively short-lived males (with an average lifespan of 3 months) to continue to mate with females over their much longer lives (15 months). The researchers also suggested that sperm storage would allow the genetic material of male guppies to survive the rainy season, when males die in much higher numbers than females. From the female perspective, the researchers proposed that using sperm from many males, both dead and alive, would produce offspring with higher genetic diversity. In a fluctuating habitat like a Trinidadian stream, this should increase the odds of producing some offspring that are genetically well suited to whatever environmental conditions the future has in store.

By the end of their year-long study, the researchers found that posthumously conceived guppies comprised almost 25% of the total reproductive population. This both highlights the importance of stored sperm to female guppies in nature and also raises red flags for measurements of genetic diversity in other species where females can store sperm: if the potential genetic contributions of dead males are not assessed, population sizes may be significantly underestimated. Apparently, you won't hear, 'Till death do us part', at a guppy wedding.

10.1242/jeb.084475

López-Sepulcre, A., Gordon, S. P., Paterson, I. G., Bentzen, P. and Reznick, D. N. (2013). Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proc. R. Soc. B* **280**, 20131116.

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MATERNAL CARE

THE SOOTHING PACE OF A CARING MOTHER

Any mother knows how much care a baby requires. Constant attention and care is essential for the survival and development of a baby during the first several months of their life. This is no easy chore for the parents, and infants do not hesitate to remind their mommies about their needs by loudly protesting and squirming when they are separated from their mom.

As most mothers will tell you, one of the most effective ways of calming a crying baby is by carrying them in her arms while walking. Although not a magic switch, this action usually relaxes the baby, who will stop crying and squirming. What moms do not know is why this works, or the physiological effects this soothing motion of carrying has on their baby. A recent study by Gianluca Esposito and colleagues, from the RIKEN Brain Science Institute in Japan and the University of Trento in Italy, explores the physiological mechanisms and effects, as well as the behavioural changes, caused by carrying human babies and mice pups.

Twelve healthy babies and their moms participated in the study. Esposito and his colleagues obtained video recordings of babies in their cribs, and of moms either holding their babies while sitting on a chair or while carrying their babies and walking around the room. The babies' heart rate was also recorded during this time using heart rate monitors.

Not surprisingly, the babies were more relaxed in their mother's arms than when they were left alone in their cribs. However, holding alone wasn't enough. Racing hearts slowed down, crying often stopped and fidgeting nearly disappeared when mommies walked while carrying their babies.

So, is this soothing effect of carrying an infant unique to humans? Hardly so. The researchers conducted similar experiments in mice. The researchers separated mice pups from their mothers by placing them in a clear cup where their mothers could still see them. As with human babies, the pup's heart rate decreased, ultrasonic vocalizations (thought to be the equivalent of crying in human babies) stopped and fidgeting disappeared when their mouse moms carried them with their teeth by the scruff of their neck out of the cup and back to the nest. Baby mice that had been slightly drugged or had their neck skin numbed continued squirming and vocalizing and their heart rates were still high when their moms carried them back to the nest. These results indicate that both touch and the rocking motion perceived by the infant are important factors in the soothing effect of mom's carrying. Interestingly, all the struggling and squirming prevented moms from effectively carrying their babies back to the nest, so it took them much longer to rescue their pups from the evil cup.

This study confirms what most moms have discovered through experience; carrying their baby while walking soothes their child. It appears that other baby mammals, like mice, also like being carried by their moms. In the wild, a mother might have to rescue her babies from danger and the quieting effect of carrying would aid in that rescue. For the first time, we have some insight into the physiological mechanisms of this type of mother–infant communication.

10.1242/jeb.084491

Esposito, G., Yoshida, S., Ohnishi, R., Tsuneoka, Y., Rostagno, M. d. C., Yokota, S., Okabe, S., Kamiya, K., Hoshino, M., Shimizu, M., et al. (2013). Infant calming responses during maternal carrying humans and mice. *Curr. Biol.* **23**, 739-745.

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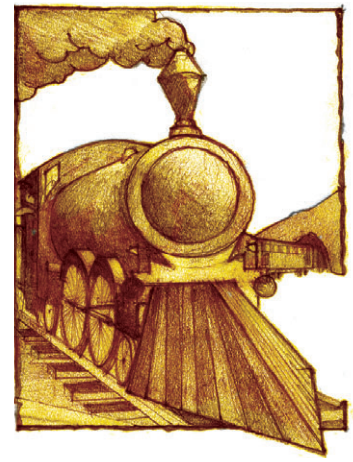
BIOMECHANICS



WHY DO SMALL ANIMALS CROUCH?

Size is one of the most important features governing how an animal functions: there are no elephant-sized mice or mouse-sized elephants for myriad reasons. Big animals have different metabolic and structural demands than small animals. One of the archetypal examples of a size-related biological trait is the posture of terrestrial vertebrates. As weight is proportional to volume (a length cubed), and strength is proportional cross-sectional area (a length squared), as size increases, weight increases faster than the ability to support it. That is why the biggest terrestrial vertebrates need stiff, thick, columnar legs and must maintain an upright posture to support their own weight. This contrasts with small animals, which usually crouch. This raises the question, 'Why?' If small animals stood upright like their bigger companions, they could have lighter limbs, with the same ability to support their weight. So why do small animals crouch?

In a recent study published in *Biology Letters*, James Usherwood of the Royal Veterinary College, London, asked just this question. By looking at existing locomotor scaling relationships and the gaits of differently sized animals, and employing numerical models, Usherwood posited that there are size-dependent differences in the demand for muscle work and muscle power for a given gait. To minimize the amount of active muscle required to produce work and power, animals of different sizes may use different gaits.

Large animals tend to use gaits that minimize the length of time each foot is in contact with the ground. This is energetically cheap: little work is wasted slowing down and speeding up with each step. However, despite the reduction of mechanical work, short ground contact time can be a problem when it comes to the demand for muscle power. As power is the rate at which work is performed, reducing the amount of contact time with the ground increases the power required for push-off.

Usherwood thought that small animals crouch to increase the amount of time the foot is in contact with the ground during each stride, reducing their power requirements. After observing that smaller animals do in fact use gaits with longer foot contact times, Usherwood used a numerical model based on data from flightless birds of different sizes to model the effects of size on the muscular requirements of different gaits.

According to Usherwood's model, if small animals were to use the upright, stiff-legged, high power gait, they would need to activate a wastefully large volume of muscle. Reducing their power requirements by having longer periods of contact with the ground allows small animals to minimize their active muscle volume. Maintaining longer contact time requires bent legs. According to this logic, the crouched posture in small animals is merely a way of giving the leg more time to push off the ground and compensating for the large power demands (relatively speaking) of small size.

This is not to say that crouching is ideal. In exchange for their lower push-off power requirements, small animals do lose energy as they change speed within the stride. That's why if you walk with crouched legs, it's more exhausting than if you walk normally. But, in the unlikely event you find yourself shrunken to mouse proportions, try crouching.

10.1242/jeb.084467

Usherwood, J. R. (2013). Constraints on muscle performance provide a novel explanation for the scaling of posture in terrestrial animals. *Biol. Lett.* **9**, 20130414.

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A NEW CLOCK FOR MOLTING

To facilitate growth and development, insects regularly shed off their old inelastic envelope and replace it with a newly synthesized, larger one. The timing of this molting process is tightly controlled by hormones, which ensures that the insects have built up sufficient energy reserves during their larval stages to fuel molting and metamorphosis (the transformation from juvenile into adult forms). Hormonal regulation of insect development has been extensively studied in the past and many details are known. However, in a recent study published in *PNAS*, Lyn Riddiford, James Truman and a team of Japanese scientists demonstrate that insects possess a previously unrecognized molting timer.

Because of their relatively large size and ease of rearing, the tobacco hornworm (*Manduca sexta*) is a widely used model organism for studying the hormonal control of insect development. The larvae of the last (fifth) instar have to reach a critical mass of about 5 g before specific hormones trigger the onset of metamorphosis, which involves a larval-to-pupal (metamorphic) molt. This metamorphic checkpoint is negatively controlled by juvenile hormone (JH), which – as indicated by its name – prevents the larvae from developing into pupal and finally adult stages.

Knowing that the larvae can undergo additional larval-to-larval molts when they don't reach the critical mass for their metamorphic moult as a result of an insufficient supply of food, the team hypothesized that a positive regulator that drives molting regardless of body size must exist. To test this, they designed a set of illuminating experiments. First, they monitored growth rates and the onset of metamorphosis in fifth instar larvae that were starved for various periods of time before being re-fed to allow the metamorphic molt. They observed that starvation is compensated for by the extension of the subsequent feeding period to reach the minimum mass necessary for metamorphosis, so the longer the larvae were starved, the longer they ate before moulting.

However, the situation was different when the negative effects of JH on metamorphosis were suppressed. For this purpose, the scientists surgically removed the glands that secrete JH from larval brains, and starved these insects as before. In line with their hypothesis, they observed that the larvae no longer compensated by increasing the subsequent feeding period, but began metamorphosis after a fixed period of 4 days regardless of whether they had reached the critical mass, as long they had fed for a minimum of 12–24 h during those 4 days. Thus, a 'molt timer', independent of body size, appears to exist and is initiated by a feeding stimulus. Performing similar experiments with younger, fourth instar larvae further showed that this timer controls not only metamorphic molts but also larval-to-larval molts.

Next, the team went on to manipulate the dietary composition, to identify which type of nutrient is needed to initiate this timer. It turned out that food quality matters when triggering this response, and that the most important component in the diet is protein. Finally, the team asked where the timer resides in the insect's body. They ligated the neck of larvae to block signals released from the brain and compared normal with JH-deficient larvae. As the timer was functional in these neck-ligated insects, they concluded that a significant part of the timer is localized outside the head region, possibly in the prothoracic gland, which is known to secrete ecdysone, the major insect molting hormone.

In summary, the study of the Riddiford/Truman team represents a fundamental breakthrough in understanding insect development. They have discovered a previously unrecognized developmental clock, which may be ubiquitously found in all molting invertebrates.

10.1242/jeb.084483

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