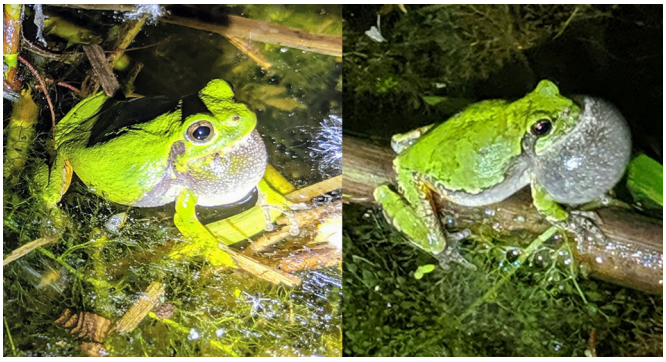
INSIDE JEB

Interval-counting neurons allow gray treefrogs to make the right choice



Male Cope's (left) and eastern (right) gray treefrogs performing mating calls.

At the right time of year across the eastern USA, from Texas to Maine, wetlands come alive with the sound of trilling eastern gray treefrogs (*Hyla versicolor*), each hoping to attract the attention of a nearby female. However, male eastern gray treefrogs are not the only vociferous suitors in the neighbourhood; they share their wetland breeding grounds with another close relative, Cope's gray treefrogs (*Hyla chrysoscelis*). 'The two species are visually indistinguishable', says Saumya Gupta from the University of Minnesota, USA. Yet, the consequences of a Cope's gray treefrog inadvertently selecting an eastern gray treefrog partner would be disastrous; their offspring rarely survive and, if they do, they are infertile. Fortunately, eastern and Cope's gray treefrogs barely make this rookie mistake, thanks to their trilling croaks. Even though the trill structures of their amorous calls are similar, they are sufficiently different – eastern gray treefrogs trill a little more slowly than their Cope's cousins – for females to avoid selecting the wrong mate. Yet, no one was sure exactly how these closely related species distinguish between each other's trills.

However, Gupta and Mark Bee (University of Minnesota) had a hunch. They knew that a unique set of neurons in frog brains – called interval-counting neurons – are often tuned to the trill rate of their own species' calls. Could these neurons hold the key to female eastern gray treefrogs rebuffing Cope's gray treefrog serenades and vice versa?

Collecting female gray treefrogs in amorous embraces with their male partners from the Tamarack Nature Center in Minnesota, USA, Gupta first had to identify which species each frog belonged to. 'I played mating calls of the eastern and Cope's gray treefrogs from two separate loudspeakers and identified the species based on which speaker it approached', she says. Next, Gupta simulated a series of gray treefrog trills composed of individual pulses – 10 ms pulses, 20 ms apart for the Cope's gray treefrogs and 30 ms pulses separated by 60 ms for the eastern gray treefrogs. After recreating the distinctive pulse signatures of both species, she played a range of trills to the females, from a single pulse up to 16 repetitions, recording the number of pulses when the female's attention switched from indifference to attraction as she hopped toward the loudspeaker.

Comparing the females' reactions, it was clear that the Cope's gray treefrogs tended to become interested in trills composed of ∼8, 10 ms pulses when the calls were softer (65 dB SPL) while trills of ∼7.5 pulses became attractive when louder (85 dB SPL). In contrast, the eastern gray treefrog females only required ∼5, 30 ms pulses to get their attention, falling to 3.5 pulses when the volume was higher. But how would the interval-counting neurons in the treefrog brain respond to each species-specific serenade?

This time, Rishi Alluri and Gary Rose from the University of Utah, USA, played simulated trills with increasing numbers of pulses to the frogs while painstakingly recording when interval-counting neurons in the frog brain began sending electrical nerve signals. Eventually, the duo identified a subset of neurons in the Cope's gray treefrog brain tuned to fire when they heard trills of at least 6–8, 10 ms pulses. Meanwhile, the eastern gray treefrogs' interval-counting neurons responded strongly when the frogs heard trills containing at least 3–5, 30 ms pulses.

'Interval-counting neurons play key roles in decoding information about species identity in frogs', says Gupta, placing the neurons at the hub of the mechanism protecting eastern and Cope's gray treefrogs from interbreeding, keeping them faithful to their own species' serenades.

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Kathryn Knight
kathryn.knight@biologists.com