Correspondence

Synthetic rainfall vibrations evoke toad emergence

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Toads occupy underground refugia during periods of daily or seasonal inactivity, emerging only during rainfall [1]. We test the hypothesis that rainfall-induced vibrations in soil are the cues that trigger the emergence of toads from underground. Using playback experiments in the absence of natural rainfall in native habitats, we observed that two Iberian toad species (*Pelobates cultripes* and *Bufo calamita*) emerged significantly earlier than controls when exposed to low-frequency soil vibrations that closely mimic those of rainfall. Our results suggest that detection of abiotic seismic events are biologically relevant and widespread in arid-zone anurans. These findings provide insights into the evolutionary role played by the two low-frequency-tuned inner-ear organs in anuran amphibians — the amphibian papilla and sacculus, both detectors of weak environmental vibrational cues.

Buried anurans regulate their depth in relation to temperature and moisture availability. While underground they are likely to be in moist soil that will not increase its saturation with rain [2]. Thus, their ability to sense rainfall may result from the detection of vibrations in the soil using alternative sensory pathways.

The inner ear of anuran amphibians contains three organs that subserve the detection of airborne and substrate-borne sounds: the amphibian papilla, the basilar papilla, and the sacculus. The basilar papilla is presumably used to detect airborne sounds such as advertisement calls clearly related to reproduction. The functions of the sacculus, and to some extent of the amphibian papilla, are not always as clear. Presumably, both organs are involved in detecting exquisitely low-level substrate-borne low-frequency vibrations [3,4]. In a pioneer study without a control group, presumed low-frequency (undefined) vibrations were associated with the emergence of *Scaphiopus* from their underground aestivation sites [5].

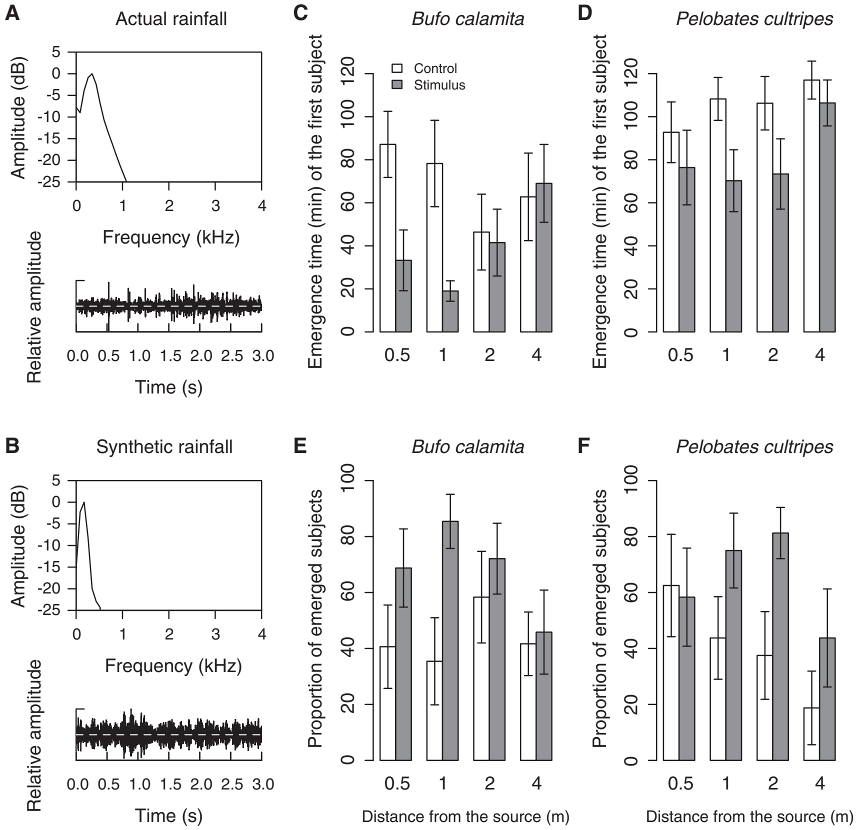

Figure 1. Rainfall vibrations, synthetic stimulus and emergence of buried toads.
(A) Spectrum and oscillogram of vibrations produced by natural rain (512 samples, 124 Hz bandwidth). (B) Sectrum and oscillogram of an actual recording of the synthetic vibration. The natural rain vibrations were recorded in El Ajolí, El Rocío, Huelva, Spain. All recordings in the field were made with a geophone, amplified by a custom-made amplifier connected to a portable digital recorder. Mean time (min) between the onset of the playback test and the emergence of the first individual of (C) *Bufo calamita* and (D) *Pelobates cultripes*. Individuals of both species were buried in the substrate within enclosures exposed to vibration stimuli (grey bars) and in control enclosures (white bars) located at four distances from the vibration source (0.5, 1, 2, and 4 m). Whiskers indicate standard errors of the means. Maximum time (120 min) was assigned to enclosures in which no individuals emerged during the playback test. (E) Mean proportion of emerged individuals of *Bufo calamita* at the end of the playback experiments. (F) Mean proportion of emerged individuals of *Pelobates cultripes* at the end of the playback experiments. Vertical bars are the proportion of individuals of both species that emerged during the two-hour playback test in enclosures exposed to vibration stimulus (grey bars) and in control enclosures (white bars) located at four distances from the vibration source (0.5, 1, 2, and 4 m). Whiskers indicate standard errors of the means.

Our study focuses on two species of anurans in a xeric sand-dune environment during their yearly period of activity. We address the following issues: first, can soil vibrations alone (that is, in the absence of rainfall), cause buried toads to emerge from underground? Second, are there differences in the response characteristics between two species of Mediterranean burrowing anurans? Last, is the distance between vibration source and receiver critical for eliciting toad emergence?

Playback experiments were conducted in coastal dunes in Southern Spain in the autumns of 2013 and 2014 when the first rainfalls occur, triggering the activity of adult toads before the formation of their temporary breeding ponds [6].

Rainfall vibrations were recorded *in situ* with a geophone and digital recorder in 2012 to determine the characteristics of the synthetic stimulus for playback tests. A 2-hour synthetic vibration stimulus was generated with Audacity 2.0.2 by low-pass filtering (threshold 200 Hz) broadband noise and applying a 'fade in', increasing linearly from 0 to 100% over the total duration. Playback vibrations were generated with a portable computer connected to a tactile-sound transducer buried 10 cm below ground (Figure 1A,B). Toads were captured and placed in two sets (experimental and control) of eight enclosures with plastic walls built in their natural habitat. Control enclosures were not exposed to vibrations. The enclosures were placed at 0.5 m, 1 m, 2 m, and 4 m from the emission point of the vibrations (see Supplemental Information).

A single test was performed each experimental (rainless) night. Four replicates of the test were conducted in the study. At sunset, all 16 enclosures were visually inspected for emergent toads; during the next 2 hours, a vibration stimulus with a linearly increasing intensity was broadcast from the tactile-sound transducer. All enclosures were visually inspected every five minutes. The emergence time of the first subject in each enclosure and the proportion of emerged subjects at the end of the playback test were recorded. Scoring the emergence of the first toad minimizes any effect of vibrations from other moving toads (expected to be similar in treatment and control). Two general linear mixed-effects models GLMM [7] were used to test the relationship between the two response variables and potential predictor factors (see Supplemental Experimental Procedures).

Toads in enclosures exposed to the vibratory stimulus emerged an average of 26.2 minutes earlier than those in the control group ($\chi^2 = 6.90$, df = 1, p = 0.009). To the best of our knowledge this is the first demonstration of significantly accelerated toad emergence in response to a controlled vibrational stimulus. In the case of *P. cultripes*, at all distances, mean emergence time was shorter in the treatment group than in the control group, and emergence time was also shorter in three of four distances in *B. calamita* (Figure 1C,D; Table S1).

The emergence times of *B. calamita* were 39.1 minutes shorter than those of *P. cultripes* ($\chi^2 = 4.33$, df = 1, p = 0.037). This may be related to the fact that only the time of emergence is being scored and not the time of initiation of emergence. In fact, after the experiments, excavation of the enclosures revealed that *B. calamita* was significantly closer to the surface (mean 9.2 cm, range 3–20 cm, n = 18) than *P. cultripes* (mean 25.3 cm, range 10–54 cm, n = 15, two sample t-test t = –5.4951, df = 18.675, p = 2.83e-05).

There was no graded difference related to the distance from the vibrational source ($\chi^2 = 2.82$, df = 3, p = 0.419), and no interactions were found between the fixed factors, treatment and species ($\chi^2 = 0.01$, df = 1, p = 0.909). On the other hand, the proportion of emerged subjects at the end of the playback tests was not significantly different between treatment and control groups for both study species (Figure 1E,F; Table S2). This may be explained by the fact that the experiments were performed during the season of high nightly adult activity after sunset.

Our results add abiotic atmospheric phenomena to the sources of vibrational signals in communication and hatching behavior in anurans [8–10]. We suggest that the evolution of emergence in response to vibrational signals in two anurans from different families may be a case of convergent evolution in the face of parallel ecological pressures. Furthermore, these results may unveil a yet unappreciated role for two anuran inner ear organs (See Supplemental Results and Discussion).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Introduction, Results (two tables), Experimental Procedures, Discussion, Author Contributions, and References, and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.11.005.

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