

of a widespread species than to be faced with the challenge of conserving every population because all are distinctive. Undoubtedly, some reptile species have already gone extinct before we even knew they existed. But a lot remain to be discovered: for example, a single square kilometre of Australian desert can contain 14 co-existing species of the lizard genus *Ctenotus* (many of them so similar that only an expert can tell them apart). On Caribbean islands, tree-dwelling anoline lizards similarly occur at remarkably high densities and diversities. Reptile conservation thus has the problem that on the one hand these small secretive animals do not attract much sympathy from the public, and on the other there are vast numbers of genetically distinct reptile populations whose loss would significantly erode biodiversity.

Many of the threats that affect all animals also affect reptiles, such as habitat degradation, new predators, and overexploitation. Some of the most worrying cases involve turtles, highly prized as traditional food and medicine in many Asian countries. Most turtles have very 'slow' life-histories, requiring several years to reach maturity, and reproducing at low rates. In such animals, even a small increase in adult mortality can cause rapid population declines. It is difficult to see how wild turtles will survive into the next century over much of Asia. Crocodylians also are under substantial hunting pressure, reflecting the economic value of their skins, but so far have proved surprisingly resilient. Many field biologists believe that reptile populations have declined precipitously over the last few decades, and fears are growing that reptiles may follow amphibians into an extinction vortex.

The central role of temperature in reptile biology suggests that climate change will have enormous impacts on many reptiles. It has already affected breeding phenology in some species. Thermal effects can ramify through all aspects of reptilian society and reproduction. For cold-climate reptiles, higher temperatures may bring benefits. For example, warmer springtime weather translates into longer mating seasons for Swedish sand lizards (*Lacerta agilis*), thereby increasing mating opportunities and thus, the average number of males

with which a female mates. This multiple mating enhances offspring viability because females of this species selectively use sperm from distantly related males to fertilise their eggs, thereby avoiding inbreeding. In contrast, global warming poses severe thermal challenges to tropical reptiles. Already forced to remain inactive in shaded shelters for most of the day to avoid lethal ambient temperatures, such animals may have fewer and fewer opportunities for activity in a warming world. In species where sex is determined by incubation temperature, increasing nest temperatures may shift offspring sex ratios and affect population growth.

Modern reptiles are the results of millions of years of evolution and comprise a diverse suite of lineages with intricate adaptations to a low-energy lifestyle. Freed from the energy-guzzling demands of endothermy, a myriad array of shapes, sizes and ecologies have evolved in reptiles. Even within a single lineage, the reptiles range from tiny pond turtles to giant Galapagos tortoises; from dwarf caimans to 6 m saltwater crocodiles; from 1 g geckos to 150 kg Komodo Dragons; and from tiny wormsnakes that feed on ant eggs to anacondas that eat capybaras. In many parts of the world, reptiles comprise a high proportion of all terrestrial vertebrate species. As we ponder the challenges of conservation in a changing world, we need to find solutions that work for these remarkable creatures.

Further reading

- Böhm, M., Collen, B., Baillie, J.E.M., Chanson, J., Cox, N., Hammerson, G., Hoffmann, M., *et al.* (2013). The conservation status of the world's reptiles. *Biol. Conserv.* 157, 372–385.
- Bull, C.M. (2000). Monogamy in lizards. *Behav. Processes* 51, 7–20.
- Greene, H.W. (1997). *Snakes. The Evolution of Mystery in Nature.* (Berkeley: University of California Press).
- Kearney, M.R., Porter, W. and Shine, R. (2009). The potential for behavioral thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl. Acad. Sci. USA* 106, 3835–3840.
- Pianka, E.R., and Vitt, L.J. (2003). *Lizards.* University of California Press, Berkeley.
- Shine, R. (2005). Life-history evolution in reptiles. *Annu. Rev. Ecol. Evol.* 36, 23–46.
- Vitt, L.J., and Caldwell, J.P. (2012). *Herpetology, Fourth Edition: An Introductory Biology of Amphibians and Reptiles.* (San Diego: Academic Press.)

Biological Sciences A08, University of Sydney, NSW 2006, Australia.
E-mail: rick.shine@sydney.edu.au

Correspondences

Circadian clock determines the timing of rooster crowing

Tsuyoshi Shimmura¹
and Takashi Yoshimura^{1,2,3,*}

Crowing of roosters is described by onomatopoeic terms such as 'cock-a-doodle-doo' (English), 'kik-ke-ri-ki' (German), and 'ko-ke-kok-koh' (Japanese). Rooster crowing is a symbol of the break of dawn in many countries. Indeed, crowing is frequently observed in the morning [1]. However, people also notice that crowing is sometimes observed at other times of day. Therefore, it is yet unclear whether crowing is under the control of an internal biological clock, or is simply caused by external stimuli. Here we show that predawn crowing is under the control of a circadian clock. Although external stimuli such as light and crowing by other individuals also induce roosters' crowing, the magnitude of this induction is also regulated by a circadian clock.

To test whether crowing of roosters is under the control of an internal biological clock (i.e., circadian clock), or is instead controlled by external stimuli such as light and other roosters' crowing, we first recorded crowing under 12-h light:12-h dim light (12L12dimL) and constant dim light (dimLL) conditions. Since crowing has been classified as a warning signal advertising territorial claims, and it challenges or threatens intruding males [2], the number of crows decreased significantly under isolated conditions (data not shown). Therefore, we housed four inbred PNP roosters in each group [3]; a total of three groups were examined. The experiments were conducted in a light- and sound-tight room. In order to avoid pecking, roosters were introduced into individual experimental cages that were placed separately within the room. Crowing was recorded all day, simultaneously using an IC recorder and a digital video camera equipped with a near-infrared illuminator. Since roosters

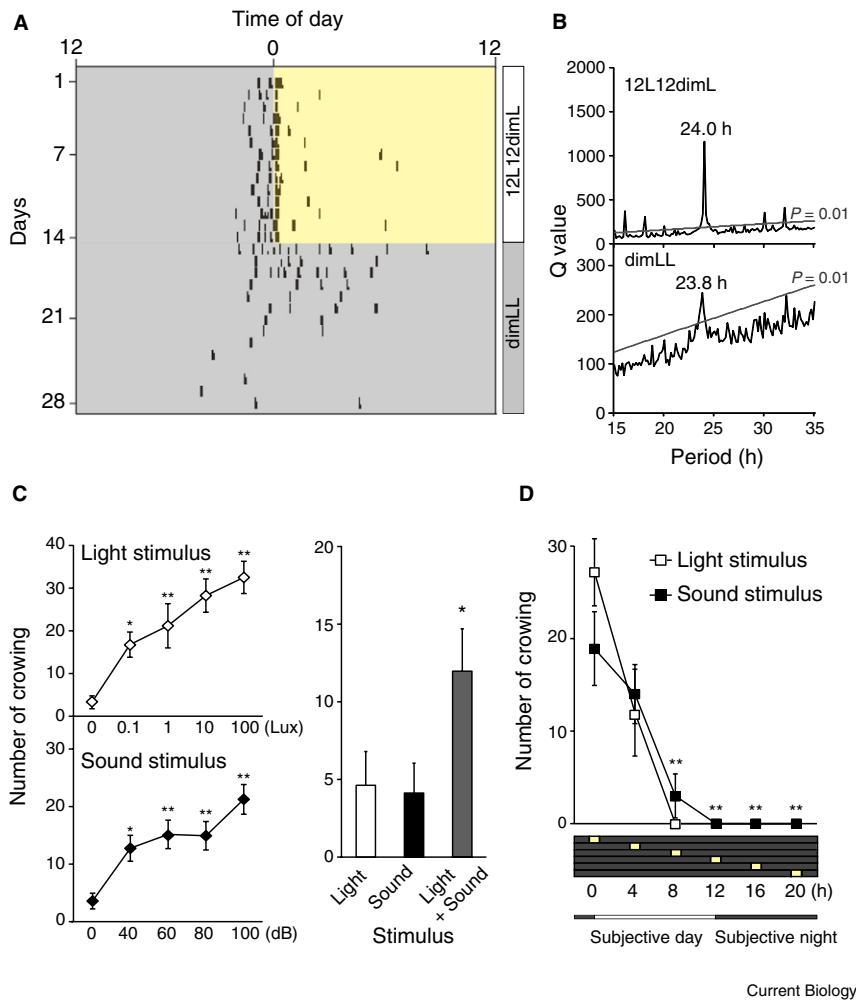


Figure 1. Regulation of morning crowing of roosters.

(A) Representative crowing record of a rooster kept under 12-h light:12-h dim light (12L12dimL) cycles and constant dim light (dimLL) conditions. The light and dim light periods are indicated by yellow and grey backgrounds, respectively. (B) Periodogram analysis of crowing under 12L12dimL and dimLL conditions. (C) Light and sound stimuli induced crowing in a dose-dependent manner ($F_{4,55} = 9.6$ [light stimulus, $P < 0.01$], $F_{4,55} = 7.9$ [sound stimulus, $P < 0.01$], ANOVA; $^*P < 0.05$, $^{**}P < 0.01$, vs. 0 lux or 0 dB, Dunnett's test; mean \pm SE, $n = 12$). Light (0.1 lux) and sound (60 dB) stimuli exhibited additive effects with regard to induction of crowing ($F_{2,18} = 4.7$ [$P < 0.05$], ANOVA; $^*P < 0.05$, vs. 0.1 lux and 60 dB, Tukey-Kramer's test; mean \pm SE, $n = 8$). (D) External stimulus (light or sound)-induced crowing was observed during early subjective day ($F_{5,30} = 16.6$ [light stimulus, $P < 0.01$], $F_{5,30} = 10.2$ [sound stimulus, $P < 0.01$], ANOVA; $^{**}P < 0.01$, vs. time 0, Dunnett's test; mean \pm SE, $n = 4-12$).

exhibit elevation and extension of the head prior to and during crowing [4], the crowing of each individual rooster could be easily distinguished from the sound and video recordings. Under 12L12dimL cycles, crowing was observed approximately 2 hours before the onset of light (i.e., anticipatory predawn crowing), consistent with observations of red jungle fowls in the wild [1] (Figure 1A). Under dimLL conditions, a free-running rhythm of crowing was observed with a period of 23.7 ± 0.1 h ($n = 4$), but this free-

running rhythmicity gradually damped out (Figure 1A,B). Since crowing is an androgen-dependent behavior [5,6], we predicted that exogenous androgen administration would enhance crowing behavior. Therefore, we next recorded the crowing rhythm of capons implanted with testosterone propionate. As expected, the free-running rhythm of the androgen-administered capons was more prominent than that of intact roosters kept under dimLL conditions (Figure S1A in Supplemental Information, published with this article online).

Although testosterone administration affects the period of free-running rhythmicity or causes splitting of locomotor activity rhythms in some species [7,8], we did not observe such differences in this study (Figure S1A-C). It seems necessary to record free-running rhythms for much longer durations in order to observe such effects of testosterone. In any case, these results clearly indicated the involvement of a circadian clock in anticipatory predawn crowing.

In addition to the anticipatory predawn crowing, we observed induction of crowing immediately after light onset (i.e., light-onset crowing) (Figures 1A and S1A). Therefore, we next investigated whether light-onset crowing is caused by an external light stimulus. When light stimuli of various intensities (0, 0.1, 1, 10, and 100 lux) were given at dawn (i.e., circadian time 0) for 30 min, they induced crowing, and the number of crows during the 30-min light exposure increased in a light dose-dependent manner (Figure 1C). It is known that when one rooster crows, nearby roosters have a high probability of crowing [9]. Therefore, we next examined the effect of a 30-min sound stimulus (i.e., the crowing sound of familiar roosters) of various doses (0, 40, 60, 80, and 100 dB) on crowing at dawn. Indeed, this sound stimulus also induced crowing in a dose-dependent manner (Figure 1C). When we gave light (0.1 lux) and sound (60 dB) stimuli in combination, they had an additive effect (Figure 1C). Also, to examine dependency of crowing on the time of day, the light (100 lux) or sound (100 dB) stimulus was given at various times of day, and we counted the number of crows when the light or sound stimulus was presented for 30 min. As shown in Figure 1D, induction of crowing by light and sound was significantly higher during the early subjective day.

Our observations prove that the rooster breaks the dawn every morning as a function of his circadian clock. It has been known for a long time that crowing is also induced by external stimuli. We conclude that not only anticipatory predawn crowing, but also external stimulus-induced crowing, is under the control of a circadian clock.

Supplemental Information

Supplemental information includes one figure and experimental procedures and can be

found with this article online at
<http://dx.doi.org/10.1016/j.cub.2013.02.015>.

Acknowledgments

We thank Drs. T. Namikawa, Y. Matsuda, M. Mizutani, and Y. Atsumi for providing PNP roosters. Takashi Yoshimura is supported by the Funding Program for Next Generation World Leading Researchers (NEXT Program) initiated by the Council for Science and Technology Policy (CSTP) and Tsuyoshi Shimmura is supported for JSPS fellows 22-4942. WPI-ITbM is supported by the World Premier International Research Center Initiative (WPI), MEXT, Japan. Authors deny any financial interest.

References

1. Collias, N.E., and Collias, E.C. (1967). A field study of the red jungle fowl in north-central India. *Condor* 69, 360–386.
2. Wood-Gush, D.G.M. (1971). *The Behaviour of the Domestic Fowl*. (London: Nimrod Press).
3. Tadano, R., Kinoshita, K., Mizutani, M., Atsumi, Y., Fujiwara, A., Saitou, T., Namikawa, T., and Tsudzuki, M. (2010). Molecular characterization reveals genetic uniformity in experimental chicken resources. *Exp. Anim.* 59, 511–514.
4. Shaw, B.K., and Kennedy, G.G. (2002). Evidence for species differences in the pattern of androgen receptor distribution in relation to species differences in an androgen-dependent behavior. *J. Neurobiol.* 52, 203–220.
5. Berthold, A.A. (1849). Transplantation der Hoden. *Arch. Anat. Physiol.* 16, 42–46.
6. Marler, P., Kreith, M., and Willis, E. (1962). An analysis of testosterone-induced crowing in young domestic cockerels. *Anim. Behav.* 10, 48–54.
7. Gwinner, E. (1974). Testosterone induces “splitting” of circadian locomotor activity rhythms in birds. *Science* 185, 72–74.
8. Daan, S., Damassa, D., Pittendrigh, C.S., and Smith, E.R. (1975). An effect of castration and testosterone replacement on a circadian pacemaker in mice (*Mus musculus*). *Proc. Natl. Acad. Sci. USA* 72, 3744–3747.
9. Koene, P. (1996). Temporal structure of red jungle fowl crow sequences: single-case analysis. *Behav. Proc.* 38, 193–202.

¹Laboratory of Animal Physiology, ²Avian Bioscience Research Center, Graduate School of Bioagricultural Sciences, ³Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan.

*E-mail: takashiy@agr.nagoya-u.ac.jp

The editors of *Current Biology* welcome correspondence on any article in the journal, but reserve the right to reduce the length of any letter to be published. All Correspondence containing data or scientific argument will be refereed. Queries about articles for consideration in this format should be sent by e-mail to cbiol@current-biology.com

Where has all the road kill gone?

Charles R. Brown^{1,*}
and Mary Bomberger Brown²

An estimated 80 million birds are killed by colliding with vehicles on U. S. roads each year [1], and millions more die annually in Europe [2] and elsewhere. Losses to vehicles are a serious problem for which various changes in roadway design and maintenance have been proposed [3]. Yet, given the magnitude of the mortality reported for some species [4], we might expect natural selection to favor individuals that either learn to avoid cars or that have other traits making them less likely to collide with vehicles. If so, the frequency of road kill should decline over time. No information is available for any species on whether the extent of road-associated mortality has changed [2]. During a 30-year study on social behavior and coloniality of cliff swallows (*Petrochelidon pyrrhonota*) in southwestern Nebraska, we found that the frequency of road-killed swallows declined sharply over the 30 years following the birds' occupancy of roadside nesting sites and that birds killed on roads had longer wings than the population at large.

We have worked on cliff swallows since 1982 in southwestern Nebraska, centered in Keith County at the Cedar Point Biological Station (41°12.591' N, 101°38.969' W), where colonially nesting cliff swallows attach their gourd-shaped mud nests in clusters on a vertical wall underneath a horizontal overhang. The birds use primarily highway bridges, overpasses and box-shaped concrete culverts underneath roads or railroad tracks as colony sites [5].

As we traveled among colonies daily in the course of our research, we stopped for each road-killed cliff swallow. We made the same effort to search for road kills and drove the same roads each year. We based our count of road kill retrospectively on the number of specimens prepared as skins and assume that the number salvageable provides a relative measure of mortality among years. We compared road kills to a sample of cliff swallows accidentally killed during mist-netting in the same years. The net fatalities (hereafter considered 'the population at large') did not differ from

living birds caught at the same time (Supplemental information).

The number of salvageable specimens each year declined significantly from 1983–2012 (Figure 1A). This result could not be explained by concurrent decreases in the cliff swallow population size around roads, because the population increased over the 30-year period (Figure 1A). The decline in road kills also could not be related to increases in the number of avian scavengers over time, as none showed significant increases in our study area (Supplemental information). Direct information is not available for mammalian scavengers within the study area, although populations of those species associated with humans probably have not changed, given that the resident human population of Keith County varied little during the study. Also, scavengers such as skunks have declined for the state of Nebraska as a whole [6]. Road-kill trends did not result from reduced vehicle traffic volume over time, which either did not change significantly or increased, depending on the metric used (Supplemental information). Sport-utility vehicles, which have probably increased during our study and offer a greater surface area for collision (relative to sedans that were more common in the 1980s), might contribute to changing bird mortality. However, road kill decreased as the larger vehicles became more common. Differences in detection likelihood did not affect our results, as the total kilometers traveled by us annually did not change significantly (Supplemental information). Thus, none of the obvious factors that confound most road-kill surveys applied to our study.

Wing length of road-killed cliff swallows was significantly longer than in the population at large (Figure 1B). Over time, wing lengths of cliff swallows killed on roads increasingly diverged from that of the population at large (Figure 1C). Average wing length of the population as a whole exhibited a significant long-term decline during the years of the study, whereas the opposite pattern held for the birds killed on roads (Figure 1C).

Cliff swallows now commonly nest on highway bridges, overpasses, and road culverts [5]. The Breeding Bird Survey that began in our study area in 1967