



Green tree frogs (*Zhangixalus arboreus*) lay tree or ground nests based on ambient conditions.

Tree Frog Nests

The tree frog *Zhangixalus arboreus*, found in forests in Japan, shows elasticity in its preference for nesting sites, sometimes nesting in trees and sometimes on the ground. Doctoral student Yukio Ichioka and his supervisor, Hisashi Kajimura, from Nagoya University in Japan, sought to find out why these tree frogs would put their offspring at risk from predators by selecting terrestrial nesting sites rather than placing them within the relative safety of the tree branches.

This species takes advantage of seasonal ponds in its forest habitat for mating, then seeks a favorable spot to lay eggs. Females secrete a frothy foam nest around the eggs as protection from predators and the elements, keeping the eggs moist until hatching. When oviposited in tree branches, a nest is usually located directly above the surface of a small forest pond. When the eggs hatch, the hatchlings drop into the water below. When oviposited on the ground, a nest is typically located near the water's edge.

Previous research found that this genus of frogs—*Zhangixalus*—ancestrally had arboreal nesting, then evolved terrestrial nesting during periods of global cooling. Thus, proposed the authors of the recent study, foam nests made on the ground may provide better insulation from the cold. They hypothesized that terrestrial foam nests would have a higher success rate than arboreal nests under relatively cool conditions.

To test their hypothesis, the researchers set up trials comparing arboreal vs terrestrial nests. They relocated a set of five arboreal nests to a standardized distance of one meter suspended over the ground, and relocated another set to the ground surface around the pond. They measured temperatures within the foam nests and ambient air temperatures, then tallied hatchling success.

The temperatures remained warmer at night in nests located on the ground, and those nests had a statistically higher hatching success rate than the arboreal nests. “These findings will be an important contribution to understanding the evolution of spawning behavior in amphibians,” said Kajimura. “They will also shed light on the adaptation strategies of forest organisms to the thermal environment.”

In their paper, the team noted that other factors influencing nest site selection may be the presence of predators and genetic differences between clades, which warrant further study. (*Ecology and Evolution*)
—Diane Miller

Mother's Milk Caecilian Style

Amphibians are not viewed as the most maternal of animals. However, a team of herpetologists have discovered that one species of amphibian, the ringed caecilian (*Siphonops annulatus*), produces a milk-like substance to feed its young.

Native to South America and measuring from twelve to eighteen inches long, ringed caecilians are a glossy dark blue and as adults eat snails, bugs, and other invertebrates. Like other caecilians, they live primarily underground, rendering them elusive and enigmatic to study.

An international group of researchers from Brazil, Germany, and the United States, by observing sixteen captive female caecilians with litters of four to thirteen, detailed how mother caecilians provide nutrition to their offspring via a liquid excreted from their vents.

All known species of caecilians care for their young, which are born altricial (helpless at birth). In ringed caecilians and many other species, infants are born with shovel-shaped teeth that they use to scrape off layers of their mother's skin, which they then consume.

Ringed caecilian mothers, the researchers found, also secrete a substance made of carbohydrates and lipids by oviductal glands to feed their offspring. This “milk” provisioning process resembles that of echidnas and platypuses, which also lack nipples but nevertheless secrete milk for offspring.

Whereas skin feeding occurred every few days, hatchlings fed multiple times a day on the mother's vent secretions. The young ringed caecilians seemingly solicit milk by making high-pitched sounds and nuzzling their mothers—behavior startlingly similar to infant mammals nosing and crying for letdown. After feeding, hatchlings were observed laying belly up, suggesting their appetites had been sated.

Alexander Kupfer, study co-author and zoologist at the University of Hohenheim, Germany, said, “Through our investigations in amphibians, we have now discovered a vertebrate system in amphibians that has developed similarly comprehensive brood care mechanisms as known for mammals.”

The finding may have broader implications than thought for the study of infant-mother communication and for parental care in vertebrates. (*Science*) —Brittany Steff

Female Brazilian caecilian (*Siphonops annulatus*) with her nest of babies



ALEXANDER KUPFER, DOI: 10.1126/SCIENCE.ADI6379

Bull Sharks May Benefit from Warmer Waters

At the Alabama Department of Conservation and Natural Resources, long-term trends over two decades reveal that juvenile bull sharks have become five times as abundant in warming, local coastal waters.

Led by Lindsay Mullins, a graduate student at Mississippi State University and research assistant at the Northern Gulf Institute, the study used a random stratified sample of gillnetted bull sharks (*Carcharhinus leucas*) to track population trends. Derived from a larger multi-species surveying effort, such long-term data that allow for assessment of climate change impacts are rare, said Mullins. In addition, “fishery-independent data remove bias,” she said.

Data collection entailed sampling fish from three major areas and smaller sub-areas at least monthly. The team then used machine learning and spatial statistics to analyze data for 440 juvenile bull sharks coupled with: satellite data on aquatic levels of chlorophyll *a*, salinity, dissolved oxygen, and temperature. The researchers also applied land use data on prevalence of forested, agricultural, bare, woody, wetland, scrub, shrub, or low- to high-intensity human development.

All bull sharks captured in the study were immature, and while Mobile Bay has not yet been formally assessed as shark nursery habitat, “we’re almost certain that is one reason why this is so significant,” said Mullins. Bull sharks are using these habitats as part of their reproductive cycle, she said. “These kinds of estuarine habitats provide a lot of safety and prey availability.”

So far, bull sharks appear resilient to warming waters, showing



Bull sharks (*Carcharhinus leucas*)

an ability to adapt their range to encompass suitable habitat, with other features of habitat suitability including salinity, riverine discharge, chlorophyll *a*, and water depth.

Mullins emphasized that just because there is a trend toward more young bull sharks on the Alabama coast does not mean more shark bites. Nor do the results, said Mullins, mean that there is no upper thermal threshold for the species. “It just means we don’t know it yet.” She also cautioned that management of fish species affected by climate change cannot be “one size fits all.”

“Contrary to the grim global outlook forecasted for many shark species,” wrote the authors, “this study illuminates the resiliency and potential benefits rendered for juvenile bull sharks in the northern Gulf of Mexico in the face of climate change and coastal urbanization.” (*Scientific Reports*) —Lesley Evans Ogden

Viruses Gone Wild

Telltale signs of plant viral infection, such as stunting and discoloration, are obvious in a uniform field of crops, but detecting them in the diverse world of wild plants is much harder. Hence, scientists long assumed wild plant communities were immune to crop viruses. But researchers at the University of California Riverside, Michigan State University, and University of Naples in Italy are helping reveal the hidden viral toll of modern

agriculture on wild plants.

In Southern California, huge tracts of melons, peppers, and other annual vegetable crops grow not far from protected areas of intact desert. To understand whether and how viruses might spread from crops into the wild, researchers examined the diversity, prevalence, and impacts of viruses on three species of native perennial plants: two in the squash and melon family, *Cucurbita foetidissima* and *C.*

palmata, and one in the pepper and tomato family, *Datura wrightii*. Over multiple years, the team took samples of leaf and stem tissues from wild plants at three natural reserves in Southern California.

In the lab, genomic sequencing was used to determine which viruses were present in the host tissue samples. Of the dozen previously characterized virus species, two non-native crop pathogens dominated the results: cucurbit yellow stunting disorder virus (CYSDV) and cucurbit aphid-borne yellows virus (CABYV). Both viruses are spread by sap-feeding insects—whiteflies (family Aleyrodidae) and aphids (family Aphidoidea). As many as 88 percent of the sampled wild squashes tested positive for CABYV. Dual infections with both CABYV and CYSDV were common.

Kerry Mauck, an entomologist and virologist with the

Native California desert plants, such as wild squash, acquire viruses from crops.

University of California Riverside, said, “Some of our data suggest that getting sick with one virus makes it easier, or more likely, that the plants will get another virus.”

To examine the impacts of CABYV infection, which could persist for years, the team grew *C. foetidissima* and *C. palmata* in a greenhouse and inoculated the wild squashes using infected aphids. They found that CABYV reduced plant vigor and shrank the size of the squashes’ long, moisture-seeking taproot by at least 25 percent. These effects could spell trouble for the desert plants and the myriad creatures relying on them, especially combined with a destabilizing climate and competition with invasives.

CABYV and CYSDV are both new to California—with a tenure of less than thirty years. “In such a short time, this ancient squash species has had its physiology completely upended by having to deal with chronic virus infections,” said Mauck. (*Phytobiomes*) —Ashley Braun



TESSA SHATES, DOI: 10.1094/PBIOMES-05-23-0033-R

SAMPLINGS

Bird Brain Barcodes

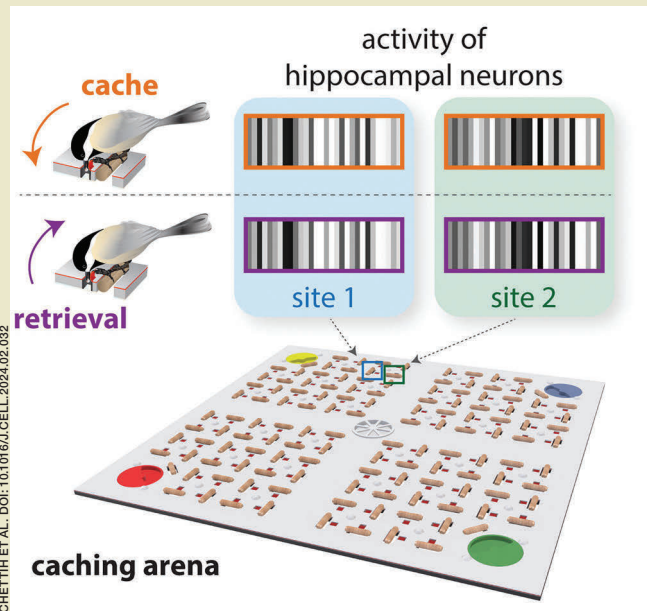
Over the course of a day, chickadees cache up to several thousand food bits. Remarkably, studies have shown that these small, busy birds can remember for weeks afterward which food items they have cached and exactly where they are stowed with centimeter precision. In birds and mammals, the hippocampus is implicated in such “episodic memory” formation. Yet identifying the neurophysiological underpinnings of episodic memory-making and retrieval has proved experimentally challenging.

Research now leverages the frenetic caching of chickadees to shed light on the phenomenon. Black-capped chickadees (*Poecile atricapillus*) were introduced to custom-built caching arenas, consisting of large acrylic grids with 128 crevices. Using their beaks and feet, the chickadees could lift flaps over these crevices to cache supplied sunflower seeds. Birds couldn't see their cached items, but the transparent crevice floors allowed cameras to track the caching.

“We put a lot of effort into optimizing these lab environments to make it really enticing to the birds,” said study co-lead author Selmaan Chettih, a postdoctoral researcher in the lab of Dmitriy Aronov, Assistant Professor of Neuroscience at Columbia University in New York. “And they seem to get very excited when they figure out how to find seeds and use the cache sites to hide them.”

Microdrives implanted in the birds' heads extended electrode probes into the hippocampus that collected neural data. The results showed that hippocampal “place cells”—which represent an animal's location and had been proposed as episodic memory-makers—did not change during caching events.

However, whenever the birds made a new cache, hippocampal neurons generated barcode-like patterns of activity. These neural firing patterns were different for each caching event, seemingly labeling the specific memory. When birds retrieved a cached item,



Schematic showing chickadee brain activity during seed-caching events

the unique hippocampal barcode re-fired, suggesting that its reactivation enabled chickadees to recall a distinct “what, where, and when” experience.

“The implication is that the brain might generate labels so that it can store lots of information about each memory, without accidentally starting to blur information from different memories together,” explained Chettih.

The researchers point to the transient hippocampal signals as the key for chickadees' episodic memory—and possibly for ours as well. “There are findings in humans in particular that are consistent with barcodes existing in the human hippocampus,” said Chettih, “although future research is needed.”
(Cell)

—Adam Hadzazy

Pint-Sized Pollinator

The orchid family (Orchidaceae) includes about 28,000 species, more than any other plant family. Orchids attract pollinators with a variety of displays and lures, from scents that mimic food or mates to actual rewards, such as nectar or places to lay eggs, as seen in the *Gastropoda foetida* orchid [“Samplings,” NH, 11/23].

Despite the vast diversity of pollination strategies that have been observed in orchids, it remains unknown how about 90 percent of orchid species are actually pollinated. In a study of the orchid *Oberonia japonica*, the pollinator has now been identified.

O. japonica is an epiphytic species native to Japan, Korea, and Taiwan. The flower stalks grow on tree trunks and contain hundreds of tiny orange flowers each only two millimeters across. A team of researchers led by biologist Yuta Sunakawa at the University of Tokyo spent over twenty-six hours across three days observing *O. japonica* orchids at a plum orchard in Aichi Prefecture, Japan.

All observed insect visits to the orchids were at night. Macro photography revealed that the minuscule insects were gall midges (family Cecidomiidae), some

the orchid's pollinaria, a lump of pollen and accessory structures, was a thrilling experience,” Sunakawa said.

The researchers collected 135 insect visitors for further identification, a third of which were carrying pollen. Almost 95 percent of the insects were gall midges. All were female, suggesting that females may receive some benefit from visiting the flowers. No females were observed laying eggs on the flowers, but gall midges may be receiving nectar from the flowers. Females might also be deceptively lured by a color or smell that mimics the presence of mates.

There are roughly 300 species of *Oberonia* orchids, and more than 5,700 species of gall midges found around the world. Further observations of *O. japonica* and related orchid species may clarify the relationship between these flies and orchids. *(Ecology)*

—Kati Moore



Flower whorls and stalks of Japanese orchid *Oberonia japonica*

of which had yellow pollen on their heads. *O. japonica* is the first orchid known to be pollinated by gall midges.

“Photographing a gall midge carrying