News & views

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Figure 1 | Australian green and golden bell frogs (*Ranoidea aurea*). Waddle *et al.*[3] constructed mini saunas using bricks inside a small plastic greenhouse. The warm environment enabled the frogs to clear a deadly fungal infection, and the animals gained resistance to reinfection by the fungus.

Conservation biology

Mini saunas help frogs survive fungal disease

Brian Gratwicke & Anna Savage

Amphibian species around the world are threatened with extinction by the deadly fungal disease chytridiomycosis. A simple, low-cost solution to provide warm conditions enables frogs to clear the infection and remain disease free. **See p.344**

At the 1989 World Congress of Herpetology in Canterbury, UK, scientists began discussing amphibian declines that were happening around the globe, but the cause of these enigmatic declines was unknown. A decade later, the fungus *Batrachochytrium dendrobatidis* (*Bd*) was described and identified as the pathogen responsible for the deaths of frogs in Australia and in Latin America[1,2]. Subsequently, many other mysterious amphibian disappearances were attributed to chytridiomycosis, the disease caused by this pathogen. As amphibians have continued to disappear, zoos and aquariums around the world have created captive colonies of imperilled amphibians to provide backup for these species and buy time until a solution is found. On page 344, Waddle *et al.*[3] point to a way to tackle this problem.

Thousands of papers have been published about *Bd* since its discovery. Amphibians can be tested for infection and be treated effectively in captive settings, but little progress has been made in controlling *Bd* in the wild. The first successful example was a herculean effort by a team of biologists in Mallorca, Spain, who captured and treated all the Mallorcan midwife toad (*Alytes muletensis*) tadpoles on the island, then drained their pools and scrubbed them out with antifungal chemicals[4]. Another

successful effort involved moving mountain yellow-legged frogs (*Rana muscosa*) from recovering populations that had presumably evolved some disease resistance and re-establishing them in places where the species had been previously wiped out[5]. Waddle *et al.* describe an innovative method to increase the resilience of a susceptible frog to *Bd* in a natural setting.

The authors designed an experiment using Australian green and golden bell frogs (*Ranoidea aurea*) on the basis of two interacting mechanisms. An exploitable weakness of many disease-causing fungi is that they cannot tolerate high temperatures; 28 °C is the thermal maximum[6] for *Bd*. Keeping frogs at 30 °C for ten days will clear most *Bd* infections[7]. Waddle and colleagues constructed mini plastic greenhouses, enclosing piles of bricks, inside naturalistic outdoor enclosures (Fig. 1). The greenhouses warmed the brick piles, creating frog 'saunas'. The frogs preferred hanging out in these little hot spots. Frogs in sunny enclosures had lower infection loads than did frogs in shaded ones. The authors also found that exposing green and golden bell frogs to *Bd* then clearing the infection improved their disease outcomes on re-exposure to *Bd*.

Together, these two mechanisms (immune responses and heat) seem to place a thumb on the scale in favour of the frogs, improving their resilience to chytridiomycosis. The authors think that, if these hotspot microhabitats are constructed in the wild, the frogs will seek them out for warmth and clear their *Bd* infections through heat treatment. In turn, their resistance to the disease during cooler times of the year will improve because of this previous exposure, creating sustainable "resistance engines".

There is much room for improvement in scientists' understanding of *Bd* susceptibility, resistance and tolerance. It might seem that the improved disease outcomes in this experiment came from a straightforward defence response from the adaptive branch of the frogs' immune system, but previous *Bd* vaccination trials found limited evidence for this[8]. Vaccination studies are further complicated because *Bd* suppresses responses from certain types of immune cell in susceptible individuals[9], whereas other types of defence system (those generated by the innate branch of the immune system, including antimicrobial peptides and a defence response called the complement system) have an important role in disease resistance[8].

Genes encoding major histocompatibility complex (MHC) molecules found on immune cells seem to be under evolutionary selection to enable resistance to *Bd*. Expression of some MHC genes soon after an infection might enable frogs to be more tolerant of future infections. Yet, sustained MHC expression is a possible cause of death[10]. Scientists lack mechanistic data on immune function in wild frogs, but research on *Xenopus laevis* – the model frog species for laboratory study – points to a key role for a type of innate defence response from immune cells called T cells that goes against the dogma in mammals that T cells are involved only in adaptive immunity[11]. In this context, confirmation that previous *Bd* exposure improves resilience to disease in a susceptible species opens research opportunities to understand *Bd* immunity.

Climatic refuges that frogs can live in but which are too hot for *Bd* have been observed in nature, and explain the persistence of susceptible species in some places[12]. Waddle and colleagues' research will probably stimulate more creative thinking about establishing artificial microhabitats that will help heat-loving amphibians to clear infections. The findings might also inspire biologists to consider climate when selecting reintroduction sites for amphibians and to try to better understand the interactions between climate and immunity.

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Structural biology

Enzyme blueprints will aid tuberculosis drug design

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Structural insights into how anti-tuberculosis drugs interact with the enzyme that makes ATP in bacteria and humans pave the way for improved drug design to treat the disease and combat antimicrobial resistance. **See p.409**

In 2005, a class of compound was discovered that could inhibit the bacterium that causes tuberculosis infections in humans – *Mycobacterium tuberculosis*[1]. Unexpectedly, the target of these drugs was ATP synthase, the enzyme responsible for producing cellular energy in the form of ATP molecules. The evolutionary conservation of ATP synthase enzymes across all domains of life had prompted concerns that antimicrobials targeting this enzyme would also target the human version, causing toxic side effects in humans. On page 409, Zhang *et al.*[2] provide the first high-resolution structural insights into how these anti-tuberculosis drugs – namely bedaquiline and its derivative TBAJ-587 – interact with the ATP synthases from *M. tuberculosis* and, crucially, humans.

Bedaquiline (Sirturo) was the first anti-tuberculosis drug to be discovered in decades[1]. It received approval from the US Food and Drug Administration (FDA) in 2012, and again in 2019 as part of an all-oral combination therapy with two other antibiotics, pretomanid and linezolid[3]. The clinical roll-out of bedaquiline had an immediate positive effect on treatment outcomes for thousands of individuals with tuberculosis. Despite this success, prolonged bedaquiline treatment shows cardiac toxicity in humans owing to its potent inhibition of the cardiac potassium channel protein hERG, and *M. tuberculosis* is already developing resistance to bedaquiline. These combined liabilities highlight an urgent need to develop improved derivatives of bedaquiline that will solidify this drug class as a crucial component of future treatment regimens.

Zhang and colleagues use a technique called cryogenic electron microscopy (cryo-EM) to solve the structures of anti-tuberculosis drugs bound to the *M. tuberculosis* and human ATP synthases. They show that bedaquiline interacts with the *M. tuberculosis* ATP synthase in a domain that is embedded in the cellular membrane. Strikingly, bedaquiline binds to a