

Host-specificity tests were conducted involving two provenances of *D. tomentosus*: one collected from *C. imbricata* in South Africa (henceforth called the imbricata provenance) and one collected from *C. rosea* in Mexico (henceforth called the rosea provenance). These provenances were reared on both *C. imbricata* and the target weed, *Cff*. On the target weed, the imbricata provenance survived poorly and insects of the rosea provenance did not complete their life cycle. However, both populations thrived on *C. imbricata*. This remarkable finding led to a reassessment of the target weed which was subsequently identified as *Cylindropuntia fulgida* var. *fulgida*, whose native range is the Sonoran desert of Arizona and Mexico.

With the correct identification of the weed, the project undertook the first study of the biology of *D. tomentosus* revealing that this species exhibited some biological and morphological aspects that differed from those of its congeners<sup>1</sup>. The next task was to search for a *D. tomentosus* provenance with the potential to cause severe damage to, and successfully control *Cff*. Further exploration by Helmuth Zimmermann led to the collection and importation of *D. tomentosus* provenances from a number of *Cylindropuntia* taxa in the New World: *Cff* and *C. f.* var. *mamillata* in the Sonoran desert, *C. rosea* and *C. tunicata* from different localities in Mexico, and *C. cholla* from different localities in Baja California. Experiments were conducted in South Africa, at the Plant Protection Research Institute, Pretoria, to assess survival and performance of the provenances on *Cff*. These studies revealed significant differences between provenances in their performance on and damage to *Cff*<sup>2</sup>. A provenance (collected from *C. cholla*, the cholla provenance) with great potential to control *Cff* was identified and is the insect used for biocontrol as described in the article above by Klein and Zimmermann. The most surprising aspect of this provenance was that it came from *C. cholla* from Baja California, Mexico whereas provenances from the weed's native range performed poorly. This may be explained in terms of the 'new association' phenomenon in which insect populations that have not co-evolved with a target weed perform better than old associations.

Based on the observed subspecific differentiation in host specificity among *D. tomentosus* provenances towards *Cff* and *C. imbricata*, the research further explored the hypothesis of the occurrence of biotypes of this insect in relation to host plant species. The results of these studies showed that the provenances fell into three categories on any host: some thrived on a particular host, others survived poorly, whereas others died before completing their life cycles. These observations demonstrated the occurrence of host-affiliated biotypes.

Although the occurrence of biotypes had now been determined, the extent of their differentiation was still an outstanding issue. This warranted some attention: as *C. imbricata* and *Cff* occur in sympatry in some areas in South Africa, it is likely that the ranges of the two insect biotypes may overlap. Therefore, cross-breeding studies were conducted between the cholla and imbricata biotypes to assess their

reproductive compatibility and the impact of hybridization on host specificity. The two biotypes cross-bred freely in the laboratory producing viable offspring that had similar or greater potential to cause damage to *Cff* and *C. imbricata* than their parents. The hybrid offspring were less host specific than their parents, implying that hybridization would not have a negative impact on biological control of either weed.

During this part of the study, a bias in hybrid performance towards their maternal parent host was observed indicating that host specificity may be genetically based. Genetic diversity among the biotypes was assessed by sequencing part of the mitochondrial cytochrome *c* oxidase subunit 1 gene. Sequences within a biotype were identical and biotypes from the same host species clustered together irrespective of place of origin. This provides further evidence for the existence of host-adapted biotypes in *D. tomentosus*.

These studies on *D. tomentosus* highlight the importance of considering the existence of biotypes when selecting insects for biological control, as the selection of an inappropriate biotype may lead to its failure in the field.

<sup>1</sup>Mathenge, C.W., Holford, P., Hoffmann, J., Spooner-Hart, R., Beattie, G.A.C. & Zimmermann, H.G. (2009) The biology of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae). *Bulletin of Entomological Research* (online 10 February 2009; doi: 10.1017/S0007485308006597).

<sup>2</sup>Mathenge, C.W., Holford, P., Hoffmann, J., Zimmermann, H.G., Spooner-Hart, R. & Beattie, G.A.C. (2009) Distinguishing suitable biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) for biological control of *Cylindropuntia fulgida* var. *fulgida* (Caryophyllales: Cactaceae) in South Africa. *Bulletin of Entomological Research* (online 23 March 2009; doi: 10.1017/S0007485309006671).

By: Catherine Mathenge & Paul Holford,  
Centre for Plant and Food Science,  
University of Western Sydney, Locked Bag 1797,  
Penrith South DC, NSW 1797, Australia.  
Email: c.mathenge@uws.edu.au  
p.holford@uws.edu.au

### Can Manipulations of Amphibians' Mutualistic Skin Bacteria Control a Lethal Skin Disease?

The current extinction crisis is not sparing amphibians. While habitat loss and destruction are the major causes of amphibian extinction, disease is also causing population declines. Chytridiomycosis is a skin disease of amphibians caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), and is the causal agent of population declines and extinctions in relatively pristine areas, such as national parks. This species was only described in 1999, yet we have learned quite a bit about its biology and ecology. *Bd* seems to have originated in South Africa and spread to other parts of the world on *Xenopus laevis*, the African clawed frog, which was exported for use in

pregnancy tests<sup>1</sup>. Despite the pathogen's role in many amphibian species' decline and extinction, relatively little research has focused on how to control the disease in nature. Our research has focused on the ecology of amphibian skin in an attempt to limit the disease.

When *Bd* colonizes amphibian skin, it encounters transient and resident skin microbiota. We have found that many of the resident bacterial species inhibit *Bd* in laboratory challenge assays. Three of the inhibitory metabolites, 2,4-diacetylphloroglucinol from *Lysobacter gummosus* and violacein and indole-3-carboxaldehyde from *Janthinobacterium lividum*, have been identified from pure bacterial cultures<sup>2</sup>. In addition, these metabolites are found on some amphibians in nature in concentrations high enough to inhibit *Bd*. To our knowledge, amphibians do not produce these metabolites themselves, so it appears that their symbiotic skin bacteria are responsible.

We have shown through a 'bacterial removal' experiment that the resident microbes on the skins of *Plethodon cinereus*, red-backed salamanders, ameliorate the symptoms of chytridiomycosis. Individuals with their skin bacteria reduced by exposure to antibiotics had greater morbidity than those salamanders left with their skin bacteria unmanipulated. Bioaugmentation is a strategy whereby we increase the proportion of individuals with a species of anti-*Bd* bacteria already found on other members of the population. In a laboratory experiment, we added *J. lividum* to skins of the mountain yellow-legged frog, *Rana muscosa*, before exposure to *Bd*<sup>3</sup>. Those treated with *J. lividum* suffered no mortality or morbidity, whereas frogs exposed to *Bd* alone lost weight and died. Frogs that had *J. lividum* added to their skins had much higher concentrations of violacein on their skins, suggesting that this bacterial-produced metabolite inhibited *Bd*. Another exciting result from this experiment was that we were able to take *J. lividum* collected from a salamander species and successfully place it on a frog species, suggesting that some bacterial species can target the larger amphibian assemblage.

In nature, populations of *R. muscosa* that co-exist with *Bd* have a higher proportion of individuals with at least one culturable anti-*Bd* bacterial species. This result suggests a threshold effect analogous to herd immunity, whereby only a fraction of the population needs to be immunized in order for the disease to die out. A bioaugmentation strategy that can increase the proportion of amphibians with anti-*Bd* bacteria may prevent epidemic outbreaks of chytridiomycosis. There are a number of remaining challenges, such as how to implement a bioaugmentation strategy in nature. Currently, we are investigating transmission of bacteria from soil and water to amphibians. We already know that in the laboratory, we can introduce skin bacteria to amphibians from bacteria in artificial pond water. Of course, tests to make sure non-target species are not affected will be of critical importance. Recent work in agricultural contexts suggests that the introduction of beneficial bacteria does not affect the resident soil microbiota, which is encouraging.

An important component of amphibian conservation efforts is the creation of survival-assurance colonies. Many species are being kept from going extinct in these breeding colonies, but they cannot be released into nature because *Bd* is endemic and persisting on resistant amphibian species. Treating susceptible species with anti-*Bd* bacteria before introduction into the field may allow successful re-introductions. One important open question asks how long bacteria stay on amphibians. Our research with *J. lividum* suggests that it stays on frogs at least 20 weeks<sup>3</sup>. In the future, we may be able to facilitate the re-introduction of amphibians from survival-assurance colonies. Population declines and extinctions due to *Bd* are ongoing and more research is urgently needed into the efficacy of a bioaugmentation strategy for amphibians.

<sup>1</sup>Weldon, C., du Preez, L.H., Hyatt, A.D., *et al.* (2004) Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10, 2100–2105.

<sup>2</sup>Brucker, R.M., Harris, R.N., Schwantes, C.R., *et al.* (2008) Amphibian chemical defense: antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*. *Journal of Chemical Ecology* 34, 1422–1429.

<sup>3</sup>Harris, R. N., Brucker, R.M., Walke, J.B., *et al.* (2009 in press) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME Journal*, doi:10.1038/ismej.2009.27.

By: Reid N. Harris & Kevin P. C. Minbiole,  
Department of Biology (RNH) & Department  
of Chemistry and Biochemistry (KPCM), James  
Madison University, Harrisonburg, VA 22807, USA.  
Email: harrisRN@jmu.edu / minbiokp@jmu.edu

## Trying to Taming Wild Gingers

*Hedychium* (heh-DIK-ee-um) or wild ginger species, as they are generally known, were extensively cultivated throughout England and Europe in the nineteenth century where their exotic forms and the heady perfumes of their magnificent blooms made them prized ornamentals in tropical 'hot-houses'. Following the paths of empire builders, a number of species were transported to warmer climates around the world and subsequently three species, *H. gardnerianum* (Kahili ginger), *H. flavescens* (yellow ginger) and *H. coronarium* (white ginger), have escaped cultivation and become naturalized. These now cause significant environmental and economic impact globally, especially in New Zealand and Hawaii (USA) in the Pacific, La Réunion (France) in the Indian Ocean, the Azores (Portugal) in the Atlantic, and Brazil. *Hedychium gardnerianum* has been nominated as among the 'World's 100 Worst Invaders' by the Invasive Species Specialist Group of the World Conservation Union.

Native to the eastern Himalayan range, these coarse perennial herbs are aggressive colonizers of indigenous forest habitats in their introduced range, but display ecological and altitudinal adaptability, persisting under closed rainforest canopies (both littoral