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Variable Susceptibility to an Emerging Infectious Disease, Chytridiomycosis, in Anurans

Picture of *Nyctimystes dayi* by N. Kenyon

Thesis submitted by

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In July 2008

For the degree of Doctor of Philosophy

in Zoology and Tropical Ecology

within the School of Marine and Tropical Biology

James Cook University

Townsville, QLD, Australia

STATEMENT OF SOURCES DECLARATION

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. All research reported in this thesis received the approval of the ethics committees and the QPW.

Signature   Date

STATEMENT OF CONTRIBUTION OF OTHERS

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Two publications have been submitted from Chapter 2. Dr. Andrea Phillott provided input on the manuscripts and generously provided her frog monitoring population to compare the true identity of the frogs via toe-tipping and photographic identification method. Professor Ross Alford gave valuable advice on statistical analysis and writing the manuscript.

Chapter 5 is in the process of being submitted to Journal of Zoology with the help of Professor Ross Alford. The design of this experiment was an ongoing development and Ross provided valuable input during that time, including the analysis.

Chapter 6 has been submitted to Journal of Herpetology with the help of Sara Bell and Professor Ross Alford. Sara Bell offered advice on culturing *Bd* and writing the manuscript. Professor Ross Alford gave valuable advice on the experimental design, statistical analysis and writing the manuscript, which is currently in revision.

The submission of several chapters to different journals required the use of different English styles and reference formats which were retained in this thesis.

Declaration of ethics

Animals were obtained and all data collected under Animal Ethics Approval A960 granted by James Cook University Animal Ethics Committee, and Scientific Purposes Permits WITK01932505 and WISP01764304 granted by Queensland Parks and Wildlife Service.

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ABSTRACT

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Chytridiomycosis is an emerging infectious amphibian disease, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), and has caused numerous amphibian declines around the globe. Chytridiomycosis can be lethal in many amphibian species but not in others, leading to three different responses to exposure, 1) the amphibian becomes infected with *Bd* and dies, 2) the amphibian becomes infected with *Bd* and survives and 3) the amphibian does not become infected even though it occurs in a habitat where *Bd* exists. My project aimed to increase our understanding of the causes of these interspecific differences. I investigated the hypotheses that they could be caused by innate immune defences (antimicrobial peptides) against *Bd*, innate or adaptive responses of individuals through microenvironment selection, or behavioural avoidance of infective water.

I found evidence for all three mechanisms. *In vitro*, antimicrobial peptides (AMPs) of *Litoria genimaculata* (vulnerable to infection with *Bd* with highland population declines followed by recovery) and *L. rheocola* (vulnerable to infection with *Bd* with severe declines at higher elevations with little or slow recolonisation) can completely inhibit *Bd* growth. I also found large seasonal variation in antimicrobial peptide defences in both species. This may result from physiological shifts driven by temperature, or may reflect adaptation to seasonal fluctuations in the risk of infection. The proportion of *L. genimaculata* from high elevation populations, which have experienced strong viability selection pressure imposed by chytridiomycosis outbreaks, that produced AMPs that effectively inhibited *Bd in vitro,* was significantly higher than in low elevation populations, which have been protected from chytridiomycosis by environmental factors. There was also evidence that high elevation populations produced AMPs that differed slightly in chemical composition from those produced by low elevation populations. However, when individuals of either frog species produced AMPs that inhibited the growth of *Bd*, the effectiveness of AMPs from high and low elevation populations did not differ significantly. This suggests that any responses to selection may have occurred through an increase in the proportion of individuals producing effective AMPs, with no change in the types of AMPs produced.

Antimicrobial peptide defences did not differ significantly between high and low elevation population of *L. rheocola*, suggesting that this species may have recolonised

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upland areas. On the other hand, *L. rheocola* had more effective antimicrobial peptide defences against *Bd* than *L. genimaculata* and may have experienced stronger selection pressure after the appearance of chytridiomycosis.

Thermal microenvironments selected in the laboratory corresponded to those expected from decline patterns observed in the wild. *Litoria caerulea* (vulnerable to infection with *Bd* but no population declines due to chytridiomycosis have been detected) selected warm and hot environments significantly more often than *L. genimaculata*. Additionally, although not significant, there was a trend that intensity of *Bd* infection in all three species was more likely to decrease over time in individuals that had a choice of hydric and thermal microenvironments than in frogs that were housed under standard environmental conditions. There was also evidence of disease avoidance behaviour; some *L. caerulea* and *L. genimaculata* chose uncontaminated water significantly more often than water that contained *Bd* zoospores. None of the frog species were able to completely avoid water containing *Bd* zoospores, possibly in part because their pond selection was also influenced by side fidelity.

My study demonstrates the complexity of host-pathogen interactions and that multiple factors, including innate immune defence, microenvironment selection and disease avoidance behaviour, can influence the progress of chytridiomycosis and should be considered when establishing species specific management plans.

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