Investigating Fine-scale Geographic Variation in the Australian Funnel-web Spider (*Atrax sutherlandi*) – Brief Introduction and Current Progress

Mark K. L. Wong, Research School of Biology, The Australian National University, markwong.research@outlook.com

Note: Mark is the recipient of the 2014 Honours and Undergraduate Student Travel Subsidy and that he will present his research at the 2015 AWMS Conference.

The Study

The newly described Australian funnel-web spider *Atrax sutherlandi* (Gray, 2010) (Figure 1) belongs to the same genus as the notorious Sydney funnel-web, and is named after the late toxicologist Professor Struan Sutherland. *A. sutherlandi* is distributed across south-eastern Australia, including the Tallaganda forests of New South Wales. Research suggests that during the cold periglacial episodes of past Pleistocene glacial-interglacial cycling (30,000–15,000BP), Tallaganda's native eucalypts would repeatedly contract into low-lying, sheltered gullies to be replaced at higher altitudes by alpine grassland (Hope *et al*., 2004). Amidst the harsh climates, these isolated forest remnants or 'refugia' preserved local habitats, thus enabling a variety of species to persist in an otherwise inhospitable region. While the forest is now continuous, there exists substantial evidence to indicate that many of Tallganda's species still reflect this ancient isolation in their genetic makeup (Garrick *et al*., 2012). For example, in water skinks, velvet worms, springtails and flatworms, several distinct genetic forms are presently recognisable across the forest and their respective distributions coincide with the locations of refugia (Garrick *et al*., 2012). This genetic biodiversity and population structuring among Tallaganda's refugia regions is also well documented for the funnel-web spider *A. sutherlandi*, where differences between six refugia populations are of a magnitude generally attributable to distinct species (Beavis *et al*., 2011).

Figure 1. *Female Atrax sutherlandi. Photographed by Mark Wong at Tallaganda National Park.*

Although the genetic differentiation and short-range endemism of Tallaganda's fauna is well documented and has shown to be unequivocally associated with historical climate change, no studies have yet sought to investigate the possibility of corresponding geographic variation in their phenotype. Presented with this opportunity, and with the aim of elucidating the historical climatic effects on a species' physical characteristics, I aim to survey geographic variation in the phenotype of *A. sutherlandi* populations at Tallaganda. These spiders have been selected as the study species owing to their extensive lifespans (>15 years), limited dispersal patterns, sensitivity to ecological disturbance and positions as top predators in many terrestrial microhabitats (King *et al*., 2002; Harvey 2002; Beavis *et al*., 2011), which together confer them a unique potential for retaining past signals of environmental and demographic change that may also be representative of the wider terrestrial invertebrate communities they predate. Three phenotypic characters deemed to be important to *A. sutherlandi* life history have been selected for study. These are venom yield, mass-specific metabolic rate and morphological structure. These characters will be studied for their specific patterns of geographic variation, which if congruent to the same patterns of six distinct populations from the spiders' genetic makeup would potentially be telling of a historical climatic effect on phenotype.

Methods & Current Progress

Sampling & Housing

A total of twelve sites in Tallaganda have been sampled. Sampling at each site involves opportunistically turning over woody debris and rocks, then scanning the ground underneath for the burrow of *A. sutherlandi* (Figure 2). For extraction of spiders, a thin stick is first inserted into the burrow to reveal the direction of descent. Next, a trowel is employed to dig away loose soil adjacent to the burrow. Finally, the burrow is then deconstructed and the exposed spider transferred into a specimen jar with metal forceps. A total of 187 female specimens have been collected thus far. Specimens selected for use in venom milking and metabolic rate measurement were first acclimatized to identical housing conditions of 10°C and 100%RH with a regular diet of *Tenebrio molitor* larvae for at least 2 months, so as to avoid obtaining variation in the results originating from the differing field conditions of separate collection dates.

Figure 2. *The distinctive silk web of Atrax sutherlandi with multiple funnel-shaped entrances. Photographed by Mark Wong at Tallaganda National Park.*

Venom Yields

Pilot tests for venom milking have been successful in establishing a standardized milking regime thus far. To obtain the maximum volume of venom from each spider, they will be milked daily in a 5-minute sitting over 3 consecutive days – this period substantially depletes the venom resources of all specimens tested and hence promises to deliver a consistent quantity for comparison across the study sample.

Continued on page 9

Investigating Fine-scale Geographic Variation in the Australian Funnel-web Spider (*Atrax sutherlandi*) – Brief Introduction and Current Progress

Continued from page 8

During each sitting, the spiders are transferred into a large glass dish and a wooden stirrer is employed to continuously antagonize the subject into adopting a defensive rearing position with both fangs extended and venom droplets forming at their tips. These droplets are collected through capillary action using a 32mm (5µL) microcapillary tube (Model: P1799, Drummond Scientific, USA). This method averts the use of close-contact intrusive electrostimulation that present risks to specimen health and investigators' safety (Nisani *et al*., 2007; Morgenstern & King, 2013), and similar milking methods have also been used with other funnel-web species (e.g. Atkinson & Walker, 1985).

Metabolic Rate

The mass-specific standard metabolic rate (ms-SMR) of each spider will be obtained using flow-through respirometry to measure CO_2 output. The setup comprises a gas exchange system (Model: LI-6400XT, Li-Cor Inc., USA) and a 70cm^3 brass animal chamber. A supply of dry ambient air (ca. 0%RH) is delivered through the chamber at 100 ml min^{-1}, and the gas analyser calculates the spider's ms-SMR (µl CO_2 g^{-1} h^{-1}) based on the CO_2 levels of air exiting the chamber and the spider's weight. Preliminary results from 30 specimens present evidence of high variation in ms-SMR ($\bar{x} = 39.6 \pm 9.99$ µl CO_2 g^{-1} h^{-1}) among *A. sutherlandi* at Tallaganda, and the congruence of this with geographic distributions of distinct genetic populations will be tested subsequently.

Morphometric Measurements

Specimens not utilized in either venom milking or metabolic rate measurement were preserved in 80% ethanol and used for morphometric measurement. These were made to the nearest 0.001mm using the software ImageJ (Abràmoff *et al*., 2004) to analyse images of morphological structures obtained from a digital microscope (Model: P-400Rv, Nikon, Japan). So far, preliminary analysis using Multivariate Analysis of Variance (MANOVA) to examine variation in 34 size-corrected linear exoskeletal measurements from 80 specimens have found 11 measurements which vary significantly among the six refugia populations ($p < 0.05$). This presents early evidence of morphological variation among the genetic populations of *A. sutherlandi*, and following morphometric measurement of the remaining specimens, Discriminant Analysis will be utilised to test the congruence between the patterns of morphological variation and genetic divergence at Tallaganda.

Acknowledgements

I would like to express my sincere appreciation to The Australasian Wildlife Management Society for not only providing me with support for this research, but also many valuable insights into the management and conservation of Australia's native species.

Mark Wong collecting a funnel-web spider at Tallaganda National Park

Mark Wong examining a large female Atrax sutherlandi in the lab

Literature cited:

Abràmoff, M. D., Magalhães, P. J. & Ram, S. J. (2004). Image Processing with ImageJ. *Biophotonics International*, *11*, 36-42.

Atkinson, W. & Walker, P. (1985). The effects of season of collection, feeding, maturation and gender on the potency of funnel-web spider (*Atrax infensus*) venom. *The Australian Journal of Experimental Biology and Medical Science, 63*(5), 555-561.

Beavis, A. S., Sunnucks, P. & Rowell, D. M. (2011). Phylogeographic disparities in two Australian funnel web spiders. *Biological Journal of the Linnean Society, 104*(4), 805-809.

Garrick, R. C., Rowell, D. M. & Sunnucks, P. (2012). Phylogeography of Saproxylic and Forest Floor Invertebrates from Tallaganda, South-eastern Australia. *Insects, 3*(4), 270-294.

Gray, M. R. (2010). A revision of the Australian funnel-web spiders (Hexathelidae: Atracinae). *Records of the Australian Museum, 62*(3), 285-392.

Harvey, M. (2002). Short-range endemism among the Australian fauna: some examples from non-marine environments. *Invertebrate Systematics, 16*, 555-570.

Hope, G. S., Kershaw, A. P., Van Der Kaar, S., Xiangjun, S., Liew, P. M., Heusser, L. E. … Moss, P.T. (2004). History of vegetation and habitat change in the Austral-Asian region. *Quaternary International 118-119*, 103-126.

King, G. F., Tedford, H. W., & Maggio, F. (2002). Structure and function of insecticidal neurotoxins from Australian funnel-web spiders. *Toxin Reviews, 21*(4), 361-389.

Morgenstern, D. & King, G. F. (2013). The venom optimization hypothesis revisited. *Toxicon, 63*, 120-128.

Nisani, Z., Dunbar, S. G. & Hayes, W. K. (2007). Cost of venom regeneration in *Parabuthus transvaalicus* (Arachnida: Buthidae). *Comparative Biochemistry and Physiology Part A, Molecular & Integrative Physiology 147*(2), 509-513.