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HONEY BEE BEHAVIOURAL EVOLUTION AND *ITPR* GENE STRUCTURE STUDIES

Thesis submitted by

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In March 2002

for the degree of Doctor of Philosophy
in Zoology and Tropical Ecology
within the School of Tropical Biology
James Cook University

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Rika Raffiudin

ABSTRACT

Honey bees (genus *Apis*) display a rich variety of fascinating traits, which can reveal considerable information about their evolution by means of analysis and investigation. Therefore, my main objective is to investigate some of these traits in order to determine food recruitment and nesting behaviour ancestral traits in *Apis*.

In my first experiment, I focused on foraging behaviour performed by the forager honey bee. Its ability to conduct food recruitment through elegant dance behaviour is facilitated by memory formation in the brain. In the first experimental study described in Chapter 2, I conducted an *itpr* gene characterisation, a highly expressed gene present in honey bee brain (Kamikouchi et al. 1998). A partial sequence of the *A. mellifera itpr* gene was obtained which comprised of 2, 091 bp and showed 62%, 60%, 33%, 56%, and 56% similarities respectively to those in *Panulurius argus* (lobster), *Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis*, and *Mus musculus itpr*-1. A phylogenetic analysis using *itpr* demonstrated that *D. melanogaster itpr* is closest to that of *A. mellifera itpr*. It has two introns showing the same positions as those of *D. melanogaster itpr* introns (Sinha and Hasan 1999) and there are four *Apis itpr* introns which appear absent in *D. melanogaster*. In my research, I investigated several conserved putative sites in *A. mellifera* IP$_3$R protein namely protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites. These particular phosphorylation sites are considered to be important in honey bee memory formation (Menzel 2001).

Certain parts of *itpr* characterised in Chapter 2 were used as molecular markers for honey bee molecular phylogenetic reconstruction, concatenated with *COII* and *lsRNA* genes.

In further analysis based on the more complex model of DNA evolution, another hypothesis of *Apis* evolution was revealed. According to this model, the monophyletic *Apis* genus is split into two lines; those are the *A. mellifera* group line and the *A. dorsata* and *A.*

florea groups line. Another outcome based on *COII* molecular phylogenetics combined with previous data, indicated the ambiguity detected in *A. koschevnikovi* by Willis et al. (1992) resulted from a possible error.

A. florea and *A. andreniformis* were confirmed as basal species in *Apis* phylogeny followed by the more derived species: *A. dorsata*, *A. laboriosa*, *A. d. binghamii*, *A. mellifera*, *A. koschevnikovi*, *A. cerana*, *A. nuluensis*, and *A. nigrocincta*. My findings suggest that *A. nuluensis* and *A. nigrocincta* are the most derived species and that they have recently speciated from *A. cerana*.

By mapping dance behaviour characteristics onto the weighted Maximum Likelihood (ML) consensus tree, an interesting result was produced. It was found that unordered trait analysis did not answer the question whether horizontal dancing or vertical dancing was the ancestral trait because species with vertical dance behaviour are monophyletic and so are those with horizontal dance behaviour. However, given that horizontal dancing behaviour is less complex, an ordered dance character state seems justified. Based on these considerations, horizontal dance behaviour seems most likely to be ancestral. Another possibility of dance behaviour evolution hypothesis revealed by applying another DNA evolution model in ML analysis, mentioned that the vertical dancer honey bees (*A. mellifera* and *A. dorsata* group) are not monophyletic; they are clustered in different clade. The latter are in the same clade with the other horizontal dancers, *A. florea* group. Hence, it turns another possibility that vertical dance could be the ancestral to that of horizontal dance.

Inferring honey bee nest behaviour by mapping onto the molecular phylogenetics tree led me to the conclusion that there were two alternative evolutionary histories accounting well for this behaviour. One scenario has the ancestral state for *Apis* being open nesting with cavity nesting being a derived state. In the other, equally parsimonious scenario, cavity nesting in *Apis* is ancestral and apparent derived directly from cavity nesting in the

Bombini and Meliponini, and open-nesting has been evolved twice (or once) in the *A. florea* and *A. dorsata* groups.

Only open nesters that construct platform at the top of their nests perform horizontal dancing. Other open nesters such as the *A. dorsata* group do not construct such platform and so it is possible that their dance behaviour has evolved into a vertical dance. Similar features namely no platform and vertical dance behaviour exists in the *A. mellifera* group. Hence, there is an adaptation of dance behaviour to the nest structure, which shows a correlation between these two behavioural character traits.

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STATEMENT ON SOURCES

DECLARATION

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.

March 27, 2002

Rika Raffiudin