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**Molecular evolution of the immune
related gene transferrin in *Polyrhachis*
ants and distantly related insect taxa**

PhD thesis submitted by
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Statement of contribution of others

All data chapters of this thesis include collaborative work with my supervisors

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General Abstract

Host proteins involved in defence against parasites are expected to evolve rapidly and adaptively due to selective pressure from pathogens. Social insect taxa, which face an increased threat from pathogens due to demographic attributes such as high genetic similarities amongst nestmates and high population densities, differ in ways that allow us to examine the impact that variable pathogen loads have on the evolution of immune system genes. Social insects which nest in subterranean habitats come into contact with a wide range and large number of parasites. In contrast, many social insects nest in arboreal or lithocolous localities and so have less contact with the soil and the microbes within it. There is mounting evidence that social insect species which nest in subterranean habitats are associated with greater levels of parasitism. It was predicted that immunity genes will have evolved at a greater rate, as evidenced by positive selection, in social insect taxa that inhabit subterranean nests, as compared to their arboreal and lithocolous counterparts.

Transferrins are single polypeptide chains that play an important role in iron metabolism and resistance to infection in a variety of organisms. In order to limit the amount of iron microbial fauna receive, transferrin is up-regulated following infection in all insects studied to date. As the ability of a microbe to grow and develop within a host's body depends upon the availability of iron, conflict has arisen between host and parasite for procuring this essential element. For example, evidence of the battle to keep and acquire iron has been detected in salmon transferrins, which are evolving rapidly and adaptively. It has been proposed that transferrins in these fish are evolving in this manner because they occupy many different habitats during their

lifecycle, supporting the notion that the type and number of parasites encountered by an organism can affect the strength of selection on their immune system genes.

Polyrhachis is one of the most species rich ant genera in the world and is very well represented in Australia. Species within this genus exhibit extensive variation in nesting habit, making them a suitable and useful model for examining the evolution of immune genes under different selective regimes. Additionally, *Polyrhachis* ants lack metapleural glands, which secrete substances with antibiotic properties able to kill a wide range of pathogenic organisms. This lack enhances their use in an analysis of immune gene evolution, as without these secretions to help eliminate pathogenic microorganisms, other aspects of their immune system are likely to have experienced stronger selective pressure from pathogens than in the case of many other ants, due to a reduced armoury.

I used different models of evolution, based on the nonsynonymous to synonymous substitution ratio (dN/dS ratio), to compare the evolution of the immune related gene transferrin in 14 *Polyrhachis* species with different nesting habits. The type of selection in this protein across lineages ranged from strong purifying selection to positive selection, demonstrating that certain species of *Polyrhachis* have experienced different selective pressure to change the amino acid composition of their transferrin. Three lineages (with consensus between models) have a dN/dS ratio greater than one, indicative of positive selection. Of these three species, two nest in subterranean and one in arboreal localities. However, while predominantly arboreal, the third species is known to also nest in the ground. I suggest that the increased level of evolution in

certain lineages was brought about by variable loads and types of pathogens encountered by *Polyrhachis* ants as they radiated.

Transferrins of five species with subterranean nesting habits have a dN/dS ratio < 1 , indicative of purifying selection. It may be that in these species other factors are more important than nesting habit in influencing parasite loads. Overall, predictions as to the parasite load a given species is likely to encounter are sound, however it seems that these predictions need to be inclusive of other factors, as many features are likely to influence parasite exposure.

As well as models designed to detect variable selective intensity across lineages, models that assign a dN/dS ratio to each site were used. Three individual sites were assigned a dN/dS ratio greater than one using consensus among models. These sites are located in regions that are likely to be bound by bacterial iron binding proteins, as they align with regions in vertebrate transferrins known to be so and that have also been subject to adaptive evolution. The occurrence of this type of evolution in equivalent sites and regions in such phylogenetically distant organisms suggests that these sites are important targets for microorganisms that seek to acquire iron from host transferrin.

Using transferrins of distantly related insect taxa, including three hymenopteran genera, I tested for positive selection across lineages and at individual sites. Positive selection was not detected at any site or in any lineage with consensus across models. Rather, the molecule appears to be under strong and significant purifying selection,

suggesting that the molecule is under selective pressure not to change its amino acid composition.

In this analysis of distantly related taxa I included the transferrin of a species of *Polyrhachis* that had a dN/dS ratio greater than one in the analysis *Polyrhachis* transferrins. I propose an explanation as to why positive selection was detected in transferrin of this species in one analysis and not another. Over time synonymous changes accumulate and can mask high rates of nonsynonymous change when it has occurred, as any detrimental nonsynonymous change is expected to be rapidly eliminated from the population. The insects used in this analysis are distantly related, therefore even if certain lineages have experienced selective pressure to change (and we know that at least one has), looking for evidence of this at this scale is likely to miss it, unless selection has been very strong and/or constant. As such, I suggest that where possible it is advisable to search for adaptive evolution in genes within shorter evolutionary time scales, such as in the analysis of *Polyrhachis* transferrins.

Most transferrins consist of two homologous lobes (the N and C lobes) and iron binding in each lobes involves six amino acid residues. With the exceptions of a termite and cockroach, insect transferrins studied to date are not generally conserved for binding motifs in their C termini, thus the capacity to bind iron in this region appears to have been lost. The degeneration of this region is thought to be due to antagonistic interactions between host transferrin and iron scavenging proteins of pathogenic bacteria. It is noteworthy that the positively selected sites in transferrin of *Polyrhachis* ants are located in the N -terminal, which is expected, if the C- terminal of transferrin in these insects is unable to bind iron. Based on alignments of

transferrins from distantly related animals (mammals to insects) doubt has been expressed as to as to whether most insect transferrins can bind iron at all {Lambert, 2005 #757}. My alignment differs to that presented by Lambert et al. {Lambert, 2005 #757}, and I conclude that there is no basis for suggesting that insect transferrins are unable to bind iron in the N-terminal.

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