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Systematics of the liverwort genus *Telaranea*: can nucleotide sequences resolve species relationships in this morphological complex?

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Species limits and relationships in the *Telaranea centipes* species complex (Sect. *Ceraceae*) were investigated using molecular phylogenetic methods and morphological phenetics. Analysis of chloroplast nucleotide sequence data indicated that *Telaranea* as currently circumscribed is polyphyletic. *Telaranea* Sect. *Ceraceae*, to which the *T. centipes* complex belongs, is shown to be sister to a clade comprising *Lepidozia* and the remaining species of *Telaranea*. It is proposed that *Telaranea* Sect. *Ceraceae* be elevated to generic status. Analysis of nuclear ribosomal nucleotide sequence data supported the monophyly of *Telaranea* Sect. *Ceraceae*, and further resolved species relationships within it. Two New Zealand species, *T. tuberifera* and *T. perfragilis*, were distinct from each other and from all other Australian taxa. Australian representatives of *T. elegans* were not monophyletic, and could not be separated phenetically from Australian representatives of *T. centipes*. However, according to the analysis of nr nucleotide sequence data, two populations of Australian *Telaranea* morphologically conspecific with *T. elegans* were sister to the remainder of *Telaranea* Sect. *Ceraceae*. These populations might represent syntopic cryptic species, a situation not yet recorded for Marchantiophyta.

Habitat Restoration for an Endangered Snake

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Habitat degradation due to anthropogenic activities threatens the persistence of many species. One such species is the Sydney Broad-Headed snake (*Hoplocephalus bungaroides*). Restricted to south-eastern Australia, this snake spends winter beneath sun-exposed sandstone exfoliations, which provide the warmest microhabitats at this time of year. Such rocks are frequently removed by bush-rock thieves, resulting in the degradation of these habitats not only for the snakes but also their main prey species the Velvet gecko (*Oedura lesueurii*) but also a variety of invertebrates and other reptiles that rely on these thermally distinctive shelters. Is habitat restoration a feasible option to restore these degraded habitats?

Previous research on this system has shown that characteristics of the exfoliated rocks that overly crevices (e.g., rock dimensions, exposure to solar radiation) influence thermal regimes, and thus the crevice's suitability for reptiles. To date, however, characteristics of the crevice itself have not been quantified. I hypothesised that details of the three-dimensional crevice space (e.g., width, number of openings) also affect reptile utilisation. Thus, I measured three-dimensional characteristics of crevices known to have been utilised by Broad-headed snakes and Velvet geckos, compared to nearby rocks not utilised by these species. The crevices used by reptiles differed in several respects from the randomly-selected unused rocks, suggesting that three-dimensional crevice structure does indeed influence reptile utilisation.

I then constructed 200 artificial rocks that matched the crevice characteristics identified from the above investigations, and deployed these rocks on outcrops. Rapid colonisation of the crevices beneath these rocks by invertebrates and lizards is encouraging for the feasibility of restoring degraded habitats for this endangered snake.

Partitioning systems of staphylococcal multiresistance plasmids

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Staphylococcus aureus is a major cause of hospital-acquired infections. Treatment and control of *S. aureus* is often confounded by plasmids, which encode resistance genes for an extensive range of antimicrobials. Staphylococcal plasmids such as pSK1 confer multiple resistances. Partitioning systems maintain plasmids in populations, allowing resistances to persist even in the absence of selection. pSK1 and related plasmids encode a novel partitioning system that comprises only a single protein-encoding gene, designated *par*. The aim of this project was to identify the functional domains of the Par protein.

A putative N-terminal helix-turn-helix (HTH) domain and central coiled-coil domain were detected by sequence analysis. Conserved residues within these putative domains were chosen for alanine-scanning mutagenesis studies, and the entire putative coiled-coil domain was eliminated by loop-out deletion for additional analysis. As expected, electrophoretic mobility shift assays revealed that a single amino acid substitution in the HTH domain abolished DNA-binding ability. They also revealed that Par binds its DNA-binding site with cooperativity, and that cooperativity was lost when mutations disrupted the coiled-coil domain. Plasmid segregational stability assays were conducted to assess the effect of the mutations on Par-mediated enhancement of plasmid segregational stability. Mutations that disrupt the coiled-coil domain resulted in a loss of this enhancement, confirming that this domain is essential.

The findings of this project will assist the development of new strategies to disrupt the carriage and spread of resistance by targeting plasmid maintenance.

Habitat use of mammals in remnant vegetation; a multi-species and multi-spatial scaled approach

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Urbanisation is the process of converting natural ecosystems into human dominated landscapes, resulting in the reduction and fragmentation of native vegetation over a landscape mosaic in which the structure and composition of the remaining vegetation is progressively altered. In Australia, native mammals are considered the fauna group most detrimentally affected by habitat changes resulting from urbanisation. For native mammals living in urban environments, identification of preferred habitats is crucial given the rate of urban expansion and the constant threat of habitat modification by humans. This includes acquiring knowledge of their essential resources at multiple spatial scales, and determining how these resources are partitioned among and within species.

Within remnant vegetation of Garigal and Ku-ring-gai Chase National Parks, the study examined the habitat use of multiple species of mammals at three spatial scales; landscape (100s-1000s ha), site (defined as broad vegetation type, < 1 ha) and microhabitat (1-10s of metres). Capture-mark-recapture data indicated that distribution and abundance of native mammal species did not significantly correlate to land uses in the landscape. However, abundances of the exotic *Rattus rattus* (Black Rat) increased with increasing coverage of residential land and decreasing coverage of remnant vegetation in the landscape. Distribution and abundances of all mammal species and population dynamics, i.e. biomass, sex ratio and survival rate, of *Antechinus stuartii* (Brown Antechinus) did not differ significantly among broad vegetation types (Scribbly Gum-Red Bloodwood Woodland, Heath, Angophora-Peppermint Open Forest and Silvertop Ash-Brown Stringybark Open Forest). Whereas, the survival rate of *R. fuscipes* (Bush Rat) was significantly lower within Scribbly-Red Bloodwood Woodland. Spool and line tracking revealed that *A. stuartii*, *Perameles nasuta* (Long-nosed Bandicoot) and *R. fuscipes* used specific microhabitat components within Heath and Ash-Brown Stringybark Open Forest. Primarily, structural microhabitat components that provided cover were utilized, such as high abundances of vertical stems. Microhabitat use of *A. stuartii*, *P. nasuta* and *R. fuscipes* in Heath, revealed partitioning of resources among species. *A. stuartii* foraged arboreally and on the ground, along logs, within deep ground litter and among flowering banksia trees, *P. nasuta* foraged solely on the ground, within ferns, in areas of high flower coverage and bare ground and *R. fuscipes* foraged on the ground and occasionally in trees, preferring habitat with a high coverage of shrubs and high abundances of rock crevices. The results of this study indicate that habitat use of native mammals was governed by processes occurring at site (broad vegetation type) and microhabitat scale. It appears that quality of habitat rather than quantity of habitat was of greater importance to native mammals. Lower survival rates of *R. fuscipes* in Scribbly-Red Bloodwood Woodland were most likely attributed to poor vegetation cover and an associated higher risk of predation. These results demonstrate the importance of targeting conservation efforts at site and microhabitat level for these species. In particular, essential microhabitat components should be maintained within their habitats.

A Novel Phylogenetic Tool with Implications for Understanding the Evolution of the Angiosperms

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Phylogenetic approaches that use nucleotide substitution models, such as Maximum likelihood and Bayesian inference, are gaining in popularity. Unknown to, or ignored by, some investigators, these models make a number of assumptions about the underlying evolutionary processes that led to the sequences being analysed. One of these assumptions is that all sites evolved at the same rate of change, though this assumption can be unrealistic. Violations of this or any of the assumptions may confound phylogenetic reconstruction.

It is, therefore, imperative that a survey of the aligned sequences is conducted, prior to inference, to assess the suitability of the various phylogenetic methods available. A novel phylogenetics tool called AlignVis was developed to detect violations of site-specific rate uniformity. AlignVis produces a dynamic visualisation of the site-specific rates from aligned sequences and, unlike some of the other survey tools, does not require a predefined phylogenetic hypothesis.

AlignVis was used to explore a contentious area of evolutionary biology: the evolution of the angiosperms. This was once described by Darwin as an “abominable mystery”, but recent molecular studies have elucidated many clues to solve this mystery. The current prominent theories place either *Amborella trichopoda* or the monocots as the most basal branch. This study aims to find the best-fitting evolutionary theory that led to the extant angiosperms.

Protein-coding genes from the chloroplast genome of 64 land plants were collected and analysed. The alignment survey suggests that these genes evolved under conditions more complex than those accommodated by many substitution models. Consequently, conclusions drawn from previous studies which did not incorporate these aspects of the evolutionary process are equivocal. Bayesian inference under more appropriate substitution models and parsimony analysis recovered a strongly supported topology congruent with the notion that *Amborella* is sister to the rest of the angiosperms.

Functional analysis of the replication initiator protein of the *Staphylococcus aureus* multiresistance plasmid pSK41

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Strains of the Gram-positive bacterium *Staphylococcus aureus* that are resistant to a large number of antimicrobial agents have become widespread in hospitals throughout the world in the past few decades. These multiresistance strains are responsible for a significant level of morbidity and mortality in immunocompromised hospital patients. A large number of the genetic determinants responsible for the wide-ranging antimicrobial resistance of these strains are carried on plasmids – extrachromosomal genetic elements which can be readily spread throughout bacterial populations by horizontal transfer. One such plasmid, the conjugative multiresistance plasmid known as pSK41, is the focus of this project. Replication of pSK41 is initiated by the actions of the cognate initiator protein, Rep, which binds to four directly repeated sequences (Rep boxes) located within the origin of replication (*oriV*). The aim of this study was to identify the region of the pSK41 Rep protein responsible for interactions with the Rep boxes.

Seven amino acid residues on the Rep protein, which were highly-conserved among a wide range of bacterial genera, were subjected to site-directed mutagenesis. Analysis of the replication proficiency of these mutants *in vivo* demonstrated that five of the residues were essential for plasmid replication. All seven mutant Rep proteins were overexpressed in *Escherichia coli* and purified by affinity chromatography. The DNA-binding capabilities of these proteins were analysed by electrophoretic shift mobility assays, revealing that three of the mutant Rep proteins were deficient in DNA-binding. Two of the corresponding amino residues were located on the C-terminal half of the Rep protein, whereas the other residue was located on the N-terminal half. These residues were concluded to be involved in the binding of the pSK41 protein to the Rep boxes.

The evolutionary of viviparity in Australian skinks: A molecular analysis

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I aimed to:

- 1) Quantify mRNA expression of hypoxia inducible factor 1 alpha (HIF-1 α) and vascular endothelial growth factor (VEGF) in the uterus of oviparous and viviparous skinks, and
- 2) Identify and describe non-classical major histocompatibility complex (MHC) class I genes in a viviparous skink.

Uterine epithelium vascularises as gestation progresses, and the uterus of viviparous species tends to be more vascularised than in oviparous species. Embryonic oxygen demand increases during development, resulting in hypoxic conditions inside the uterus that trigger blood vessel proliferation. Regulation of two genes, hypoxia inducible factor 1 alpha (HIF-1 α) and vascular endothelial growth factor (VEGF), are responsible for hypoxia-induced vascularisation in mammalian uterus. I have demonstrated mRNA expression of HIF-1 α and VEGF in the uterus of oviparous and viviparous skinks during both non-reproductive and late gravid/pregnant stages. No change in uterine VEGF expression was detected between non-reproductive and late gravid/pregnant females in either oviparous or viviparous species.

In the absence of an eggshell, embryos of viviparous skinks are in close apposition with maternal tissues. This apposition is enough to allow maternal recognition of pregnancy, as cytokines occur at the materno-fetal interface of viviparous skinks. Once recognised by the maternal immune system, the embryo is in danger of immune rejection, as it presents surface antigens that are different from those on maternal tissues. Non-classical MHC class I genes are expressed by mammalian embryos, 'hiding' the embryo from immunological attack by killer T-cells. I have cloned class I genes expressed in the uterus of a viviparous skink and described two loci with non-classical features. The next step will be to compare non-classical gene expression in oviparous and viviparous embryos to investigate whether the evolution of immunological interactions between mother and embryo is a major step in the transition to viviparity.

***Asparagus aethiopicus* in urban fragments: effects on ant and beetle communities and myrmecochory**

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Asparagus aethiopicus, or “asparagus fern” (family: Liliaceae) has become established in many fragments of native habitat in the Sydney region. This species drastically alters microhabitat characteristics and can create an “invasional meltdown”, at which point a habitat becomes unsuitable for many native species. Asparagus fern has the potential to adversely affect ground invertebrate assemblages and as a consequence of a disrupted ant community may disrupt myrmecochory in infested sites.

I aim to investigate the effects of asparagus fern on the species diversity, abundance and structure of ant and beetle assemblages in fragments of dry sclerophyll sandstone forest at ten sites in the Sydney Harbour region. I also aim to investigate the effect asparagus fern has on seed removal within these sites.

Ant and beetle assemblages were surveyed using pitfall traps at five sites in which a high proportion of the ground layer was dominated by the weed and five sites in which asparagus fern was not dominant in the ground layer. Individuals were identified to morphospecies and then characterized into functional groups based on each species’ ecological role, habitat requirements and competitive abilities. Carbohydrate and protein bait studies were used to determine whether asparagus fern altered the competitive interactions and behavioural dominance relationships in ant communities.

The disruption to seed removal by the presence of asparagus fern was studied at six sites. Five seed species representing a range of sizes and masses (*Bossiaea obcordata*, *Eriostemon australasius*, *Dillwynia retorta*, *Acacia longifolia* and *Acacia suaveolens*) were used to determine whether the disruption to seed removal effects which seeds were taken. A pilot study was performed to determine the appropriate number of seeds to be used in each depot.

My results will be discussed in the context of how structural measures of diversity can be used to predict the functional consequences of weed invasion in urban fragments.

Regulation of myoglobin expression as a mechanism for hypoxia tolerance in the Mozambique Mouth-brooder (*Oreochromis mossambicus*)

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Animals are dependent on a sufficient supply of oxygen to produce energy and maintain body function. Due to the low oxygen capacity and diffusibility of water, aqueous environments are especially prone to low oxygen levels, or hypoxia. As a result, many aquatic animals, such as fish, have developed physiological mechanisms to survive wide fluctuations in dissolved oxygen content. One possible hypoxia tolerance mechanism is the up-regulation of myoglobin expression. Myoglobin is a respiratory pigment in striated muscle that acts as an oxygen storage molecule and also facilitates oxygen diffusion.

The aim of this project was to determine if regulation of myoglobin expression occurred as a response to low oxygen levels in *O. mossambicus*, a hypoxia tolerant invasive fish. Fish were acclimated to three different regimes: environmental hypoxia, exercise induced hypoxia, and normoxia control (normal dissolved oxygen concentration). Critical sustained swimming speed (U_{crit}), metabolic scope (recovery/resting $\dot{V}O_2$), myoglobin protein concentration, and myoglobin gene expression were measured in response to acclimation.

A significant effect of hypoxia acclimation was displayed in U_{crit} , but not in metabolic scope. Also, myoglobin protein concentration was significantly higher in the hypoxia treatments than in the control. However, myoglobin gene expression was not significantly different between the three treatments. The difference in swimming performance shows that tolerance of hypoxia in *O. mossambicus* does involve a physiological mechanism, and elevated myoglobin protein levels are related to this. Interestingly, the discrepancy between the myoglobin protein and gene expression suggests that a post-transcriptional control might be the source of myoglobin expression regulation in response to hypoxia.