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Title: 'Avian migration and movement of pathogens in the Australo-Papuan context: Developing novel methods for accurate assessment of relevant connectivity between populations.'

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Summary: This project has been successful in terms of field collection of samples and identification of infectious organisms in the target host species, the Pied Imperial-pigeon. A limitation of funding has meant that the selection of field sites has had to be reduced and that sampling in Papua New Guinea has been postponed until late 2011. Despite this, many of the key milestones in this project have been fulfilled.

Objectives, Key Performance Indicators & Milestones:

The collection of appropriate samples from a model migratory bird species at nine sites representing a range of biogeographical and ecological situations.

At least twenty individuals to be sampled at each of nine planned sites.

By the end of 2010, Pied Imperial-pigeons had been trapped and sampled at the following sites: Low Isles, QLD (n=130); Iron Range, QLD (n=80); Calvert River, NT (n=4); Darwin, NT (n=4). The third site in QLD, near Mackay, had to be abandoned due to cyclone damage in 2010. The development of methods to trap birds with minimal disturbance at offshore colony breeding sites and in arboreal feeding sites (>10m high) has been a significant achievement of the project and a description of this methodology is being drafted for publication. Further field sites have been identified for future sampling (late 2011) at Double Cone Island, QLD, and the Bonaparte archipelago, WA.

The examination of samples from all studied populations to identify target organisms.

Identification and epidemiological description of at least two of six potential infectious organisms from hosts at each of five, and then all sampled sites;

Infectious organisms have been identified in samples from Pied Imperial-pigeons collected at four sites across QLD and NT. These include a previously undescribed species of *Trichomonas* that exists at a high prevalence; three species of haemosporidians which have not been described before, including

Haemoproteus and *Parahaemoproteus* isolates; a hippoboscid Dipteran (which has not been identified yet); and a new species of *Staphylococcus*, for which prevalence is being determined. Further work is being done to describe these species and to investigate their prevalence, however the identification of four groups of infectious organisms represents significant progress ahead of the milestones described.

The finding of a related trichomonad in Pied imperial-pigeons and two other species of Australian Columbiformes (the White-quilled and Chestnut-quilled Rock-pigeons) raises interesting questions about the previous transmission of this organism between its migratory host and resident hosts along its flyway. Further samples are needed from diverse species of pigeons and doves in New Guinea before the significance of this is realised. Detailed genomic investigation into the Pied Imperial-pigeon trichomonad is also likely to shed light on previous host-switch events that may be relevant to analyses of the risk of such events during incursions of novel pathogens into the region through wildlife.

The high-resolution genotyping of multiple organisms within the host species.

Identification of the most appropriate technique for high-resolution genotyping of at least one identified organism and implementation of this technique in the analysis of organisms originating at each of five, and then all nine sampled sites.

Progress towards this milestone is being made, with the development of microsatellite libraries for the organism *Trichomonas* and for the host species, the Pied Imperial-pigeon. This represents the first time the population structure of a protozoan and its host will be compared at such a fine scale. The laborious task of selecting loci with sufficient allelic diversity is being undertaken and will be followed by a comparison of host and parasite across the four sites sampled to date, prior to the end of 2011.

Statistical analysis and interpretation of population within host and infectious organisms.

Production of detailed phylogenetic dendrograms and identification of clades with strong likelihoods to represent populations of both host and at least one infectious organism across the sampled sites, and interpretation of these to determine populations involved in horizontal transmission of infectious organisms.

Progress towards this key performance indicator is time-dependent on accomplishment of microsatellite analysis of *Trichomonas* and its host (as described above). Training in the use of phylogenetic inference software has occurred in the last twelve months, and the software PHYLIP, CLUSTALX and SEQUENCHER are already being used to investigate the evolution of this newly discovered trichomonad species in the Pied Imperial-pigeon.

Publication of findings with recommendations for this technique’s practical future implementation.

Submission for publication in leading scientific journals of relevant findings.

Papers detailing the methodology used in the capture of the Pied Imperial-pigeon and describing the new species of *Trichomonas* identified in this migrant bird are being prepared. Two papers, "A Lightweight, Adjustable Pole System for the Mist Netting of Birds in the Low and Mid Canopy" and "Catching the Pigeons and Doves of Australia" are being submitted to the Journal of Field Ornithology and Emu: Austral Ornithology respectively. A paper describing the new trichomonad, "*Trichomonas thorsborneae*, a novel trichomonad in the Pied Imperial-pigeon (*Ducula bicolour*)", is in draft stage currently. A technical paper is being prepared on the use of real-time PCR and high resolution melt analysis to detect, identify to species level and quantitate abundance of trichomonads in birds. This technique offers a much more useful tool to researchers investigating factors associated with increased parasitism in wildlife than traditional 'presence or absence of infection' prevalence data. A review of the possible role of Pied Imperial-pigeons in the expansion and contraction of rain forest in northern Australia in response to Holocene climatic events is also in draft. Further publications will be prepared as the results of the above objectives come to fruition over the remainder of 2011.

Conclusion: The milestones described in the project proposal have been met or are being worked towards in a timely manner. The methodology that has been developed in this project represents significant innovation in the study of infectious disease in migratory birds in the Australasian region. The initial findings suggest the successful development of a powerful tool for the modelling of infectious organism movement along migratory fly-ways.