The population and epidemiological dynamics associated with recent decline of woylies (*Bettongia penicillata*) in Australia.

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Photo: Sabrina Trocini

To my wife and friend,

Sabrina

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

…….

Carlo Pacioni

Preface

Chapters 5, 6, 7 and 8 are either published papers or manuscripts intended for publication in scientific journals as stand-alone pieces of work. Consequently, some repetition was unavoidable. In addition, some differences in style are due to the requirements of the targeted journal. The reference style of the remaining chapters follows the current guidelines for the journal of *Conservation Biology*.

The intellectual development and writing of this thesis was carried out by the author. Inclusion of co-authors in the papers is to acknowledge the contributions of collaborators who provided tissue samples, demographic data, preliminary analysis and/or background information, as well as helpful discussions and editorial comments.

Abstract

The woylie or brush-tailed bettong (*Bettongia penicillata ogilbyi*) has recently undergone a dramatic decline (approximately 80% between 2001 and 2006). The Woylie Conservation and Research Project (WCRP) was established to investigate possible causes of this decline. It was hypothesised that predators and/or a disease may be a concomitant cause if not the primary cause(s) of the decline, based on the peculiar temporal and spatial characteristics of the decline and available associative evidence.

This research project is an integrated and collaborative component of the WCRP and its broad aim was to contribute to the knowledge on the general health and ecological attributes of woylie populations that were considered directly relevant for the conservation and recovery of the species.

Initially, the WCRP in collaboration with several researchers supported the investigation of specific pathogens. These projects were ongoing when the research described in this thesis began, however there had been no disease risk assessment prior to these ongoing pathogen studies. Therefore, a formal qualitative assessment of the disease risks potentially relevant to the woylie declines was undertaken in this study to ensure a systematic evaluation and to prioritise allocation of resources. Several pathogens were identified as a high priority for further investigation including, but not limited to, Macropod Herpesvirus (MaHV), Macropod Orbivirus (Wallal and Warrego serogroups), and Encephalomyocarditis virus (EMCV).

A haematological investigation was carried out and reference ranges were established. An overall increase of the leukocytic response in animals trapped in Upper Warren (8%, n=23)

compared to woylies in Karakamia (0%) was demonstrated. Gender differences were also recorded, namely males had higher red blood cell, white blood cell and lymphocyte counts than females.

No clear evidence was found that supported an association between changes in the health status of woylies and the decline. Nevertheless, the increased proportion of lymphocytosis ($p<0.0005$) in Perup, which includes two forest blocks that underwent a decline during sample collection, and the higher prevalence of health problems identified during the physical examinations of animals trapped in Upper Warren (41.3%, n=557) as compared to those from Karakamia (10%, n=80, $p<0.0005$. Odds Ratio=6.33, 95% CI 2.99-13.40), justified further disease investigations.

Based on the results of the disease risk assessment and haematological analysis, the serological response to Macropod Herpesvirus (MaHV 1 and 2), Encephalomyocarditis virus (EMCV) and Orbivirus (Wallal and Warrego serogroups) was investigated. There was no serological evidence of any of these viruses affecting woylie populations. Nevertheless, due to sample size limitations, it was not possible to confirm the absence of these diseases with a high level of confidence (i.e. >90%). Additionally, the absence of detection of seropositive individuals does not necessarily imply absence of the pathogen in the population.

Genetic profiles of indigenous (extant wild populations) and translocated woylie populations were examined in order to assess whether woylie populations were suffering from a reduced genetic “health”, as a consequence of the bottleneck that occurred after European settlement. In order to do this a preliminary investigation of the cross-species performance of 32 primer pairs was carried out to assess their suitability for the aims of this study. Twelve microsatellite primer sets were identified as polymorphic and reliable for genetic analysis in woylie.

Additionally, the cross-species performance of the 32 primer pairs was analysed within the potoroines species to facilitate future ecological and genetic studies in bettongs and potoroos. A 50% reduction in amplification success of polymorphic loci for every 1 million years of evolutionary distance from taxa was found and a "priority-list" of markers for use in potoroines was identified.

Genetics does not appear to be a contributing factor to the present woylie decline. Expected heterozygosity (H_E) was around 80%, ranging from 42.3% to 83.6% and the allelic richness (N_{AR}) was around 6, ranging from 2.67 to 9.72. Nevertheless, among the indigenous populations particular concern was raised for woylies at Tutanning Nature Reserve, and for the translocated populations on the South Australian islands. These populations have a substantially reduced genetic diversity (Tutanning: H_E = 0.64; St Peter island: H_E = 0.631; Wedge island: H_E = 0.602; Venus Bay island "A": H_E = 0.423).

Important insights were gained into woylie population structure and dynamics through the analysis of molecular data. Four genetically distinct indigenous populations were identified (i.e. Dryandra woodland and Tutanning Nature Reserve in the wheatbelt region and two discrete populations in the Upper Warren in the south-west forests of Western Australia). The mtDNA analysis showed historical connections between populations in Dryandra and the Upper Warren region (Kingston and Perup). These connections no longer exist as a result of habitat fragmentation caused by agriculture and farming land use. Additionally, substantial gene flow was identified between Kingston and Perup and was supported and quantified by microsatellite analyses in the order of 2-3% migration rate. The evidence of current gene flow within and between populations (i.e. up to 60 km) signifies that direct transmission of an aetiological agent would be possible throughout the whole Upper Warren region within the time frame experienced in the decline.

Analysis of genetic data indicated also that the woylie population in Kingston had already undergone a decline. As a consequence of this change in population abundance, the spatial genetic structure of this population changed, generating a significant correlogram up to 6 km. In other words, in this population, two woylies trapped within a radius of 6 km are likely to be related as opposed to other populations where the genetic signal drops between 1 and 3 km. Additionally, and consistent with previous ecological studies, female philopatry was confirmed and genetic consequences of this behaviour were identified.

Population viability analysis (PVA) demonstrated that the main threatening process for woylie populations is the result of the interaction of various variables (in particular predation and inbreeding) that acquire a considerable strength together, whilst not being greatly significant by themselves. It also quantified the minimum mortality rates necessary for the decline to occur (an average juvenile and subadult mortality rate of 28% and 22% for adults per 91 day time period). The minimum viable population size (MVP) estimated through PVA was consistent with the empirical evaluation based on molecular data (i.e. 1,000-2,000 individuals). As a consequence of the inherent inability of satisfactorily predicting stochastic events and incomplete knowledge on important factors that may affect population size a conservative approach should be adopted. On this basis, a population size of more than 8,000 individuals should be targeted to maximise the likelihood of positive conservation outcomes.

In light of the results of this research project, disease can not be completely dismissed as a possible cause of decline, in particular in association with predation. Haematological, serological and genetic information generated by this study greatly improved the available knowledge on the health and viability of woylie populations and represent baseline data that

will enable monitoring and detection of changes in the health status in these populations, as well as contribute to the refinement of the disease risk assessment and quarantine protocols.

The haematological data will also facilitate and improve the interpretation of disease investigations carried out by the additional collaborative components of the WCRP. Moreover, information obtained on woylie ecology through the analysis of genetic molecular data will assist such interpretations, for example by conveying indications on the frequency and extent of animal movements.

This research also provided suggestions for critical management decisions. For example, the identification of woylie populations at risk of substantial loss of genetic diversity and possibly inbreeding depression calls for appropriate management actions. Where there is no indication of any factor limiting the demographic growth of the populations (i.e. populations on South Australian islands) supplementation was identified as the most suitable management option. Based on the detailed knowledge obtained on the spatial organization of woylie populations, it is now possible to adequately source animals from indigenous populations to augment genetic diversity. Animals should be trapped at a distance of at least 1-3 km in order to maximise the probability that individuals are unrelated. On the other hand, it is critical to identify the causes currently limiting population growth prior to the implementation of these management actions, especially where limited population size (in respect to the carrying capacity) and consequent genetic drift is the main reason for the poor genetic profile of the population rather than isolation.

The PVA also helped to determine critical requirements for the establishment of new, and maintenance of, populations; more specifically that sites should be able to support a minimum

population size of 8,000 individuals and that the average mortality rate should be maintained below 22% for juveniles and 28% for adults per 91 day period.

Finally, this research helped to identify important future areas of investigation. These include longitudinal studies of the health status of individual woylies; epidemiological analysis of the data generated by this study integrated with those generated by other WCRP researchers and a quantification of the influence of ecological factors, such as rainfall and diet, on general health parameters. Additionally, regular genetic monitoring is recommended because the baseline data produced in this study and the associated ecological and demographic data available would provide an optimal opportunity to improve our understanding of genetic consequences of rapid population declines. This monitoring may help to quantify the genetic loss associated with the decline and evaluate the accuracy of PVA predictions. It might be possible to assess the success of management actions (e.g. supplementations) and detect if and when inbreeding depression becomes manifest in populations at lower genetic diversity (e.g. Tutanning).

In addition, the molecular genetic data represents the background work needed to establish the interplay between individual (host) genetic profile and disease susceptibility or fitness (including fecundity and survival). The fact that cross-species primers were used would make the utility of the knowledge acquired easily and directly applicable to other species of the superfamily Macropodoidae.

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Abbreviations

BBN	Balban
BCP	Boycup
CBL	Corbal
CHCM	Corpuscular haemoglobin concentration mean
CHP	Chariup
CMR	Camelar
DEC	Department of Environment and Conservation
EMCV	Encephalomyocarditis virus
FBG	Fibrinogen
HDW	Haemoglobin distribution width
HGB	Haemoglobin concentration
KAR	Karakamia
KING	Kingston
KNP	Keninup
MaHV	macropod herpes virus
MCH	Mean corpuscular haemoglobin
MCV	Mean cell volume
MPN	Moopinup
PCV	Packed cell volume
PER	Perup
PVA	population viability analysis
RBC	Red blood cells
RDW	Red cell distribution width

r_{mt}	Mantel test correlation coefficient
r_{SAc}	Spatial autocorrelation correlation coefficient
SA_C	Spatial autocorrelation
TP	Total protein
VNT	Virus neutralization test
VNTR	Variable Number Tandem Repeat
WBC	Total white blood cells
WCRP	Woylie Conservation Research Project
WDRC	Woylie Disease Reference Council
WJP	Winnejup
WRP	Warrup