

EXECUTIVE SUMMARY

Project Title: Phylogeography and genetic variation in the Common Wombat

The Common Wombat is an important ecosystem engineer of South-Eastern Australia's mesic environments, yet it is vastly understudied. Contemporary distributions are only vaguely known (with increasing reports of local population declines/extinctions) and there is no information on the genetic structuring and diversity of populations, which includes three recognised subspecies. Based on the fossil record, paleoclimatic data and current distributions, it appears that common wombat diversification and population structuring has been vastly influenced by past geographical/meteorological events. It is also apparent that contemporary populations are in decline across their range by work of factors other than climatic influences. It is imperative that areas which represent evolutionary significance/and or distinctiveness within the species are identified and conserved in order to counter possible significant losses of diversity in the species, as well as in other species which may share a similar biogeographic history.

This project aims to assess the biogeographic structuring of genetic diversity within this ecologically important and highly evolutionarily distinct marsupial, and to identify how key population processes have altered over time in relation to past climatic changes. This effort will provide an understanding not only of how species diversification has taken place in South-Eastern Australia's mesic environments (which is hugely understudied), but will also allow inferences of how population processes may alter in the face of future climatic change. Through determining whether geographic patterns of population-genetic diversity are driven by the history of habitats or by factors unique to the species biology, conservation strategies can be devised to counter loss of genetic diversity and biodiversity.

Project duration

July'08- July'11

Student's enrollment classification

Ph.D. student (first year).

Co-supervised by Professor Steven Harris of Bristol University and Dr Andrea Taylor at Monash University.

Detailed Project Description

Title: Phylogeography and genetic variation in the Common Wombat

Background

Common wombats are important in maintaining ecosystem productivity, as native grazers and ecosystem engineers (Jones et al 1997); they increase soil turnover thus impacting soil nutrition (Kinlaw 1999), as well as being an enigmatic symbol of the unique fauna of Australia. However, despite their functional importance and conservation value, Common Wombats remain largely unstudied across their range in South Eastern Australia. In contrast to many well-studied mammal species, they exhibit female-biased dispersal (the processes of which between populations is not yet fully understood), and may exhibit unique patterns of population-genetic differentiation over small and large scales. Thus conservation strategies based on well-studied co-distributed mammals may be insufficient for the maintenance of genetic diversity within this species.

A primary goal of conservation biology is to ensure the maintenance of biodiversity (Stiling, 1999), of which genetic diversity is a fundamental component (Moritz & Faith, 1998), and effective conservation programs depend on the identification of unambiguous management units that represent evolutionarily distinct lineages (Avice, 1994). However, much more information about Australian mesic (wet environment) mammal population dynamics and species distributions is required, given that basic conservation questions such as how often, how large, and how far apart species habitat patches should be, remain largely unanswered (Thomas & Morris, 1994). Through determining whether the mesic habitat induces consistent and predictable outcomes in population structure of mammals, management programs can be directed towards the most effective means of conserving biodiversity.

Phylogeography (the investigation of processes influencing geographic distributions of genealogical lineages within species or between *closely related* ones) provides a means of (i) distinguishing between habitat-specific and species-specific processes, and (ii) determining the relative influence of extrinsic and intrinsic processes upon the present-day distribution and abundance of genetic diversity (Avice, 2000). Ultimately, this approach yields information about both individual species and regional biota that is of critical importance to conservation, and provides a framework for prioritizing geographic regions in need of protection (Kuchta & Meyer, 2001).

The Project

By studying many populations over the entire range (Fig 1), this project will aim to identify the factors that influence the spatial distribution of genetic variation within the Common Wombat. The fundamental question to this study is whether the geographic patterns of specific and sub-specific differentiation are driven by the history of habitats or by factors characteristic to mammalian species. This question carries considerable importance in both evolutionary biology and in designing biodiversity conservation protocols. Using a phylogeographic approach I will identify the relative influence of habitat characteristics (e.g. physical landscape features and historic climatic changes) and species biology (e.g. dispersal processes) upon the population-genetic structure of this ecologically and evolutionarily important marsupial species. This novel research is directly related to gaining a deeper understanding of how past geological/meteorological events have influenced

diversification in South-Eastern Australia (which remains largely under-studied, especially in mammals), as well as providing the first investigation into the identification of locations important in Common Wombat diversification.

Previous work on Common Wombats has revealed information on their finescale dispersal behaviour (i.e. female-mediated) as well as altered population dynamics between populations of varying sizes (Banks 2002 *1). Banks (2002*2) found that in a single isolated population, genetic variation was significantly reduced compared to that of a continuous population. However, data were insufficient to determine whether this lack of variation is due to recent events such as a genetic bottleneck, or long term effects i.e. the population has always been low in genetic variability because it is at the edge of the species' range and thus has restricted effective population size (N_e) compared to more central populations (Gapare et al 1995; Lonn &, Prentice 2002). The localities of the three extant Common Wombat subspecies: *Vombatus ursinus hirsutus* (Mainland subspecies), *V. u. tasmaniensis* (Tasmania) and *V. u. ursinus* (Flinders Island), suggest that climatic effects during the Pleistocene (last glacial maxim) i.e. range contractions and expansions, have had a significant effect on the diversification of the species. These points suggest a strong influence of habitat upon phylogeographic patterning through a complex biogeographic history (Avice, 2000). Hence, Common Wombats may exhibit consistent, predictable, spatial patterns of genetic diversity.

I will use a combination of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) markers in a phylogenetic analysis to identify the geographical locations of the deepest genetic divergences and the chronology of branching order along lineages and divergence times. The study has wide implications across many taxa in inferring the role of past geographic/meteorological events in the diversification of wide spread fauna in South-Eastern Australia. It will also allow identification of evolutionary significant populations, so conservation efforts can be focussed on those most genetically/evolutionary unique, and threatened by decline.

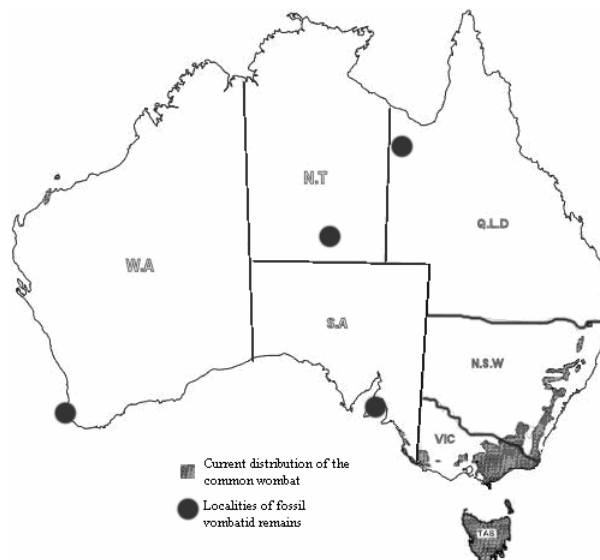


Figure 1: The current known distribution of the common wombat is shown in grey, with dots indicating localities where fossil Vombatid remains have been found. This implies wide-scale continental range contractions.

Wombat synopsis

Wombats are Australian marsupials, and the largest extant burrowing mammal in the world. They are notoriously hard to study in the wild due to their nocturnal and burrowing life style. Three extant species exist; the common wombat (*Vombatus ursinus*) the southern hairy-nosed wombat (SHN) (*Lasiorhinus latifrons*); and the northern hairy-nosed wombat (NHN) (*Lasiorhinus krefftii*).

Common wombats are mesic (i.e. their ecological requirements are set in wet/temperate environments). As a consequence they are expected to have been dramatically affected by the contraction of mesic environments during, and after the Quaternary period, inferred from the fossil and paleoclimatic record. They are now extant in South Eastern Australia, Tasmania and Flinders Island and may show genetic signatures of simultaneous range contractions (continent-wide) and expansions (to Tasmania and Flinders Island) as a result of past climate fluctuations, facilitating diversification in the species.

Previous DNA analysis has focused mainly on a few populations in Victoria, revealing reductions in genetic diversity in an isolated population. To date no genetic analysis has taken place on either the Flinders Island or Tasmanian sub-species. In order to permit spatially explicit population analysis such as Nested Clade Phylogenetic Analysis (Templeton, 1998), much larger sample sizes and more inclusive spatial representation of sampling sites are required.

Objective 1

- *Increase Common Wombat sample size and number of sampling sites across the species entire range*

Current knowledge of Common Wombat population processes comes from a small number of sampling sites in Victoria, without focusing on an adequate number of populations on a spatial scale representative of the species contemporary distribution. Even within the small number of sites studied, there has been evidence of altered population processes over a small spatial scale (Banks 2002*2). In order to gain an understanding of contemporary population processes of the species as a whole, and differentiation within, a much larger spatial scale of sampled populations needs to be achieved.

Method

For populations not currently represented in museum or other collections, hair samples will be collected at burrow entrances via “sticky-hair traps” (Sloane et al. 2000), and DNA extracted in the field stored at 4°C to prevent degradation and maintain sample quality. Wombat hair follicles are unusual in being relatively easy to extract large quantities of DNA from, and have been shown to be able to provide adequate information to make inferences on population processes (Banks et al 2002 *1) and identification of individuals (Sloane et al 2000). This method of sampling provides an excellent way to monitor rare or elusive species, as well as alleviating the stress caused by trapping. In year 1, sampling efforts will be concentrated on identified populations in South-West Victoria, South Australia, and Flinders Island. At each sample site ecological variables (e.g. soil type, vegetation cover) will be recorded along with GPS coordinates to facilitate spatial recognition of sites, and analysis of genetic architecture in a geographical context.

Objective 2

- *Assess genetic diversity and differentiation between individuals and populations*

Genetic analysis will focus on both mtDNA (d-loop and the cyt b coding region) and nDNA (microsatellites). Identification of sexes will take place by PCR amplification of a 158 base pair (bp) fragment of the Y-linked *Ube1Y* gene. The genetic data will be used to evaluate and infer past and present population processes and aim to answer the following questions:

1. How are populations related to each other and when and where did divergences take place?
2. Do smaller/isolated/island/peripheral populations have less diverse gene pools than do large populations, and is the amount of variation a result of recent or long-term effects?
3. Are the sub-species classifications of *V. ursinus* confirmed by significant genetic divergence?

Aims summary

My work will answer questions that have not been attempted in the past and will provide a new insight into the evolution and biology of this important endemic species. The aims of the project are:

- 1) To investigate the phylogenetic structure of common wombats, identifying the geographical locations of the deepest genetic divergences and the chronology of branching order along lineages and divergence times; essentially the history of the formation of the species.
- 2) To investigate the genetic variation of common wombats across the entire range. This will allow the study of patterns of contemporary gene flow and identify whether or not population isolation events are a cause of recent human activity. This will also allow the identification of MUs and ESUs and thus enable conservation efforts to be realized and focused on significant populations.
- 3) To gain a deeper understanding of the population genetic consequences of existing in relative isolation on the periphery of the species range, and/or on islands.
- 4) To investigate the level of genetic divergence between the three currently recognized subspecies of common wombat.

Literature cited

- Avise, J. C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge.
- Avise, J. C. 1994. *Molecular markers, natural history and evolution*. Chapman & Hall, USA.
- Banks, S. C. et al., Wombat coprogenetics: enumerating a common wombat population by microsatellite analysis of faecal DNA. *Australian Journal of Zoology* 50 (2), 193 (2002). (1)
- Banks, S. C., Skerratt, L. F., and Taylor, A. C., Female dispersal and relatedness structure in common wombats (*Vombatus ursinus*). *Journal of Zoology* 256, 389 (2002). (2)
- Gapare, W. J., Aitken, S. N., Ritland, C. E., and Lg, Genetic diversity of core and peripheral Sitka spruce (*Picea sitchensis* (Bong.) Carr) populations: implications for conservation of widespread species. *Biological Conservation* 123 (1), 113 (2005).
- Garrick, R. C. et al., Catchments catch all: long-term population history of a giant springtail from the southeast Australian highlands - a multigene approach. *Molecular Ecology*, 16 (9), 1865 (2007).
- Kinlaw, A. and Av, A review of burrowing by semi-fossorial vertebrates in arid environments. *Journal of Arid Environments* 41 (2), 127 (1999).
- Kuchta, S. R. & Meyer, D. 2001. A genealogical view of geographic variation. *Molecular Ecology*, 10:2569-2576.
- Lonn, M., Prentice, H. C., and Yx, Gene diversity and demographic turnover in central and peripheral populations of the perennial herb *Gypsophila fastigiata*. *Oikos* 99 (3), 489 (2002).
- Moritz, C. & Faith, D. P. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* 7:419-429.
- Sloane, M. A. et al., Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. *Molecular Ecology* 9 (9), 1233 (2000).
- Stiling, P. 1999. *Ecology: theories and applications* 3 rd ed. Prentice-Hall Inc, New Jersey.
- Templeton, A. R. 1998. Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7:381-398.
- Thomas, J. A. & Morris, M. G. 1994. Patterns, mechanisms and rates of extinction among invertebrates in the United Kingdom. *Philosophical Transactions of the Royal Society of London Series B* 344:47-54.

Start and Completion dates

Start: July'08. Completion July'11

Fieldwork schedule

Oct'08: Two-month sampling of isolated populations in Victoria and South Australia.

Jan'08: One-month sampling of Flinders Island populations.

Total Budget

University fees, PhD stipend and research funding

My Bristol University tuition fees and living stipend are paid for by a grant received by Professor Steven Harris. All research costs (e.g. laboratory consumables, molecular analysis, field work) will be sought from additional external agencies. To date I have received \$1,500 from Australian Geographic, \$600 from the M.A. Ingram Trust and continue to seek funding for the project, additional to this application.

University support

I will be co-supervised by Professor Steven Harris, registered at Bristol University, and Dr Andrea Taylor at Monash University. Professor Steven Harris is an expert in mammal ecology and population biology, and has a keen interest in collaborating in a project that focuses on marsupial evolution.

I will be based at The Molecular Ecology Research Laboratory at Monash University, which specializes in research into molecular population biology. My supervisor here (Dr Andrea Taylor) and the rest of the team have a wide range of experience in applying diverse molecular techniques to answering ecological and evolutionary questions, and as such the laboratory is very well equipped for the needs of this project.

Research budget (July 2008 - July 2009)

The estimated costing for the first year of my research is shown below.

1. Field collections

- Travel to study area (by car) and travel between and within study areas.
\$0.75 per km x 3000km \$ 2250
 - Return flight from Melbourne to Flinders Island \$ 420
 - Purchase of portable in-car refrigerator:
Bushman 42ltr Fridge/Freezer \$1000
 - Purchase of ENGLE dual battery:
for powering in-car freezer where applicable \$260
- \$3930**

2. Laboratory consumables

- Genotyping (including PCR and LiCor gel runs):
2 PCRs x 12 loci x 240 hair samples @ \$2.00 per reaction \$ 11,520

TOTAL	\$ 15,450
	= £ 7, 330

Budget Justification

Because of the lack of available samples from isolated and island populations of Common Wombats, intensive sampling is required across South Australia, parts of Victoria, and Flinders Island. Data from these samples will provide the preliminary snapshot of how genetic diversity is structured in relation to population size and geographic location. I expect to find altered population processes between island, isolated, and continuous populations (which will be sampled in the following year).

The purchase of a portable in-car fridge/freezer is critical for the transport and storage of DNA extraction resin, and DNA specimens, at 4°C, to prevent degradation and maintain high sample quality, before being stored at -20°C at the laboratory. The purchase of the stand-alone dual battery is also crucial for powering the fridge/freezer where applicable and mitigating the risk of failed power from the car battery.

Repeat PCRs per locus per specimen may be required to ensure accurate genotyping. The number of specimens to be analysed is based on the estimate of gaining samples from 20 individuals per population, per week (or less).

Other Information

Academic Supervisors

Dr Andrea Taylor
Senior Research Fellow
School of Biological Sciences
Monash University
VIC 3800
Telephone: 9905 5623
Email: andrea.taylor@sci.monash.edu.au

Professor Stephen Harris
School of Biological Sciences
University of Bristol
Woodland Road
Bristol, BS8 1UG
Phone: +44 (0)117 928 7479
Email: s.harris16@talktalk.net

Referees

Dr Paul Sunnucks
Reader
School of Biological Sciences
Monash University
VIC 3800
Telephone: 9905 9593
Email: paul.sunnucks@sci.monash.edu.au

Dr Alan Lill
Associate Professor
School of Biological Sciences
Monash University
VIC 3800
Telephone: 9905 5664
Email: alan.lill@sci.monash.edu.au