The science behind the conservation of endangered species:

My experiences with Australian and Japanese bats

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This talk

1. Why I am a Zoologist / Conservation Biologist.
2. Scientists usually specialise.
3. An introduction to bats.
4. The various methods I use to study bats:
   ~ Field surveys
   ~ Distribution modelling in GIS
   ~ Morphometric comparisons of skeletons
   ~ Echolocation
   ~ DNA markers
5. What happens to my data.
This talk has been modified heavily for display on the internet.

Many pictures have been removed, but in their place I have included text so that the reader can follow the main points.
Japanese people appreciate natural beauty very much, and one way they express this is through their gardens. The *Teien* 庭園 concept of the Japanese garden expresses a balance between man-made and natural beauty.

Unfortunately, real, undisturbed natural beauty is being destroyed all over the world. Zoologists can play a part in helping to preserve our ecosystems by creating knowledge through scientific research.
To follow are some slides of undisturbed natural areas in the arid north of Western Australia, where I have done most of my work.

Part of the value of these areas, in comparison to Japanese gardens, is that they have a complexity and self-sufficiency that gardens do not.

Rare species are one of the special elements of ecosystems. Rarity can be the result of natural processes, or human disturbance. Thus rare species embody the quality, function and history of the ecosystem.
Zoologists....

Some specialise on one particular group of animals, or one concept.

Others use a range of techniques, or apply one main technique to various animal groups.

I have worked on several animal groups in the past, but specialise on bats, using a range of techniques.
We often need a range of skills, including training in safety …
And we sometimes work with other types of industry, including large mining companies.
An introduction to bats.....

Order Chiroptera (Latin for “hand wing”)
About 1100 species worldwide (1/4 of all mammals)
Morphologically very diverse

One bat can eat 1200 insects in a night
Important pollinators of trees, especially in tropical forests
Vulnerable to extinction because of their low reproductive rate

More at www.Batcon.org
Here I showed pictures of bats, their skeletons and echolocation to illustrate:

• The two main types of bat (megabats: e.g. flying-foxes, and microbats).

• How different bat species look: some have large ears, some large eyes, some ornate and complex noseleaves, colourful fur, presence or absence of a tail. See www.batcon.org

• Diversity of diet (insects, fruit, nectar, small vertebrates, blood (but only 3 of those 1100 species!).

• Diversity of skull shape and how you can tell what the bat species eats, and other aspects of ecology just by looking at skull morphology.

• Each species has its own echolocation signature.
Flying dinosaurs

Bird

Bat
The second part of my talk showed some of my past research on bats...
Field surveys

Where do the bats live?
Pilbara
Field surveys in remote areas
Trapping bats.....
Ghost Bat
Macroderma gigas
GIS computer models

Predicting distribution and gene flow based on geology and topography
Satellite image of the Pilbara
Modelling distributions using GIS
Morphometrics

Measuring skeletal specimens to determine species or subspecies groups
Skulls and teeth
Digital measurements from live animals
Multivariate analysis
Echolocation

Bats use it for:
- navigating and feeding in darkness

Scientists study it to:
- determine species, understand bat ecology,
- evolution and species boundaries
• The echolocation call of each bat species is unique, and can be used to tell them apart.

• Sometimes there are significant differences in echolocation variables between populations of the same species.

• This tells us something about the evolution occurring within that species.

• I showed some examples from two Australian and Japanese bats.
This the echolocation call of a Japanese *Rhinolophus* bat. I measure the frequency of the flat part of the call from a bat ‘at rest’, and compare the average between populations/groups.
DNA markers

Short pieces of DNA that tell us about evolution, and many other things …
DNA sampling without killing the bat (an endangered species!)

We remove a small piece of skin from the wing membrane with a Biopsy punch, and then release the bat back into the cave.

This hole completely heals in 3-4 weeks.

We preserve the DNA in a special salt solution.
Parts of the mitochondrial DNA genome are commonly used as markers for animal movement, population structure, and species level determinations.

First we purify the DNA from the skin.

Then we design short pieces of DNA (‘primers’; usually about 20-24 base pairs long) that match the sequence either side of the target marker.
PCR and DNA Sequencing

Next we make many copies of the target marker (which is usually about 1000-2000 base pairs long) by a process called Polymerase Chain Reaction.

You can actually see the DNA if you run it on a gel.

Then we use a sequencing machine to read the exact sequence of A, C, G and T in each sample.

Part of the genetic code of one bat
## DNA sequence alignment

| Sequence | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 |
|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|    |
| Bat1     | A | A | A | A | A | A | A | A | G | T | A | T | G | C | T | C | C | A | G | T | A | T | C | - | A | T | A | T | A | T | A | T | G | C | T |    |
| Bat2     | - | G | - | A | - | - | T | - | - | G | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat3     | - | - | - | - | - | - | - | - | G | - | - | C | - | - | G | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat4     | - | - | - | - | - | - | - | - | - | - | - | - | - | - | A | G | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat5     | - | G | - | A | - | - | T | - | - | G | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat6     | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | G | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat7     | - | - | - | - | - | - | - | - | - | - | - | - | - | - | G | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat8     | - | - | - | - | - | - | - | - | A | - | - | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat9     | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | G | G | G | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat10    | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat11    | - | - | - | - | - | - | - | - | A | - | - | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat12    | - | - | - | - | - | - | - | - | - | - | - | - | - | - | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat13    | - | - | - | - | - | - | - | - | G | - | A | - | G | T | - | C | G | C | G | - | C | G | A | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat14    | - | - | - | - | - | - | - | - | G | A | C | G | - | A | G | T | - | C | G | - | C | G | A | T | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat15    | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |
| Bat16    | - | - | - | - | - | - | - | - | G | - | A | - | G | T | - | C | G | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |    |

Then we align the DNA sequences so we can compare the bases at the same positions on the DNA. The dots above represent the same base as the first sequence.
Distribution of DNA types

This figure shows that bats with different DNA sequences were found in different parts of the Pilbara (each colour a unique sequence, each square an individual bat). This is a phylogeographic pattern indicating past female movements.
Testing models …..
Has the population size: increased, decreased, or stayed constant?

Expected if population size constant

Observed
Evolution is most commonly studied at the level of species or below.

Using DNA markers is one way of determining evolutionary relationships.

We usually visualise evolutionary relationships and genetic distances on several types of ‘tree’, such as this one. Each branch is one bat. It suggests that there are several distinct groups that are different at the level of either species or subspecies.
There are many types of analysis that can tell you about genetic diversity, inbreeding levels, evolutionary network relationships, genetic differentiation, times since two populations diverged or were separated, male and female migration rates, and more.

Some are based on pieces of DNA in the mitochondria, and others are found in the nuclear genome.
What happens to the data?

1. Statistical analysis
2. Write a journal article
3. Reviewed and published
4. Government departments, other researchers
   - Taxonomy updated
   - Conservation status updated
   - Information used in recovery plans
   - Guide actions for new developments
   - Stimulate further research