Genetic Considerations and the Release of Rehabilitated Australian Wildlife

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Introduction:

The biological diversity of our planet is rapidly being depleted by human activities at an enormous scale. The burgeoning human population and environmentally severely costly living standards over the last century have significantly altered all environments, even those of the distant and harsh continent of Antarctica to the extent that there are no pristine wild habitats remaining. This understanding is underpinned by the increasingly more obvious effects human pollution has and continues to have on global warming.

Many species now require human intervention to ensure their survival. The IUCN recognises the need to conserve genetic diversity as one of the three fundamental levels of biodiversity. Most wild populations have decreased greatly in size or are artificially fragmented by habitat destruction into small isolated populations. Breeding in small populations increases the rate of inbreeding and the consequent deleterious effects inbreeding has on almost all normally outbreeding species. Crossing inbred animals from unrelated populations often leads to heterosis or "hybrid vigour", due mostly to favourable dominance. Repeated generations at small population size may also lead to the loss of rare alleles and even some common alleles through genetic drift, and thus less genetic diversity (Frankham et al 2004).

However, outbreeding between distantly related populations can break up locally co-adapted gene complexes or mix incompatible karyotypes, resulting in maladaptations or infertility that may threaten the survival of small populations (Edmands 2007). The release of rehabilitated animals should also address animal welfare and disease implications.

How can these often seemingly incompatible considerations be resolved when releasing rehabilitated wildlife?

Basic Genetic Principles

I will deal with the genetics of vertebrates as this is the class of animals overwhelmingly cared for by wildlife rehabilitators. All vertebrates possess a set of paired (diploid) chromosomes that constitute their genotype, e.g. humans have 23 pairs (2n = 46) of chromosomes, donkeys have 2n = 62, horses have 2n = 64 (and mules and hinnies have 63 chromosomes which is why they are infertile), tammar wallaby 8 pairs (2n = 16). One chromosome of each pair is inherited from the male parent and the other from the female.

Chromosomes are essentially huge DNA molecules with thousands of genetic loci, and each locus consists of nucleotides that code for certain proteins that affect various

phenotypic characteristics such as hair or eye colour, height, etc. At least two copies of a locus code for each gene – one from each parent. Different forms at the same locus are called alleles. The effects (beneficial or deleterious) of each allele often depend on the current environment and may change as the environment changes. Thus the more varied the alleles in a population the more circumstances it can evolve to cope with, and survive.

If the forms at the paired locus are different on each chromosome the animal is said to be heterozygous for that locus (or characteristic). If the coding is the same – it is homozygous. Certain codes result in deleterious observable characteristics (the phenotype), e.g. lack of pigment formation: albinism (a). Most deleterious alleles are recessive because dominant deleterious alleles are quickly eliminated by selection. If an animal is heterozygous at the locus (e.g. Aa) the recessive allele (a) is largely suppressed by the dominant allele (A) and its effects are not expressed. In the homozygous form (e.g. aa) the deleterious allele is expressed ?   albinism.

The more homozygous alleles the genotype contains the more deleterious alleles will be expressed. The resultant poor growth, reduced fertility, reduced disease resistance, etc is called "inbreeding depression". Repeated generations at small population size may also lead to the loss of rare alleles and even common alleles through genetic drift, reducing genetic diversity. Crossing two unrelated highly inbred animals usually produces offspring with high heterozygosity and increased growth rate, greater size, better immune function, higher fertility and improved survival: "hybrid vigour".

However, animals may evolve particular suites of alleles to cope with their local environment. If animals from distant populations and environments mate with these animals this can disrupt locally adapted genotypes, the offspring are then unsuited to the current environment and fare poorly. This observation is called "outbreeding depression".

Thus, crossing conspecifics (same species) from different populations can:
1. improve offspring survival if the parents are inbred (due to hybrid vigour and increasing genetic diversity of the population), or
2. impair offspring survival if the suite of alleles their parental populations developed to cope with their environment is disrupted by the mixing of new alleles (outbreeding depression)

Habitat Destruction and Population Fragmentation
The massive scale of human population and industrial growth has resulted in the destruction of from 35% (mangroves – MEA 2005) to 98% (old growth forests – Primack 2006) of different natural habitats and the fragmentation of most remaining habitats into isolated parcels that are often too small to sustain their natural species diversity. Innumerable species now have fragmented distributions. Much of this destruction has been recent: North and South American environments have been devastated over the last 500 years, South Africa the last 350 years and Australia the last 200 years. Prior to this recent worldwide assault most wild populations could be divided into meta-populations

that were united by the flow of genetic material through the regular or irregular migration of individuals or groups between sub-populations.

The destruction and subsequent fragmentation of most natural habitats prevents natural gene flow, isolating small populations genetically as well as physically. This artificial separation increases the level of inbreeding within small populations and leads to the loss of genetic diversity through genetic drift in small populations.

Each subpopulation becomes increasingly more inbred through matings of more related animals. Each subpopulation also loses alleles through the random sampling of parental alleles each generation (genetic drift). Smaller groups ? increased mating of related animals ? inbreeding ? less fit individuals and a less fit population as a whole. The loss of parental alleles present in the original population means reduced genetic diversity. Inbreeding and reduced genetic diversity both increase the risk of local extinction and thus a greater risk of species extinction. Loss of genetic diversity and increased inbreeding have both been shown to be detrimental to the survival of populations and are considered to be significant in pushing a population into an extinction vortex.

Release guidelines

South Australian government: when relocating brushtail possums –

- Keep the possum in the trap and release at sunset on the day of capture, on the same property, within 50 metres of the capture site. Possums must be released within 24 hours of capture.

- Releasing possums away from the capture site is illegal. Possums are highly territorial so relocating possums elsewhere will only result in the released animal being attacked by possums already in the area. This method is considered inhumane, as it is very stressful for the animal and will result in the death of the possum regardless of where you release them. It may also spread disease if the animal is sick.

NPWS of NSW:

- In the interests of genetic integrity of native animal populations, a rehabilitated or hand-raised animal should be returned to a suitable natural environment at or near the locality of the original encounter
- An animal should not be transported to a release point across a geographic or physical barrier it would not normally cross

Justifications for these guidelines or regulations include:

- Animals can be highly adapted to a particular environment (e.g., dry eucalypt woodland vs. closed rainforest) and releasing them into another environment may jeopardise their welfare and survival.
- Even if the habitat is suitable, some species are highly territorial, especially during a breeding season, so relocating animals into a conspecific's territory is

likely to result in the released animal being attacked and possibly killed. Thus releasing an animal away from its territory can be considered inhumane.

- The released animal may harbour potential pathogens that are new to the release site, introducing new diseases into the community at the release site.
- Animals released away from their birth habitat may carry a genetic complement different to those of the local conspecific population. This may:
  - ➢ render it unsuited to the new site, reducing its chances of survival
  - ➢ introduce a genotype into the resident population that is unsuited to the local environment, reducing the population's overall genetic fitness ("outbreeding depression")
  - ➢ introduce alleles not present in the resident population, artificially corrupting the 'genetic purity' of the resident population

Genetic management

One of the ways the detrimental effects of artificial population fragmentation can be mitigated is to move animals between separated populations. This can restore the former natural flow of genes and the previous level of genetic diversity, improving the fitness of the population (e.g, Delgado et al 2004, Hogg et al 2006, Parker 2008, Procházka et al 2008), thereby reducing the risk of local and species' extinction One of the greatest risks of such actions is the transfer of pathogens to the recipient habitat and community. This risk can be minimised by thorough quarantine procedures to ensure there is no evidence of disease in the animals to be transferred. The most effective and easiest method of doing this is using hand-raised juveniles that have been in care for weeks or even months. This will afford the greatest confidence that any potential pathogen has been detected through overt signs of disease in the juvenile or a failure to grow and develop as expected.

Another objection voiced to the translocation of animals is the risk of outbreeding depression. However, this risk is highly unlikely over short distances where the habitat is similar and populations have natural gene flow, or had it prior to European settlement of Australia (e.g., < 200 km for many far ranging species). Further, especially in Australia, natural habitats have been severely altered by:

1. widespread habitat destruction
2. increased soil salinity
3. polluted fresh water
4. polluted marine and estuarine waters
5. introduced exotic predators (feral cat, red fox, feral pig)
6. introduced exotic herbivores (rabbit, goat, horses, etc)
7. introduced exotic diseases
8. global warming!!!

Thus many co-adapted genetic complexes may no longer be effective in helping animals survive in the current greatly changed environments so that there is a much reduced risk (which was already limited) of outbreeding depression when mixing genotypes from

different environments. Also, red kangaroos and western grey kangaroos disperse up to 323 km (Priddel et al 1988), eastern grey kangaroo dispersal may be inferred up to 230 km (Zenger et al 2003) and brushtail possums disperse 3 – 25 km (Cown et al 1984) so that moving these species at least twice these distances poses no risk of outbreeding depression.

Compared with the numerous examples of inbreeding depression in wild and domesticated species there are only about 9 cases of outbreeding depression in plants or animals. There are many examples where increased genetic flow has improved the overall fitness of wild populations. For example:

- Greater prairie chicken (*Tympanuchus cupido*)
- Swedish adder (*Viper berus*)
- Florida panther (*Felis concolor coryl*)
- topminnow fish
- scarlet gilias
- Lakeside daisy (*Hymenoxy acaulis var. glabra*)
- Mauna Kea silversword (*Argyroxiphium sandwicense ssp. sandwicense*))

A third objection is the artificial 'contamination' of the genetic 'integrity, of the 'natural' genetic pool of the target population. As described above, the massive scale of human expansion has artificially fragmented most if not all wild populations which, as a result, are becoming increasingly inbred and losing genetic diversity through genetic drift. Thus, if there ever was anything significant in the concept of the natural 'genetic purity' or integrity of wild populations (debatable), it is no longer valid. The detrimental effects of fragmented populations significantly increase the risk of extinction and introducing genetic material from outside the population will actually restore the genetic pool to a more natural state. Plus, the increased genetic diversity will help the population evolve to cope with continually changing environments.

The more specific and significant co-adapted gene complexes are for survival in a local environment, the greater the risk of outbreeding depression. However, the more specific adaptations are to a particular environment, the less adaptive they become when the environment changes. Thus, the stronger the argument for the risk of outbreeding depression that may affect populations by moving animals between different stable environments, the stronger is the argument for introducing novel genotypes once the environment changes. The more the environment changes, the greater the need for newer alleles and thus increased genetic flow.

Populations isolated in the same or similar environments have shown no development of outbreeding depression in *Drosophila melanogaster* over 1,000 generations and in fish stocks over 3,000 generations (Frankham, pers. comm.). Thus there is little risk of outbreeding depression in populations of Australian animals that previously had genetic flow before becoming isolated in the same environment by land clearing and habitat destruction since European settlement.

There are also a multitude of examples of the survival and extraordinary population growth of many species translocated far outside their natural distribution, so much so that many have become serious pests in their new environment. Of 35 mammalian species introduced into Australia 24 (69%) have established exotic populations (Myers 1986). In Europe 32 of 47 mammalian species have (68%) (Udvardy 1969, Jarvis 1979). Other data include 20 of 48 (42%) avian species in Australia and 170 of 486 (35%) avian species worldwide (Long 1981, Lockwood 1999). These data indicate that, for many and possibly the majority of species, genetic complexes co-adapted to specific local environments are not significant to their survival.

Cane toads have had and continue to have devastating effects on the native fauna of tropical and subtropical Australia, especially on native carnivorous species such as the northern quoll. Many populations of quolls have become extinct in areas the cane toad now occupies. Those that have managed to survive where cane toads have lived for some time may have done so due to particular genes that protect them from the lethal effects of the toad (see Phillips & Shine 2004; Phillips & Shine 2006). It matters little whether this is due to behavioural, physiological or other characteristics. If they survived due to particular genotypes then translocating some of these to areas where the cane toad is just beginning its devastating impacts, their adaptive genotypes may reduce the risk of extinction the local populations face. Of course this would require thorough quarantine procedures to minimise the possibility of transferring potential novel pathogens but it is unlikely that any of these would be as devastating as the cane toad. The most obvious choice for this would be hand-raised juveniles that have been in care for some time which will afford the greatest confidence that they are disease free.

Island populations have become significant refuges for some endangered species. However, many island populations are genetically depauperate and inbred due to a small number of founders or after many generations at small population size (e.g. Eldridge et al 1999). Small population size and low genetic diversity render them highly vulnerable to local extinction. Mixing animals from different island populations or from the mainland is highly likely to result in hybrid vigour and increase the genetic diversity of the offspring and future generations, increasing the populations' chances of survival and adaptation to the environment, especially if it changes. If island populations are used to repopulate dwindling mainland populations it is highly desirable to hybridise animals from as many island or remnant mainland populations as is possible to maximise the genetic diversity of the final population. In all cases the risks of pathogen transfer and outbreeding depression should first be addressed, but should not preclude the option of re-establishing more natural levels of genetic variation.

Summary
- No pristine habitats or "pure" wild populations exist
- Many populations and species require supportive management to survive
- Habitat destruction has fragmented wild populations and disrupted gene flow
- Gene flow is necessary to maintain genetic diversity and species' survival

- Maximising genetic diversity, especially of inbred and genetically depauperate populations, can be critical to ma ximise a population's or species' chances of surviving
- Releasing rehabilitated wildlife away from their birth site is one way that should be considered to help restore more natural gene flows and help native species survive

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