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# Analysis and Conservation Implications of Koala Genetics

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**Abstract:** *Koalas are the only living member of their family and therefore deserve serious conservation consideration. Koalas have low levels of genetic variation within and among populations in the southern part of their range, where they have experienced many relocations and population crashes since European colonization of Australia. The importance of this change in variation is underlined by preliminary indications that levels of genetic variation may affect fitness in koalas. Techniques have been developed to help identify and monitor genetic problems in koalas and to provide the information and tools to make genetic management an integral part of koala conservation. The koala is currently at an appropriate point for conservation intervention: there is clear evidence of decline in some populations, but the existence of other robust populations offers the possibility of creative solutions to their conservation problems. Managers should aim to maintain this species' current ecological amplitude (the range of environments in which populations are found) and minimize the loss, fragmentation, or decline of populations. There are no data to suggest that any population requires genetic supplementation. The concepts of evolutionarily significant unit (ESU) and management unit (MU) can be useful in the genetic management of koalas, including monitoring and management regimes. But ESUs and MUs can also be misleading if they are not interpreted carefully in terms of population history and the ultimate goal of management. Translocations should not involve extensive use of stock from a single source, especially those with low genetic variation, and they require careful management to avoid possible problems when individuals encounter novel strains of the pathogen *Chlamydia pecorum*, because several genetically distinct strains have been found in koalas, some of which may derive from introduced species. Genetic indicators can and must make considerable contributions to koala management, but they require careful interpretation.*

## Análisis y Consecuencias de la Conservación Genética del Koala

**Resumen:** *Los koalas son el único miembro viviente de su familia y por lo tanto merecen serias consideraciones de conservación. Los koalas tienen niveles bajos de variación genética dentro y entre poblaciones en la parte sur de su rango de distribución, donde han experimentado muchas reubicaciones y colapsos poblacionales desde la colonización europea de Australia. La importancia de este cambio en la variación es subrayada por indicaciones preliminares de que los niveles de variación genética pueden afectar la adaptabilidad del koala. Se han desarrollado técnicas para ayudar a identificar y monitorear problemas genéticos en koalas y para proveer información y herramientas que hagan del manejo genético una parte integral de la conservación del koala. El koala se encuentra actualmente en un punto adecuado para intervenir con medidas de conservación: existen pruebas evidentes de una disminución de algunas de sus poblaciones, pero existen otras poblaciones robustas que ofrecen la posibilidad de una gran variedad de soluciones creativas a sus problemas de conservación. Los manejadores deberían intentar mantener la amplitud ecológica actual de la especie (rango de ambientes en los cuales se encuentran las poblaciones) y minimizar las pérdidas, la fragmentación o la disminución de las poblaciones. No existen datos que sugieran que alguna de las pobla-*

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*ciones requiera suplemento genético. Los conceptos de unidades evolutivamente significativas (ESU) y unidades de manejo (MU) pueden ser guías útiles para el manejo genético de koalas, incluyendo regímenes de seguimiento y manejo. Sin embargo, los ESU y MU pueden llevar a conclusiones erróneas si no son interpretadas cuidadosamente con relación a la historia poblacional y a la meta final del manejo. Las translocaciones deberán evitar el uso extensivo de grupos de una sola fuente, especialmente aquellas fuentes con baja variación genética, y requieren de un manejo cuidadoso para evitar posibles problemas cuando los individuos se enfrenten a nuevas líneas del patógeno Chlamydia pecorum, puesto que han sido encontradas muchas líneas genéticamente diferentes en koalas, algunas de las cuáles pueden derivar de especies introducidas. Los indicadores genéticos pueden y deben aportar contribuciones considerables al manejo del koala, pero se requiere de una interpretación cuidadosa.*

## Introduction

The family Phascolarctidae has been in existence for at least 15 million years, and as its only living member the koala rates highly on some criteria for conservation efforts (Vane-Wright et al. 1991). Another criterion is the likelihood that the fate of the taxon will be altered by management intervention, and from this point of view the koala seems an appropriate choice for conservation action. Although some koala populations are in decline (Reed & Lunney 1990) and some have been perturbed by hunting, overbrowsing, and relocations, there are other populations that appear secure or even too dense. Thus the conservation manager's options are not limited by low numbers across the species. We summarize the history of koala populations, analyze the likely genetic effects of this history, and compare these predictions with current knowledge of koala genetics. Finally, we offer a perspective on the current genetic status of koalas and its implications for conservation management.

When a species begins to decline or populations become fragmented, the species may lose genetic variation among populations, individuals, or genes within individuals. Low variation may result in reduced reproduction or survival and therefore in a worsened conservation outlook for the population. This problem can be either serious (Madsen et al. 1996) or relatively trivial compared with other conservation problems (Lande 1988). The importance of genetic variation depends on the biology of the species concerned, with some species surviving with little variation (Reeve 1990). Therefore, a conservation biologist must gauge how seriously any loss of variation may affect the future of particular populations and must consider how best to avert or remedy loss of variation. In doing this, managers can use a variety of genetic indicators, ranging from those with high variability and known inheritance to rougher measures such as counts of individuals or populations (A. Brown et al. 1997).

The highest level at which the genetics of a single species needs to be analyzed is the variation among populations. Measures of among-population variation include numbers of subspecies, interpopulation genetic struc-

ture of marker loci, and the environmental amplitude of populations—the range of environments in which different populations are found, which may reflect differences of adaptation (Brown et al. 1997). Evolutionarily significant units (ESUs) are geographically discrete sets of populations that have been identified by genetic studies as having evolved separately for a substantial period of time (Ryder 1986; Moritz 1994). They relate to long-term conservation needs, such as “defining conservation priorities and setting strategy, although in the short term it may be prudent to avoid translocating individuals between ESUs” (Moritz 1994). Separate management of subspecies or ESUs increases costs, however, and in captivity may lead to inbreeding in limited stocks from a single ESU.

On a finer geographic scale than ESUs, the appropriate units for short-term conservation are termed “management units” (MUs; Moritz 1994). Populations in different MUs show significant differentiation in their frequencies of nuclear alleles or mitochondrial haplotypes (Moritz 1994). The differentiation between MUs is taken to indicate some degree of demographic independence, at least in the short term. For example, if a particular MU becomes extinct, it is unlikely to be quickly repopulated by a neighboring MU. Despite their low natural exchange of genetic material, MUs from the same ESU may be subject to deliberate exchange of migrants for conservation purposes such as demographic or genetic support.

Genetic variation among individuals within populations is likely to be sharply reduced by bottlenecks (periods of small population size) or fragmentation (Frankel & Soulé 1981). Loss of genetic variation usually (but not always) leads to short-term reduction of fitness components such as survival, reproductive output, growth rates (Allendorf & Leary 1986), and to impaired ability to adapt to long-term changes in the environment. The variety of relationships between genetic variation and fitness underlines the importance of studying this relationship in a wide range of managed species. Suitable indicators for management of variation at this level range from crude measures such as numbers of individuals or population size and isolation to more techni-

cally demanding indicators such as within-population variation in marker loci or the genetic component of quantitative traits such as morphology and reproductive rate (Brown et al. 1997).

Finally, threatening processes may alter the mating system so that inbreeding becomes more frequent, exacerbating any increase in homozygosity caused by decreased variation between individuals. Detectable changes can occur in a single generation, so this can be a sensitive indicator in some species (Brown et al. 1997). Inbreeding depression—reduced fitness resulting from inbreeding—is frequently seen in captive and wild populations (Ralls et al. 1988; Madsen et al. 1996), but it may not be seen in all species or under all environmental conditions (Jimenez et al. 1994; Frankham 1995). It is therefore important for a conservation biologist to be able to determine the normal level of inbreeding for any species and the possible consequences of changes to this inbreeding level caused by threatening processes.

The history of disturbances to koala populations and the potential genetic consequences of disturbance encourage us to monitor and possibly manage genetic variation in koalas. We summarize development of the more technically demanding indicators and application of these indicators to historical and current data on koalas. We then evaluate the relationships between indicators; for example, we will assess whether the reductions in population size in the southern part of the range have resulted in reduced genetic variation, and whether reduced genetic variation at any level is associated with lowered fitness.

## Genetic Indicators

Morphological variation may be genetically based and can be of direct relevance to adaptation, so conservation of these variants may be important. In koalas there are reports of local polymorphism such as sporadic light-colored animals (Lewis 1934, 1954) and of regional variation such as shorter limbs and longer fur in the cooler, southern part of the range (Melzer et al., this issue). Whether or not morphological variation is genetically based has not been investigated because this would require measurements on hundreds of same-age, pairs of relatives or reciprocal transfers of animals within different parts of the range. Although appropriate transfers have occurred (Robinson 1978), genetic conclusions are precluded by the lack of monitoring and by likely hybridization with other stocks. Fluctuating asymmetry in morphological traits can give early warning that genetic or pollution problems are affecting normal development (Clarke 1995). Living koalas have few of the necessary accurately measurable bilateral characters, however, and some asymmetries may be due to injury rather than development. Koala dermal ridges show left-right asym-

metries and may provide suitable indicators after further research (Henneberg et al. 1997). In summary, it is currently difficult to use morphological variation for genetic monitoring or management, but morphology has been used as the basis for definition of subspecies.

Researchers have used a variety of techniques to study genetic variation in koalas. Early analyses such as allozymes (S. J. O'Brien & S. Ramus, personal communication) showed low variation in koalas, and for many years it was not statistically possible to assess whether different koala populations had different levels of variation. Many DNA techniques have been applied to koalas (Table 1), and some show considerable variation. Four techniques have revealed high levels of variation in koalas (Table 1): mitochondrial control-region analysis (Houlden et al. 1999), major histocompatibility loci (Houlden et al. 1996c), randomly amplified polymorphic DNA (RAPDs) (Fowler et al. 1998a, 1998b, 2000), and microsatellites (Houlden et al. 1996a, 1996b). In contrast to some other species, RAPD bands are highly repeatable in analyses of koalas, showing apparent autosomal-dominant inheritance (only disproved for 1.9% of bands; Fowler et al. 1998a). In two captive populations, all 25 individuals had unique RAPD profiles, which allowed parentage determination in 90% of cases. Koala microsatellites show a high level of allelic diversity and codominant autosomal inheritance, making them ideal genetic markers for paternity exclusion, pedigree analysis, and population studies (Houlden et al. 1996a, 1996b). Table 1 shows a variety of laboratory and statistical methods which ranges in geographic scope from a few individuals in a few populations to comprehensive surveys.

## Genetic Variation among Koala Populations: ESUs and MUs

The number of subspecific taxa is an easily implemented genetic indicator, and loss of subspecies is likely to represent loss of important genetic variants. The existing subspecific classification of koalas may not accurately reflect genetic diversity, however, so conservation priorities based on currently recognized subspecies may be deficient. Currently, three subspecies of koalas are recognized: *P. c. adustus* from northern Queensland, *P. c. cinereus* from New South Wales, and *P. c. victor* from Victoria. Taxonomic classification is based on characteristics that include size and color of three type specimens, but the distribution of each subspecies has not been adequately defined (Martin 1983). At present, the ranges of each subspecies are delineated by state political borders. There have been suggestions that this variation may be due to a gradual latitudinal cline, but this simple interpretation has been disputed recently after further characterization of koala populations from Queens-

Table 1. Analyses of DNA variation in koalas.

Method	Code	Level of variation <sup>a</sup>	
		within populations	among populations
Minisatellite	MN <sup>b</sup>	S: $\geq 75\%$ band-sharing (I2, P1)	SP/S: 75–100% band-sharing (I1–2, P4)
	MN <sup>c</sup>	N: band-sharing 71–92% (I36, P1), all individuals unique, parentage determined	
	MN <sup>d</sup>	SP: band-sharing 76–95% (I9–16, P2), other results complicated by sex differences and broad confidence intervals	
Mitochondrial restriction fragment length polymorphism (RFLP)	MT <sup>e</sup>	S: 2 haplotypes (I20, P1) SP: 1–2 haplotypes (I10–21, P4)	S/SP: significant differences of haplotype frequency (I10–21, P5)
	MT <sup>f</sup>	N: 2 haplotypes (I10–12, P2)	N: significant differences of haplotype frequency (I10–12, P2)
Mitochondrial control-region sequence	CR <sup>g</sup>	N: 1–3 haplotypes (I8–22, P8) S: 1–3 haplotypes (I1–19, P3) SP: 1 haplotype (I5–15, P5)	N: significant frequency differences between all populations (I8–22, P8) N/S/SP: no distinct ESUs SP: no significant differentiation (I5–15, P5)
Major histocompatibility loci	MHC <sup>b</sup>	N: (captive): $\geq 9$ sequences $\geq 3$ loci; further analysis difficult until allelism resolved	
RAPD	RA <sup>i</sup>	N: 60–85% bands polymorphic (I8–23 P4) S: 35% bands polymorphic (I11, P1) SP: 20–35% bands polymorphic (I10–20 P3)	N: 19.7% of variation between populations (I8–23 P4) N vs. S/SP: 33.9% of variation between regions S/SP: 8.8% of variation between populations (I10–20 P4)
Microsatellite	MS <sup>j</sup>	N: $H_e$ 0.67–0.83 (I14–27, P4) S: $H_e$ 0.48 (I47, P1) SP: $H_e$ 0.33–0.48 (I17–43, P5)	N: delta 3.5–6.9 (I14–27, P4) NvsS/SP: delta 9.1–31.0 (I14–47, P10) S/SP: delta 0.02–15.4 (I17–47, P6)

<sup>a</sup>N, northern populations (Queensland and New South Wales); SP, southern populations that apparently have been seriously perturbed in the past 200 years (most Victorian and South Australian populations); and S, less perturbed southern populations (Victoria); I, number of individuals sampled per population; P, number of populations sampled.

<sup>b</sup>Probes M13, Jeffreys33.6, Jeffreys33.15, pUCJ, pSP.2.5.EI, pHVR6, enzymes EcoRI, TaqI, MspI, Sau3A, BamHI (Taylor et al. 1991).

<sup>c</sup>Probe M13, enzymes BamHI, MspI (Coccolone & Timms 1992; Timms et al. 1993).

<sup>d</sup>Probe(GGAT)<sub>4</sub>, enzyme HinfI (Emmins 1996).

<sup>e</sup>Probe feline mtDNA, enzyme TaqI (Taylor et al. 1997).

<sup>f</sup>Probe mtDNA, enzyme EcoRI (Worthington-Wilmer et al. 1993).

<sup>g</sup>Mitochondrial control region sequence variation using outgroup heteroduplex analysis (Houlden et al. 1999).

<sup>h</sup>Major histocompatibility (MHC) system class I sequences (partial exon 2 and 3; Houlden et al. 1996c); also, there appear to be at least two polymorphic MHC class II loci and a pseudogene (Greville, personal communication).

<sup>i</sup>Analysis of molecular variance (AMOVA) analysis of 20 autosomal dominant, randomly amplified polymorphic DNA loci (Fowler et al. 1998a, 1998b, 2000).

<sup>j</sup>Six codominant autosomal microsatellite (CA)n loci.  $H_e$  is expected heterozygosity and delta is delta mu, a measure of population differentiation for microsatellite loci (Houlden et al. 1996a, 1996b).

land (Melzer 1995; Melzer et al., this issue). The translocations of koalas in southern Australia over the past 200 years (Houlden et al. 1996b; Melzer et al., this issue) indicates that mixing of stocks within this area is at least partially successful, as would be expected for members of a single ESU.

Genetic analysis can be used to refine the definition of ESUs. Mitochondrial DNA analysis has not revealed any clear boundaries for subspecies or other ESUs within the distribution of koalas (Houlden et al. 1999). Rather, there appears to be a broad cline from north to south,

with terminal populations strongly genetically differentiated, which is consistent with the morphological analyses of Melzer (Melzer 1995; Melzer et al., this issue). These latitudinal clines may reflect important differences of adaptation to factors such as temperature, and there may also be east-west differences in adaptation. Therefore, loss of all the populations in any one part of the range could reduce the ecological amplitude of the species and would certainly diminish the genetic variation.

Identification of management units requires studies of gene frequency differences among populations. In ko-

**Table 2.** Tests for significant differentiation of gene frequencies among southern populations of koalas.

Population	Population <sup>a</sup>				
	Stony Rises	Brisbane Ranges	French Island	Phillip Island	Kangaroo Island
South Gippsland	MT,CR – <sup>b</sup> ,MS	MT,CR – <sup>b</sup> ,MS <sup>c</sup>	MT,CR RA,MS <sup>c</sup>	– <sup>b</sup> ,CR RA,MS	MT <sup>c</sup> ,CR RA,MS <sup>c</sup>
Stony Rises		MT,CR – <sup>b</sup> ,MS	MT,CR – <sup>b</sup> ,MS	– <sup>b</sup> ,CR – <sup>b</sup> ,MS <sup>c</sup>	MT <sup>c</sup> ,CR – <sup>b</sup> ,MS <sup>c</sup>
Brisbane Ranges			MT,CR – <sup>b</sup> ,MS	– <sup>b</sup> ,CR – <sup>b</sup> ,MS	MT <sup>c</sup> ,CR – <sup>b</sup> ,MS <sup>c</sup>
French Island				– <sup>b</sup> ,CR RA,MS <sup>c</sup>	MT,CR RA,MS <sup>c</sup>
Phillip Island					– <sup>b</sup> ,CR RA <sup>c</sup> ,MS <sup>c</sup>

<sup>a</sup>South Gippsland, unperturbed population; Stony Rises and Brisbane Ranges, perturbed mainland populations; French Island, Phillip Island, and Kangaroo Island, perturbed island populations. Codes and references for techniques follow Table 1: MT, data reanalysed by pairwise contingency chi-square with correction for multiple testing; MS, microsatellite differentiation summarized as theta.

<sup>b</sup>Omitted comparison.

<sup>c</sup> $p < 0.05$ .

alas, gene frequency studies by different researchers have not used the same suites of populations and have applied genetic analyses that are amenable to different statistical analyses (Table 1). Despite this diversity, most studies indicate strong differentiation between the northern and southern populations and the existence of multiple management units within the northern range of koalas. These management units require separate monitoring and management because the low gene flow among them indicates contemporary demographic independence. None of the studies have enough detail to allow mapping of the boundaries between management units. In some cases there may not be a clear boundary, only a broad cline of gene frequencies among adjacent management units, which shows genetic isolation by distance. In these cases there will be some limited demographic interaction among populations while the intervening habitat is intact, but if the habitat is fragmented, gene flow will be disrupted and they will become independent management units.

The interpretation of gene frequency studies in southern Australia is more complex. Levels of differentiation between these populations are low. For pairs of northern populations, 19.7% of RAPD variation was among populations, and Delta mu ranged from 3.5 to 6.9 (Table 1). The comparable values for pairs of southern populations were 8.8% and 0.02–1.634, respectively (latter value calculated after exclusion of Kangaroo Island). Most pairs of southern populations do not show consistent patterns of significant differentiation (Table 2). Kangaroo Island, however, showed significant differentiation from each of the other southern populations in at least one analysis. Kangaroo Island was even significantly differentiated from French Island, the source of the Kangaroo Island stock. This high differentiation presumably reflects genetic drift during the founding bottleneck or thereafter. The next most differentiated south-

ern population is South Gippsland (Table 1), which is probably the least perturbed by crashes and artificial immigration.

Thus, the data do not strongly support a hypothesis of multiple management units in the southern range, but this interpretation must be viewed with caution. First, low within-population variation reduced the power of tests to detect differentiation among southern populations. Second, the apparent single management unit may not be a reflection of continuing high gene flow among most southern populations but rather a reflection of evolutionarily recent expansion or recent artificial relocations. Koalas are absent from the large island of Tasmania, which was connected to the southern part of the continent until about 10,000 years ago. This absence may be indicative of recent expansion to southern Australia, which could explain the low variation (N. Murray, personal communication). It is also likely that any historical pattern of multiple management units in southern Australia has been lost as a result of extensive relocations within this region during the last 200 years (Houlden et al. 1996b; Melzer et al., this issue).

## Genetic Variation within Populations

Population size, numbers of populations, and their physical isolation may affect genetic diversity. The existence of appreciable genetic variation within koala populations can be inferred from their evolutionary history. Sometimes species such as the koala, which are the only representative of their family, are regarded as “evolutionary dead ends” (Archer et al. 1993). The history of koala-like animals, however, suggests that they are not a dead end but have the ability to adapt to changing environments. Koala-like animals (family Phascolarctidae) have lived in Australia for over 15 million years. At any

one time there was usually only one species, yet successive species have shown various body sizes and have inhabited environments that were very different from one another and from those currently occupied by koalas (Archer & Clayton 1984; Archer et al. 1991). Thus, the koala's predecessors apparently could adapt to environmental changes that occurred over this enormous time scale, suggesting that they had ample within-species genetic variation. Whether the modern koala has the same level of variation as its predecessors will probably never be known, but it is certainly not without genetic variation.

There have been suggestions that analysis of the number of populations and range changes can be used as surrogate indicators of genetic processes (Brown et al. 1997). Over the last 200 years, many koala populations have experienced multiple bottlenecks due to crashes or relocation of small numbers of animals. The severity of these bottlenecks probably resulted in loss of genetic diversity. There may be a sharp contrast between the recent histories of northern and southern koala populations, making the comparison between the two regions an interesting test case for conservation genetics theories. It is thought that the more northern populations (Queensland and New South Wales) largely escaped severe bottlenecks, despite heavy hunting by European Australians, but this assertion is difficult to verify.

In contrast, most populations of koalas in southern Australia (Victoria and South Australia) have been perturbed in the last 200 years in ways that could affect their genetics, (i.e., by large fluctuations of numbers or by addition of island stock [Kershaw 1934; Lewis 1934, 1954; Currie 1937]). The southern populations of koalas have experienced extensive and repeated bottlenecks as a result of overhunting, relocations of small numbers of individuals, and population crashes due to overbrowsing. Many southern populations of koalas were thought to have become extinct by the early 1900s and were restocked this century with over 10,000 animals from island populations (reviewed by Houliden et al. 1996b). The immigrants may have entirely replaced the original stock. In some cases, populations were established outside the historic range of koalas (e.g., Kangaroo Island in South Australia). Although some of the translocations were small, records of the Victorian Department of Natural Resources and Environment and its predecessors show that many locations received hundreds of animals over a decade or longer. It is not known how well the relocated individuals survived or bred, but if even 10% of them did, then there would be little loss of genetic variation in most transfers.

The source populations themselves had a history of bottlenecks resulting from crashes and relocations of small numbers, which would be expected to reduce genetic variation. Most of the animals used for repopulation or founding new colonies derived from two artificial populations on Phillip and French Islands and from a

derivative colony on Quail Island. Late in the 1800s, the island populations of koalas were established by the relocation of small numbers of animals from the surrounding mainland—tens of animals to Phillip Island in the 1870s (Lewis 1954) and two or three individuals to French Island in the 1880s (Handasyde et al. 1988). By the 1920s, however, the Phillip Island population was in a steep decline, and 50 animals were relocated from neighboring French Island (Lewis 1934, 1954). As well as these bottlenecks, the French Island population is free of *Chlamydia* (Handasyde et al. 1988) and consequently has experienced additional genetic bottlenecks because of cycles of population boom, overbrowsing, and decline.

Interpretation of population and distribution changes as indicators of genetic processes is open to error; thus, it is desirable to investigate whether past management of koalas has actually reduced genetic variation within or between koala populations. As expected from this history of bottlenecks and translocations, the level of genetic variation seen within and among southern koala populations is low (Houliden et al. 1996b; Taylor et al. 1997; Fowler et al. 1998b). Microsatellite genotyping at six loci was used to determine the levels of genetic variation within and the extent of genetic differentiation between six southern populations, which were compared to four northern populations that were thought to have experienced less perturbation (Houliden et al. 1996b). As expected, a significantly lower level of variation was present in populations from southern Australia (Table 1; Houliden et al. 1996b). Similarly, analysis of 20 polymorphic RAPD markers showed higher variation in the northern populations than in the southern ones (Table 1; Fowler et al. 1998b). The number of mitochondrial haplotypes was also lower in southern populations than in northern populations analyzed by the same method (Houliden et al. 1999).

## Mating System

Any use of mating system as an environmental indicator in koalas must take into account the natural rate of inbreeding. Mitchell (1990) showed that male koalas disperse, whereas females remain near their natal site and could mate with the dominant male. If this dominant male is the father of most females born in the area, then inbreeding could occur; thus, the natural level of inbreeding of koalas could be relatively high. There is, however, no guarantee that behavioral dominance correlates with paternity. If there is considerable inbreeding, it should result in consistent deficits of heterozygotes relative to random-mating expectations. None of the studies of codominant genetic markers (Table 1) have shown such deficits, possibly because inbreeding levels are low, but also possibly because heterozygote

deficits are not a sensitive method for detecting inbreeding. Therefore, it is important to use variable markers to investigate paternity in wild koalas. Researchers are using microsatellites (Houlden et al. 1996a; Sharkey et al., personal communication) and RAPDs (Fowler et al. 1998a) in ongoing paternity studies.

### Genetic Variation and Fitness

Given that the association between levels of genetic variation and fitness is not the same in every species, it is important to analyze whether there is such a relationship in koalas. Population-level studies are needed to establish whether genetic effects are important for population persistence in this species, but individual-based studies can also be useful. At the population level, it is not obvious that koala populations with low variation, such as those in the south, are declining or showing low recruitment as a result of lowered genetic variation. Some of these populations, such as on Kangaroo Island, are even overpopulated. In this case and others, it is possible that any association between low genetic variation and fitness is obscured by the absence of reproductive disease, *Chlamydia*. It will be important to separate the effects of disease and low variation in future studies. At the individual level of analysis, studies of inbreeding depression can provide indications of whether increased homozygosity adversely affects the fitness of individuals. Worthington-Wilmer et al. (1993) did not find evidence of inbreeding depression in a study of the growth rate of 17 captive koalas, but they suggest that this may have been because of the small sample size. This captive colony did show a significant excess of male births, which is possibly a correlate of inbreeding, but this cannot be confirmed in the absence of a comparison with a less-inbred group of koalas under the same management regime.

A larger study of inbreeding in captive koalas showed an association between elevated juvenile mortality and increased inbreeding. One of us (J.W.) analyzed published records of 216 known-age, captive-bred koalas of Victorian origin (Vaartjes 1998). The average age of sexual maturity in koalas is estimated at 730 days (Martin & Handasayde 1991), and mortality prior to this age was assessed. Ages in days were calculated from estimated birthdate (following Bach 1998) to the date of final record (death, release, loss, or last date recorded). Data for animals currently living, living at 730 days, released, or lost were treated as censored data. For each animal, inbreeding coefficient ( $F$ ) was calculated relative to the founding animals by analysis of pedigrees using SPARKS 1.42 (Scobie 1997). Levels of inbreeding in the koalas ranged from zero to  $F = 0.25$ , the equivalent of full-sibling mating. The analysis was stratified to take into account any sex-specific difference in underlying survival

function. Also, improvement of husbandry and veterinary regimes between 1968 and 1998 (the range of records included) may have systematically affected survivorship. A proportional hazard regression model (Cox 1972) tested for an effect on mortality of both birth date and inbreeding coefficient. The model assumed a log-linear relationship between independent variables and an underlying hazard function. The regression model explained a significant portion of the variation in mortality ( $\chi^2 = 4.22$ ,  $df = 1$ ,  $p = 0.04$ ). Date of birth in captivity by itself did not have a significant effect on juvenile mortality in koalas ( $p = 0.15$ ), but mortality showed a significant regression on inbreeding coefficient (slope,  $\beta = 3.77$ , with  $SE = 1.66$ ,  $t = 2.28$ , and  $p = 0.0229$ ). The hazard ratio indicated that inbreeding at the 0.25 level is expected to be associated with a 2.57-fold increase in juvenile mortality. This data set contained only a small number [23] of inbred individuals, and many of them [13] were in one particular captive region [Australia]. Although it would be useful to stratify the test by captive region to account for possible environmental effects, stratification would leave little statistical power unless larger datasets were obtained.

In evaluation of the importance of these results for wild populations of koalas, inbreeding depression may be more severe in wild populations than in captivity (Jimenez et al. 1994). Thus it is possible that wild populations that have had increases in homozygosity equivalent to one generation of full-sibling mating would have poor juvenile survival. This simplistic argument ignores other differences between the wild and captive populations, such as differing rates of inbreeding and prior levels of variation. Also, it is apparent that high juvenile survival occurs in the less variable populations of koalas; in fact, some of these populations are overabundant (Melzer et al., this issue).

There has been little direct study of the relationship between genetic variation and fitness in wild koalas. Because variation at major histocompatibility (MHC) loci affects reproduction and survival, their analysis may be particularly relevant to conservation management (Sanjayan et al. 1996). Incomplete characterization of the variation, however, has precluded analysis of variation at the population level (Houlden et al. 1996c). The only other investigation of the relationship between genetic variation and fitness in wild koalas is a study of seminal quality. In southern populations with low genetic variation, semen samples from wild males showed relatively low levels of normal, active sperm (Wildt et al. 1991). It is possible that this is a correlate of the low variation, as is seen in lions (Wildt et al. 1987), but no conclusions can be drawn without a comparative sample from other koala populations.

Genetic variation is thought to be particularly important in adaptation to continually varying parasite infestations (Lively et al. 1990). Four major factors might affect the severity of parasitism in koalas, especially when

there is natural or artificial movement of koalas or parasites: (1) genetic differences among the parasite strains infecting koalas, (2) genetic differences among koalas, resulting in either partial resistance to infection or predisposition to severe pathogenesis, (3) environmental factors such as suitable habitat, and (4) prior exposure of individuals. The most common and serious infections of koalas are those caused by the intracellular bacterium *Chlamydia* (S. Brown et al. 1987). Almost all Australian koala populations are affected, with infection rates ranging from 20% to 100% (Jackson et al. 1999). Genetic differences in the koala's response to *Chlamydia* are suggested because the level of clinical disease is often much lower than infection rates in a population (Jackson et al. 1999). We will soon be able to address the question of whether reduced genetic diversity in some koala populations is associated with increased parasite burden and disease, as seen in other species (Lively et al. 1990). It is likely, however, that prior exposure also modifies the severity of the disease, because koalas from *Chlamydia*-free areas translocated to *Chlamydia*-infected areas are devastated by the disease (Lee et al. 1990).

Genetic work has revealed the origins of chlamydial strains, resulting in important management implications. The DNA sequencing of two outer-membrane protein genes showed that the koala chlamydial strains were not *C. psittaci*, as previously thought, but were either *Chlamydia pecorum* or *Chlamydia pneumoniae* (Glassick et al. 1996). *C. pneumoniae* primarily causes a respiratory infection in humans. In two free-range koala populations, *C. pneumoniae* was never associated with koalas showing outward clinical signs of disease (Jackson et al. 1997); thus, this species might be considered as primarily commensal in koalas. *C. pecorum* causes infections in sheep, cattle, and pigs. In four free-range populations of koalas, *C. pecorum* was always the most common chlamydial infection, with levels ranging from 20% (one Queensland population) to 100% (one South Australian population) (Jackson et al. 1999; A. Fowler, personal communication). *C. pecorum* causes infections of the eyes and the urogenital tract of male and female koalas alike without obvious bias. Also, an unexpectedly high level of mother-to-young transmission of *C. pecorum* infection has been recorded (58%; Jackson et al. 1999; A. Fowler, personal communication).

The genetic diversity of koala *Chlamydia* must be considered in conservation planning. Different geographical locations are known to have their own unique genotype of *C. pecorum*, which may have varying site preferences and disease-causing potential. Thus the level of intergenotype cross-protection among these strains may be critical in predicting the success of translocated animals. The high genetic diversity of koala *C. pecorum* (Jackson et al. 1997) also suggests multiple origins of this parasite in koalas. Of six distinct genotypes of *C. pecorum* in koalas, several are koala-specific, whereas two

are almost identical to the *C. pecorum* strains found in Australian sheep or cattle. This strongly suggests that koalas have become infected by a series of cross-species transmission events, presumably from sheep and cattle. In contrast to the diversity of koala *C. pecorum*, 10 strains of *C. pneumoniae* from koalas showed no variation at four loci (Wardrop et al. 1999). This clonality might suggest that this parasite has only recently infected the koala (not enough time for parasite evolution to occur within this host) or that its interaction with this host does not lead to evolution of surface antigens. This low variation, and the apparent low pathogenicity of this species in koalas, suggests that it is not critical to assess *C. pneumoniae* variation before translocations of koalas.

## Conclusions and Conservation Implications

Available information on koalas is incomplete but leads us to a number of detailed conclusions, many of which affect management policies, especially concerning habitat preservation and translocation.

- (1) Techniques are available to identify and monitor genetic problems and to make genetic management an integral part of koala conservation, as recommended in the national koala conservation strategy (Australia and New Zealand Environment and Conservation Council 1998).
- (2) There should be no reductions in the numbers of individuals in natural populations or increase in the isolation of populations from one another. Koalas have been subject to continuing population crashes, translocation, and fragmentation, which may be reducing genetic variation within and among populations, especially in the south.
- (3) Changes in genetic variation may result in adverse conservation outcomes for koalas. There is preliminary evidence that koalas sometimes have lowered fitness if heterozygosity is reduced, but further research is needed on wild populations.
- (4) The current ecological amplitude of the species should be maintained by conservation of representative populations throughout the range. There are no clear genetic boundaries in koalas (Houlden et al. 1999), but populations from the extremities of the range should not be mixed during translocation and captive management. Genetic and morphological data and contrasting environmental conditions suggest that these populations may be adapted to different levels of rainfall, temperatures, and so forth.
- (5) Ecological monitoring and management of koalas should take into account the fact that genetic data show multiple management units in koalas. There



are multiple management units in northern Australia (New South Wales and Queensland; Houlden et al. 1996b, 1999). The apparent demographic independence of these units indicates they require separate management and monitoring. The low differentiation between most southern populations could lead to their characterization as a single management unit. This low differentiation probably does not reflect current natural dispersal but is more likely the result of recent translocations; thus, independent managing and monitoring is required for discrete southern koala populations. Genetic tools are available for investigating whether any designated pair of populations belong to the same management unit, but there have not yet been detailed studies to determine whether clear boundaries exist among management units in koalas.

- (6) Artificial immigration for genetic reasons may need to be considered in the future. Despite low variation in some populations, no population within the natural range of koalas that could be identified unambiguously as declining due to low genetic variation. To justify augmentation in particular cases, it is necessary to define a level of variation that is low enough to significantly affect fitness. Therefore, there is a need for further studies of the relationship between fitness and genetic variation in koalas. In defining criteria for augmentation, it should be remembered that the relationship between fitness and genetic variation may be nonlinear, so adverse outcomes may appear only after variation drops below a threshold (Frankham 1995).
- (7) Translocations should avoid protocols that could reduce variation within or among populations. Translocation programs have led to successful restocking, but the resulting populations are genetically undifferentiated and are derived from over-represented founding stock. If further translocations are necessary for ecological or genetic reasons, they should use stock from the same or nearby management units. Translocation programs should avoid extensive use of stock from a single source, especially source populations that have been used extensively or have low genetic variation (e.g., some island populations).
- (8) Further translocations of koalas should be preceded by genetic detection and typing surveys of the chlamydial strains present in both source and recipient populations to help predict the outcome of such translocations. Further studies are required to investigate possible correlation between levels of genetic diversity in koalas and chlamydial infection and symptoms. These studies will allow a search for genetic factors that may predispose some koalas to severe disease or protect others.
- (9) In areas where koalas share habitat with sheep and

cattle, managers must consider the possibility that these hosts represent a source of *C. pecorum* infection for koalas. It may be possible to use *Chlamydia* as a control agent in koala populations that are overbrowsing their food trees. The justification for this suggestion is that the disease is a natural one, to which the koalas are adapted, and that infection maintains their fertility within the bounds that can be supported by the environment. We cannot support this suggestion, however, because of the likelihood that several chlamydial strains in koalas are recently derived from other species and because of ethical implications.

- (10) Future translocations of koalas should be planned with appropriate controls and monitoring to provide needed data on the long-term success of translocation and its influence on the genetic composition of populations.
- (11) Management of captive populations that are to be part of a wild management program should not conflict with the guidelines for translocation of wild populations discussed in this paper.
- (12) The study of evolutionarily significant units and management units highlights the need for care in interpretation of these studies. No ESU boundaries have been identified, but because of the extensive genetic differentiation between populations, Houlden et al. (1999) suggest that distant populations should not be artificially mixed—a management recommendation appropriate for separate ESUs (Moritz 1994). Likewise, the use of the concept of management units is inappropriate in heavily perturbed populations such as those in southern Australia. Low differentiation among these populations does not necessarily mean that high dispersal is ongoing. Therefore, adjacent southern populations will not necessarily act as a source of rapid natural recolonization of one another.

In summary, there is a need to make genetic management an integral part of koala management. The necessary genetic indicators are available but often require careful interpretation when being used to derive management advice.

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