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Beyond Roadkill, Radiotracking, Recapture and F_{ST}—a Review of Some Genetic Methods to Improve Understanding of the Influence of Roads on Wildlife

Jody M. Simmons [1,2], *Paul Sunnucks* [1,2], *Andrea C. Taylor* [1,2], and *Rodney van der Ree* [3]

ABSTRACT. Habitat fragmentation continues to occur despite increasing evidence of its adverse effects on ecosystems. One of the major detrimental effects of roads and traffic is the creation of barriers or filters to the movement of wildlife, ultimately disconnecting some populations. Our understanding of the extent to which roads reduce the movement of biota is mostly based on field-based observational methods of inferring animal movement, and to a much smaller extent, on allele frequency-based genetic analyses. Field-based methods, as it is typically feasible to apply them, tend to be informative at fine temporal and spatial scales. Allele frequency-based genetic methods are informative at broad geographic scales but at timescales usually greater than recent disturbance events. Contemporary analyses based on genotypes of individual organisms (called "genotypic" approaches herein) can augment these other approaches. They can be informative at fine spatial and temporal scales, are readily scaled up, and are complementary to the other field-based approaches. In genotypic analyses, every capture can be effectively a recapture, relieving a major limitation in sample size. They can evaluate the influence of even recently constructed roads on movements and their emergent effects on important population processes at the spatial and temporal scales of interest to wildlife and infrastructure managers. Information derived from genetic and field-based methods can be used to model the viability of populations influenced by roads and to evaluate and monitor mitigation efforts. Despite some excellent examples, we suggest that such applications are still rare relative to their potential. This paper emphasizes some of the detailed inferences that can be made using different types of genetic analyses, and suggests paths by which researchers in road ecology can incorporate genetic approaches. We recommend that the proven capacities of genetic techniques be routinely explored as approaches to quantify the diverse influences of roads on wildlife populations. With appropriate expertise, molecular ecology can be done extremely inexpensively. It is conducted within the same funding frameworks as field-based approaches and, in budgeting funding applications, molecular ecology maintenance costs are about 20–30% of payroll, in line with other disciplines and approaches. This and other common arguments against application of genetic approaches are often based on misconceptions, or limitations that no longer apply.

Key Words: *barrier; dispersal; gene flow; genotypic analyses; habitat fragmentation; road ecology*

INTRODUCTION

The length and area of transport networks (including roads, railways, utility easements, and canals) are rapidly increasing, and roads are characteristic of almost all of the earth's landscapes (Forman et al. 2002). Among the many adverse and widespread effects that roads and associated traffic inflict upon the environment (Trombulak and Frissell 2000,

Forman et al. 2002), habitat fragmentation is considered one of the greatest (Forman and Alexander 1998). Roads act as barriers that prevent or reduce the movement of biota, known to include mammals, birds, amphibians, reptiles, and invertebrates (e.g., Develey and Stouffer 2001, Goosem 2001, Andrews and Gibbons 2005, Koivula and Vermeulen 2005, van der Ree 2006, McGregor et al. 2008). In addition to reductions in the total

[1]Monash University, [2]Australian Centre for Biodiversity, [3]Australian Research Centre for Urban Ecology, Royal Botanic Gardens Melbourne

amount of habitat, patch size, and habitat quality (Fahrig 2003), isolation of remnant populations resulting from reduced connectivity among habitat patches is considered one of the greatest threats to species persistence posed by habitat fragmentation (Reed 2004). Reduced rates of dispersal of wildlife can increase the risk of local extinction by disrupting metapopulation dynamics, inhibiting recolonization, preventing access to additional resources, and harmfully altering genetic structures of populations and individuals (Koenig et al. 1996, Colas et al. 1997, Boudjemadi et al. 1999, Thomas 2000, Rodriguez and Delibes 2003, Jansen and Mushinsky 2008, Masso et al. 2008). Important population processes that rely on dispersal and movement, such as annual migrations (Mansergh and Scotts 1989), may also be disrupted. Therefore, understanding the influence of roads on the dispersal and gene flow of wildlife is essential for appropriate management of infrastructure and wildlife populations.

To date, many of the studies focusing on the barrier effect of roads have relied on field-based methods such as capture-mark-recapture (CMR) and radiotelemetry. The much smaller number of studies applying genetic techniques have relied largely on traditional population-level analyses, often based on FST (see Appendix 1 for a glossary of common genetic terms) to quantify population differentiation based on differences in frequencies of genetic variants among population samples. Only a small number of studies have taken advantage of more recently developed analyses based on genotypic data (sensu Sunnucks 2000, i.e., based on multiple genetic markers per individual). However, there are significant advantages to adding these approaches to the toolkit because of their ability to identify dispersal at multiple temporal and spatial scales including short/fine ones and in principle for any organism (Table 1). This paper does not aim to provide an exhaustive review of techniques used in road ecology. Rather, we aim to demonstrate how genotypic analyses can substantially increase the information content of genetic data sets compared with traditional analyses, and also in the context of available field-based methods. Our approach is to outline ways in which inferences about activities of individual organisms and their population processes can be made with different types of contemporary genotypic analyses and examine how they can augment field-based and allele-frequency-based genetic methods.

PERSPECTIVES ON APPROACHES TO ASSESSING POPULATION BIOLOGY IN THE CONTEXT OF ROADS

Field-Based Methods of Inferring Movement— Uses and Limitations

For the purposes of this paper, "field-based methods" refer to non-genetic methods of inferring or observing the movement of wildlife, although genetic approaches clearly have a field component also. The most common method of quantifying the barrier effect of roads on wildlife is field-based observations, especially CMR (e.g., Baur and Baur 1990, Burnett 1992, Goosem 2001, McDonald and St. Clair 2004, Andrews and Gibbons 2005, Marsh et al. 2005, Rico et al. 2007, Wilson et al. 2007, McGregor et al. 2008). This approach has been useful in providing information about day-to-day movements of animals and how roads and landscape features influence them. For example, Bhattacharya et al. (2003) used CMR to study the influence of roads on bumblebees (*Bombus impatiens*, *B. affinis,* and *Xylocopa* species), and found that although they rarely cross roads, they do have the ability to do so. Despite their value for studying types of animals that can be trapped and marked, CMR usually requires substantial equipment and personnel, not least because recapture rates fall multiplicatively with the number of captures required. There are also significant safety issues for researchers working on and adjacent to roads. For animals that can be potentially harmed by reduced feeding or injury in traps, ethical considerations require frequent checking of traps and few fieldworkers would deny the intensive nature of the work and sometimes modest returns. The intensive nature may mean reduced trapping effort, which ultimately reduces the potential to detect dispersal events (e.g., Rico et al. 2007, McGregor et al. 2008). Burnett (1992) used a trapping approach to investigate the effect of a sealed road 6–12 m in width on the native rodent *Melomys cervinipes* in north Queensland. No individuals were recorded crossing the road in a total of 15 nights of effort (3 trapping nights across 5 months when baited traps were placed on both sides of the road).

Approaches based on the growing array of increasingly effective electronic devices may alleviate some of the above drawbacks of CMR, as well as provide more spatially and temporally detailed information than CMR because data are not limited to the points of capture associated with

Table 1. Summary of the main characteristics of field-based and genetic methods.

Field-based methods	Genetic methods
Include capture-mark-recapture, radio/satellite tracking	Include allele frequency- and genotype-based analyses
Generally provide only a snapshot of processes or events at the time of the study	Can infer movement or dispersal over several temporal scales
Limited to animals that can be marked or are large enough and otherwise appropriate to carry transmitter	Virtually no limitations on the type of wildlife that can be studied, if amenable to genetic analysis
Good for examining effect of recently and historically constructed roads	With contemporary fine-scale analyses, good for examining effect of recently constructed roads
Can miss rare or long-range dispersal	Powerful for detecting rare or long-distance dispersal and sex-biased dispersal
Low sample size due to low recapture rate, loss of radio-collars, mortality etc.	No need to recapture to infer fine-scale and population-level movement
Generally invasive—capture at least once, usually more often	Usually at most single capture, or zero captures when non-invasive techniques are appropriate and feasible

subsets of behavior, notably feeding on bait (see Wheatley and Larsen (2008) for comparison of habitat use when data are derived from trapping with bait and radiotracking). Radiotracking is very long established and well used (Rondinini and Doncaster 2002, McCleery et al. 2006, van der Ree 2006, Sheppard et al. 2008; Cesarini et al., unpublished data). Devices are becoming miniaturized to the point of suitability for even some invertebrates (e. g., robust beetles; Hedin et al. 2008) and the limitations of battery life are being reduced. Data retrieval may be made less arduous and expensive by emerging technologies, for example via global positioning system (GPS) collars that generate data retrievable by recapture or satellite (Dyer et al. 2002, Dodds et al. 2007, Coulon et al. 2008). These exciting developments are becoming more generally applicable as the significant cost and weight of the systems diminishes. Systems based on radio-frequency identification (RFID) tags and related transponders that deposit data to a detector can be very effective.

Notwithstanding their contributions to understanding mobility of organisms, the physical tracking systems briefly surveyed above are subject to some limitations. The devices necessarily trade off weight against longevity and are currently more suited to larger animals. Costs of equipment including tracking devices and detection systems are generally high. Coupled with the challenges of capturing (and usually recapturing to retrieve devices and/or data) enough suitable individuals of interest that can be treated ethically (e.g., allow room for growth inside comfortable collars), sample sizes tend to be modest to small. There is usually attrition of numbers throughout a study due to mortality or dispersal, as well as the loss and failure of devices. These issues also generally combine to lead to temporally narrow windows of observation relative to the potential dispersal period of individuals. Thus, current electronic tracking studies may fail to detect dispersal events that are life-stage specific or rare (Koenig et al. 1996, Stow et al. 2001, Berry et al. 2004).

There is substantial need for approaches that are less dependent on recapturing, are applicable to more kinds of organisms, and can be conducted simultaneously on numbers of individuals in the hundreds or thousands, rather than the tens. Genetic, and more specifically genotypic, approaches can make contributions here.

Inferring Road-Related Behavior from Genetic Techniques—Features and Inferences

Genetic analyses can be applied to any organism with obtainable genetic material and tailored to a range of temporal and spatial scales (Sunnucks 2000, Pearse and Crandall 2004, DeYoung and Honeycutt 2005). With appropriate sampling, they can provide important information about movements of individuals and their gametes (including dispersal, habitat use, and gene flow) among locations, and a broad range of management-related questions can also be addressed by censusing individuals within a population. These applied questions include the design and planning of landscapes, habitat restoration, and mitigation (a non-exhaustive list of tasks, approaches, and analytical software is in Sunnucks and Taylor 2008, along with some key overview citations).

The influence of roads at the population level and intermediate time scale

Genetically effective dispersal within natural populations can be inferred using genetic methods (Slatkin 1985). A range of genetic markers (heritable characters with multiple states at each character) and statistical analyses are available for use in population biology (Sunnucks 2000, Pearse and Crandall 2004, DeYoung and Honeycutt 2005). Inferences on dispersal have generally been based on measures of genetic sub-structure (influenced by gene flow) such as F_{ST}, using allele frequencies to estimate gene flow and geneticallyeffective dispersal among populations (Holderegger and Wagner 2006). These analyses focus on the population level, and there is a large literature applying them to estimate demographic and population genetic effects of fragmentation (e.g., Hitchings and Beebee 1998, Fang et al. 2003, Williams et al. 2003). They have been used to detect the barrier effect of roads in taxa including invertebrates, amphibians, reptiles, and mammals (Reh and Seitz 1990, Gerlach and Musolf 2000, Keller and Largiadèr 2003, Epps et al. 2005, Kuehn et al. 2007). However, for this to be effective, there must be sufficient time since disturbance and effective population sizes must be sufficiently small for allele frequencies to become spatially patterned to practically detectable levels. Thus, frequency-based methods can fail to detect reduction in dispersal over time scales shorter than several generations (Berry et al. 2005). This has been noted in the context of road ecology because many large roads (e.g., motorways) are relatively young (Sheppard et al. 2008). Much of the criticism about using genetic approaches is based on these limitations of genetic analyses. However, for the last 15 years or so, genetic methods for inferring dispersal or movement at shorter time scales and levels (population and individual level) have been practical and are beginning to be taken up more widely in applied situations.

Influences of recently constructed roads can be detected—genetic analyses at the individual level

Individual-based approaches for inferring dispersal have been very widely adopted for studies in habitat alteration unrelated to roads (e.g., Stow et al. 2001, Kraaijeveld-Smit et al. 2002, Manel et al. 2003, Banks et al. 2005, Berg and Vigilant 2007, Taylor et al. 2007), yet relatively few road-ecology studies have applied these methods. Rather than assess allele frequencies of groups of individuals, genotypic arrays of individuals are used and often consist of genotypes at multiple hypervariable microsatellite loci (Selkoe and Toonen 2006). Together with the appropriate statistical tests, such as assignment tests and parentage analysis (Sunnucks and Taylor 2008; Appendix 1), individual-based approaches can detect altered dispersal patterns in fragmented habitat where allele frequency-based approaches cannot (Castric and Bernatchez 2004, Latch et al. 2006). For example, genotypic data indicated philopatry in females and male-biased dispersal in the agile antechinus (*Antechinus agilis*), despite the slight genetic structure evident from low F_{ST} values (Kraaijeveld-Smit et al. 2002). Thus, with a modest array of microsatellite loci (in the order of five to ten for a typical outbred species), there do not have to be significant allele frequency differences in order to detect mobility changes at the individual level, nor must several generations elapse. Every individual has a unique genotype referable to its parents. Therefore, genotypes are suitable for detecting alterations in movement patterns due to recent disturbances, including roads (Keller et al. 2004, Proctor et al. 2005, Riley et al. 2006, Schmuki et al. 2006b, Millions and Swanson 2007, Pérez–Espona et al. 2008).

A recent example of a road study using genotypic data is that by Riley et al. (2006), who studied the influence of the southern Californian Ventura Freeway on coyotes (*Canis latrans*) and bobcats (*Lynx rufus*). These two species typically live for 10

and 12 years, respectively (Kurt 1995) and the freeway was constructed only 47 years before the study began in 1996. Using data from 14 microsatellite loci, Riley et al. (2006) conducted both F_{ST}-based and genotypic analyses. The genotypic analyses assigned individuals to clusters of genetically similar individuals based on genotypes and independent of sampling locality. They also radiotracked 110 coyotes and 87 bobcats between 1996 and 2003. Based on radiotracking data, they found that the freeway was a significant barrier for both species (only 4.5% of radiotracked coyotes and 11.5% of radiotracked bobcats crossed in the 7-year period), and that the home ranges of bobcats did not cross the freeway (data were insufficient for coyotes). The genotypic analyses detected additional movements across the freeway: for the coyotes, two populations (north and south of the freeway) were identified, and two individuals caught south of the freeway were assigned genetically to the northern population. The radiotelemetry conducted on one of these individuals only ever recorded it on the south side, suggesting it had already crossed the freeway before tracking began. Of the 49 individuals assigned genetically to the southern population identified by STRUCTURE, only 20 were actually captured north of the freeway. Again, 18 of these 20 were radiotracked and none were shown to have crossed the freeway, suggesting they had crossed before being fitted with radio-transmitters. For bobcats, the final estimate of the number of migrants was similar using genetic techniques (12.3%) and radiotelemetry (11.5%), but assignment tests still identified some of the apparent non-road-crossing individuals as originating from the population on the opposite side of the freeway to where they were caught. Most importantly, despite 20 migrants across the freeway (5%–32% of sampled carnivores), there was still significant genetic differentiation across the freeway. The estimated migration fraction was less than 0.5% per generation (estimated by coalescent simulation modeling), consistent with the idea that most of the migrations across the freeway must have been reproductively ineffective. In other words, animals crossed the freeway and did not successfully mate and reproduce after arrival. Individuals that cross barriers may be genetically ineffective for several reasons including the presence of local demes, competition for partners, and seasonal breeding (Oxley et al. 1974). This study illustrates how genetic analyses at different

levels in addition to field-based observations can provide detailed insight into the influence of roads on wildlife. This is important given that several population processes rely on dispersal and movement of individuals.

The Riley et al. (2006) study also exemplifies another problem with many field-based studies: it is hard to know what rate of crossing would be expected if roads were not barriers. For the bobcats, home ranges bordering a freeway were smaller than those further away. Although this comparison indicates what can be expected near and away from freeways for home ranges, it is hard to estimate what percentage of animals crossing the freeway would be expected if the freeway was not a barrier. Genetic analyses can assist in identifying expected rates of movement in the absence of the barrier. For example, several studies have collected genetic material at a range of distances to determine the relationship between genetic distance and geographic distance, and then compare this with genetic distance in animals either side of a barrier. The extent to which a road acts as a barrier can then be expressed as additional geographic distance (e. g., Gerlach and Musolf 2000). Trapping could also be conducted at varying distances away from roads to gain an idea of the sorts of movements that can occur away from roads and compare this with the movements across roads. However, because trapping provides only a snapshot view of the movements occurring, it lacks resolution for detecting medium- to long-term movement patterns.

Landscape genetics ("an amalgamation of molecular population genetics and landscape ecology"; Manel et al. 2003) is relevant in road ecology because inferences can be made about the movements of animals in fragmented and non-fragmented areas. Landscape genetics incorporates fine-scale genetic with precise geographic information about where each sample was collected and is informative about the way different landscape features influence dispersal and gene flow (Manel et al. 2003, Schmuki et al. 2006b, Lada et al. 2008b). Generally, individuals are the operational unit and populations are not defined in advance; rather, they are inferred by using genotypes to assign individuals to genetic groups that can be examined for geographic locality and associated landscape features (Manel et al. 2003, Riley et al. 2006).

Detecting alterations to important population processes

With appropriate study designs, genotypic approaches can identify important within-population processes, such as kin and social structure, demographic history, sex-biased dispersal, and the influence of anthropogenic disturbances on them (e.g., Stow et al. 2001, Kraaijeveld-Smit et al. 2002, Stow and Sunnucks 2004a, b, Banks et al. 2005, Lada et al. 2007). The powerful insight into population processes is exemplified by three companion papers on Cunningham's skink (*Egernia cunninghami*), exploring how dispersal among rocky outcrops was inhibited by deforestation (Stow et al. 2001, Stow and Sunnucks 2004a, b). Despite a dramatic increase in pairwise relatedness among individuals within deforested sites, incidence of inbred matings was low and not significantly different from forested sites, attributed to inbreeding avoidance. However, reduced reproductive success due to mate limitation was evidenced by the significantly higher number of unmated males within the fragmented sites, which may have long-term implications for population persistence (Stow et al. 2001, Stow and Sunnucks 2004 a, b). Genotypically detectable impacts of fragmentation on the mating system of agile antechinus have also been reported (Banks et al. 2005).

Comparing pre- and post-road construction movement patterns and evaluating effectiveness of barrier mitigation

In addition to inferences about dispersal parameters at the most recent and intermediate time scales, genetic analyses have also been used to infer pre-fragmentation population structure, gene flow, and genetic diversity (Trizio et al. 2005, Taylor et al. 2007, Lada et al. 2008a). This provides an understanding of how habitat alterations have impacted populations and altered natural movement patterns, and potentially may predict future outcomes for populations. Lada et al. (2008a) used a combination of microsatellite markers and mtDNA sequence data to assess current and past gene flow in the Yellow-footed antechinus (*Antechinus flavipes*) along the Murray River in southeastern Australia. They constructed a network of relationships among mtDNA variants and conducted simulations in order to identify migration models that may have led to the present-day mtDNA variant frequencies. They then compared this information with the contemporary gene flow inferred from microsatellite data and a simulation to identify scenarios that may have led to the current genetic structure. The data suggested historical gene flow along the river, but anthropogenic inhibition of gene flow over the past 50 years along the river through habitat loss, intensive agricultural practices, urban construction, and altered river flow regimes. These approaches are completely transferable to road ecology in all important details including spatial scale, time scale, and technical aspects.

A powerful additional approach to estimating pre-disturbance gene flow and genetic diversity is through the use of preserved material such as museum specimens collected before disturbance. For example, Taylor et al. (2007) compared genetic diversity in current samples of the greater glider (*Petauroides volans*) in southeastern Australia with museum samples collected in 1966. The contemporary samples were collected from patches of eucalypt forest separated from each other by pine plantations and logging roads, and the 1966 samples were collected from the same locality at the time of clearing for these pine plantations. Genetic diversity of individuals in the most isolated (temporally and geographically) patches was significantly lower than the 1966 samples, although diversity was not as low as would be expected under total isolation. This was compatible with the results of assignment tests, which detected rare, long-distance movements that were completely unexpected on the basis of classic assumptions about the biology of the species. Interestingly, a 12-mo radiotracking study conducted at the time of the collection of the contemporary samples detected only one occurrence of inter-patch movement (1 km between patches) (Pope et al. 2004), whereas the genotypic analyses identified five dispersers, one of which was 7 km from the patch in which its genetically identified parents were sampled (Taylor et al. 2007).

Pre-disturbance vs. post-disturbance can also be studied on the most recently constructed roads and also roads that are about to be built or widened. With individual-level analyses, if pre-construction samples are available, or if sampling is conducted at regular intervals following construction (e.g., annually, bi-annually etc.), any changes in movement patterns can be monitored. Likewise, evaluation of the effectiveness of recently built or planned mitigating devices can be done. In fact, thorough monitoring programs for mitigating

devices are rare and do not adequately use genetic analyses (van der Ree et al. 2007, Corlatti et al. 2009). This is not because of technical difficulty or insurmountable practical issues: non-invasive tracking (through genetic identification from hairs) of indidivual grizzly bears applied to populations in Montana (Kasworm et al. 2007) is directly transferable to the situation of monitoring reconnection through a mitigation structure. Comprehensive assessments of dispersal patterns before and/or at regular times after wildlife crossing structures are installed would be valuable, and should be conducted given the large financial investment involved in mitigation works. Allele frequency-based approaches are not as suitable because population-level differentiation would not be detectable immediately after the device is implemented. Tracking movement at the individual level using genotypic analyses and techniques such as radiotelemetry would be the first step in the assessment of mitigation success. With genotypic analyses, it would be possible to identify individuals that have crossed away from their natal population. Then, as generation time since the installation of mitigation increases, changes to population-level gene flow would be detectable. Again, although it is difficult to find examples where this has been conducted for road ecology (or for any other types of mitigation for habitat disruption), analogs are found in broader fragmentation biology. After 70 years of agricultural use and deforestation, gene flow in Cunningham's skinks was reduced to the extent that spatial autocorrelation of alleles was created, even though there was still strong local genetic structure at finer scales, and low levels of on-going dispersal (Stow et al. 2001). Under successful mitigation, we would expect to see such structuring of allele frequencies dissipate.

Powerful new modeling methods that incorporate genetic and GIS information to infer how wildlife move throughout the landscape have been adopted recently (e.g., Coulon et al. 2004, Vignieri 2005, Epps et al. 2007, McRae and Beier 2007). For example, Epps et al. (2007) and Lada et al. (2008a) incorporated information about dispersal inferred with high-resolution genetic data into least-cost models to predict the relative resistance to mobility (cost) of various routes throughout the landscape (see Epps et al. 2007 for overview). McRae and Beier (2007) developed an "isolation-by-resistance" model based on electrical circuit theory that considers all possible pathways connecting populations. These models are able to identify landscape features that impede or facilitate gene flow, and thus are highly relevant to road-related fragmentation studies. Yet, only a small number of road studies have modeled mobility, let alone incorporated genetic data to infer the influence of roads as barriers to wildlife (Coulon et al. 2004, Andrews and Gibbons 2005, Jaeger et al. 2005).

Potential challanges with genotypic analyses, and their solutions

Genetic approaches for a given application in population biology are dependent on sufficient genetic variation existing within or among individuals or samples. Multiple variable markers may not be available for a given target species, in which case marker development may be necessary. This can be challenging for certain taxa (e.g., Tenebrionid beetles, Schmuki et al. 2006a) and costly. However, even the very effective modern methods for developing microsatellite markers (e. g., Edwards et al. 1996) are rapidly being supplanted by inexpensive "next-generation" genome sequencing (Ellegren 2008, Abdelkrin et al. 2009). For example, provision of 10–20 microsatellites based on this latter technology is currently in the order of US$2900(= Euro 2000 = UK pounds 1700), and declining rapidly. In any case, the requirement for marker development is declining rapidly owing to the exponential growth in species for which microsatellites have been developed and accumulation of massive generalizable genetic resources (e.g., Backström et al. 2008, Kimball et al. 2009).

A potential limitation arises if source populations are extremely similar in frequencies: it becomes more difficult to genetically assign individuals as frequencies become more similar. Nonetheless, it is only under very flat genetic gradients that this becomes problematic for many kinds of assignment test (Latch et al. 2006). This is because it is very rare for locality samples to be truly panmictic, so local "signature" allelic correlations are typically evident. Even where assignment tests are ineffective, this does not prevent much useful information being derivable from other analyses based on individual genotypes, including spatial autocorrelation of genotypes, parentage or relatedness, tracking of an individual's unique genotype in space, and its reproductive success (Castric and Bernatchez 2004, Stow and Sunnucks 2004a b, Latch et al. 2006, Saenz-Agudelo et al. 2009). In a study of banner-tailed kangaroo rats (*Dipodomys spectabilis*), offspring were assigned

genetically to parents, which identified individuals that had moved out of their birth sites or populations before first capture. Based on trapping alone, it would not have been apparent that the offspring captured had already moved out of their natal sites (Waser et al. 2006).

Naturally, sample size can also limit the resolution of analyses that can be conducted, and sampling design can be critically important for correct biological interpretations (Latch and Rhodes 2006). Low capture success can be less of an issue for genetic than some other approaches because, for most genetic approaches, recaptures are not necessary for inferences of gene flow and dispersal (i.e., the maxim "Few birds have leg bands, but all have genotypes" is relevant here; Waser and Strobeck 1998). To assign individuals to source populations, there needs to be sufficient sampling of potential sources, although even without this, more limited inferences can be made (e.g., an individual is locally born as opposed to not) with assignment tests (Manel et al. 2005).

Although there are ethical considerations associated with taking genetic samples, the procedures are often no more invasive than those used for marking individuals. In any case, such concerns are mitigated to some extent by the fact that only one sample (one capture) is required for most purposes, and in some cases, samples can be obtained non-invasively. For instance, Berg and Vigilant (2007) identified three dispersal events in the long-lived Cross gorilla (*Gorilla gorilla diehli*) in a recently fragmented landscape. They did so using DNA extracted from feces, which highlights another feature of genetic analyses—it can be non-intrusive (see also Banks et al. 2003, Piggot et al. 2006, Epps et al. 2005). In fact, complex inferences have been made from a combination of non-invasively sampled DNA and genotypic analyses, including social interactions and changes in demographic processes as a consequence of resource structure in the southern hairy-nosed wombat (*Lasiorhinus latifrons*) (Walker et al. 2006, 2007, 2008).

Genetic analyses are accessible to non-geneticists

A reason sometimes advanced for not using molecular ecology approaches in landscape and wildlife management questions is a perception that the methods are "expensive." Being active in combining field and genetic approaches including a collaborative project on road ecology, we do not share this perception and are not aware of data in support of it. Owing to complexities and idiosyncrasies, formal cost–benefit analyses comparing approaches would be complex, but in any case, we regard field and genetic approaches as complementary rather than competitive. In advance of costed case studies, we offer the following observations in support of the contention that molecular approaches are not more expensive than "equivalent" field-based approaches. In budgeting funding applications and research agreements with end-users, molecular ecology maintenance costs are about 20%–25% of payroll, in line with other disciplines and approaches. In terms of some major equipment and consumables: a four-wheel-drive vehicle costs about the same as an automated genotyper, a single GPS collar (the variety that must be retrieved and downloaded, with about a 50% chance of loss or failure) for use on a 4-kg mammal costs as much as one person's supply of *Taq* polymerase for a year. We further illustrate the comparative costs based on studies of the northern hairy-nosed wombat. After years of monitoring by expensive and disruptive field effort, managers opted for non-invasive genotyping of hairs collected at burrow entrances as soon as it was available (Banks et al. 2003), on the basis that these are considerably cheaper and more informative than the entirely field-based alternatives. Five censuses of the entire species have been conducted, each costing AU$15,000–20,000 (US$13,000–17,500) in combined laboratory consumables and salary. In 2000, a genetic census produced 95% confidence intervals of 96–150 individuals within the population, compared with 42–186 for the 1993 trapping census (Hoyle et al. 1995). The hair census revealed twice as many individuals as the trapping it followed (Banks et al. 2003). That program has yielded many insights that would not have arisen from trapping, and the approach has been completely transferable to a congener, for which the biology is now much better understood as a result (Walker et al. 2006, 2007, 2008).

The genetic techniques described here are often cost effective, efficient, and uniquely informative when applied by appropriate practitioners. For researchers or management agencies without access to a genetic laboratory, collaboration with a university or commercial research laboratory with a proven, directly relevant track record can provide a means to accomplishing valuable genetic analyses and interpretation (Sunnucks and Taylor 2008). The first step in designing a study using genetics is to identify

the species at risk or of interest, often achieved by engaging local ecologists. Once target species have been identified, it is important to get appropriate population genetic input at the outset (i.e., when designing the study) to determine the availability of suitable genetic markers for the time scale and questions concerned, and to develop or adapt them from existing materials. The next step is to design the study and collect the tissue samples. The importance of spatial distribution and the number of samples required will depend on the age of the road, the likely spatial scale of operation of the organism(s), and the type of information desired. Data collection, analysis, and interpretation can then be conducted. Feasibility and pilot studies may be appropriate for testing the effectiveness of the system (e.g., Sloane et al. 2000) and for determining the spatial scale and intensity at which to sample. An ideal situation is to have samples that pre-date road construction, which can then be followed up with collection of samples immediately and longer term following construction.

Combining genetic approaches with study designs to increase inferential power

Roedenbeck et al. (2007) suggested that there is a general trend for low inferential power in most studies of the effects of roads and traffic on the natural environment. They outline a range of study designs with different levels of inferential power to quantify the road effects, ranging from a replicated manipulative design, where a thorough before-and-after study of the population can be conducted, to a simpler design involving control sites and after-impact sites. We propose that whichever of Roedenbeck's approaches is adopted, future studies should make full use of the blending of genetic and field-based methods with statistical modeling. Genetic approaches can answer the five questions that Roedenbeck et al. (2007) consider critical to fully understanding the ecological effects of roads and traffic: (1) Good designs can incorporate genetic data on movement and population-level effects and demographic data from field-based methods into models to assess the viability of populations impacted by roads. (2) The influences of roads compared with other anthropogenic impacts can be assessed with carefully designed studies. (3) The effectiveness of mitigation devices can be assessed in manipulative experiments and carefully designed pseudo-experiments (i.e., using treatments not specifically conducted for the experiment). For example, movement patterns, different kinds of genetic diversity, and gene flow can be assessed before and after installation of a mitigation device. If a "before" assessment cannot be made, populations located in close proximity to mitigation devices can be monitored and compared with more distant populations and genetic data are often quite effective at estimating prior conditions (e.g., Lada et al. 2008a above). (4) Genetic and demographic monitoring with and without mitigation devices is needed to parameterize models of the long-term viability of populations impacted by human activities. (5) The importance and extent of the barrier effect on wildlife can be assessed at the individual-, population-, and landscape- scales.

CONCLUSION

Although field-based observations of the influence of roads on the movement of wildlife can be informative about short-term movements and the potential for different species to move over distances equivalent to the width of roads, they are often limited in sample size and temporal or spatial scale of observation and inference. Conducting comprehensive genetic analyses at appropriate scales can contribute to a more detailed and broader understanding of how roads influence wildlife. This is especially true for long-lived animals and in areas where roads are a relatively recent introduction to the landscape—here, individual-based analyses will provide far more resolution than population-based genetic analyses. Genetic techniques can be used to identify disruptions to important population features such as dispersal and any associated sex bias, input directly into habitat connectivity modeling, and can also be used to examine the influence of roads on kin, mating, and social structure of wildlife that are important to persistence of populations. This type of information is logistically difficult to obtain using other methods, and can be incorporated into models predicting the long-term viability of populations affected by roads. Road networks are continually expanding. Using all available science to advance our understanding of the influence of roads on wildlife at different time scales, and predicting the associated long-term effects, is therefore crucial.

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APPENDIX 1. Glossary of Some Common Population Genetic Terms

Alleles: Alternative forms of the same gene region/locus.

Assignment test: A statistical approach to ascribing individuals to their most probable natal populations on the basis of multiple DNA markers (Manel et al.2005).

FST: A classic measure of population genetic differentiation based on differences in frequencies of genetic polymorphisms (Neigel 2002). It varies between 0 (no differentiation) and 1 (completely different).

Genotypic arrays: combinations of genotypes across multiple loci (Sunnucks 2000).

Landscape genetics: a discipline combining landscape ecology and population genetics (Manel et al. 2003).

Locus (plural loci): A defined DNA region that can be compared among samples.

Microsatellite: A class of highly resolving DNA locus often applied in molecular population biology (Selkoe and Toonen 2006).

Mitochondrial DNA (mtDNA): The DNA within the mitochondria found within cells, typically inherited through the maternal line in animals.

Parentage analysis: Attribution of offspring to parents based on genotype data (Marshall et al. 1998).

Polymorphism: A polymorphic locus has more than one allele.