

## INVITED REVIEW

# Molecular road ecology: exploring the potential of genetics for investigating transportation impacts on wildlife

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## Abstract

Transportation infrastructures such as roads, railroads and canals can have major environmental impacts. Ecological road effects include the destruction and fragmentation of habitat, the interruption of ecological processes and increased erosion and pollution. Growing concern about these ecological road effects has led to the emergence of a new scientific discipline called road ecology. The goal of road ecology is to provide planners with scientific advice on how to avoid, minimize or mitigate negative environmental impacts of transportation. In this review, we explore the potential of molecular genetics to contribute to road ecology. First, we summarize general findings from road ecology and review studies that investigate road effects using genetic data. These studies generally focus only on barrier effects of roads on local genetic diversity and structure and only use a fraction of available molecular approaches. Thus, we propose additional molecular applications that can be used to evaluate road effects across multiple scales and dimensions of the biodiversity hierarchy. Finally, we make recommendations for future research questions and study designs that would advance molecular road ecology. Our review demonstrates that molecular approaches can substantially contribute to road ecology research and that interdisciplinary, long-term collaborations will be particularly important for realizing the full potential of molecular road ecology.

*Keywords:* BACI study design, biodiversity hierarchy, interdisciplinary collaboration, landscape genetics, noninvasive genetic sampling

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## Introduction

Transportation infrastructures such as roads, railroads and canals have become omnipresent features of contemporary landscapes. While they cover seemingly small proportions of the land surface, their ecological impacts reach far. For example, the ~6.3 million kilometres (km) of roads in the US cover only about 1% of the land, yet they affect an estimated 20% of the landscape (Forman 2000; Forman *et al.* 2003). A similar percentage of the Netherlands is impacted by roads (Reijnen & Foppen 2006) and road densities in many other developed countries, such as the United Kingdom, Germany

and Japan are 2.5–4 times higher than that in the USA (Forman *et al.* 2003). Jaeger *et al.* (2007) found that road development has decreased unfragmented areas larger than 100 km<sup>2</sup> to about 2% of the overall landscape in a West German state, and Riitters & Wickham (2003) estimated that only about 17% of US lands are more than a kilometre from the closest road. Even in developing countries, transportation networks can impact substantial percentages of the landmass (Kirsten 2006). With an estimated 28 million km of roads worldwide (CIA 2005), transportation infrastructures play a prominent role in shaping the environment, and only few areas on earth remain truly unaffected by roads.

Transportation infrastructures (hereafter: roads) affect the environment in various ways, with tremendous ecological implications. These direct and indirect road

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effects have been recognized and described for more than 60 years (e.g. Huey 1941; Hodson 1966; Ellenberg *et al.* 1981; Mader 1984) and they include the alteration of habitat, the interruption of ecological flows and increased erosion and pollution. The increasing need to understand and respond to the specific ecological impacts of roads ultimately led to the emergence of a new scientific discipline, entitled road ecology (Forman *et al.* 2003). Road ecology provides an integrated and solution-oriented framework for addressing the environmental effects of roads. Particularly, the goal of road ecology is to provide planners with practical advice on how to avoid, minimize or mitigate negative environmental impacts of transportation.

The emergence of road ecology has been paralleled by an increased interest in the application of molecular genetic approaches in ecology and natural resource management (e.g. DeYoung 2007; Geffen *et al.* 2007; Schwartz *et al.* 2007). Genetic approaches are highly valuable for applied wildlife management and general ecological research, and genetic applications for road ecology research have been proposed for several years (e.g. Hardy *et al.* 2003; Clevenger 2005; Shepard *et al.* 2008). However, molecular ecologists are not always aware of all possible road effects on animals and they often only assess whether roads affect genetic diversity and structure. On the other hand, many road ecologists are unfamiliar with recent advances in molecular ecology and often cannot evaluate how genetic approaches can help address a broader range of research questions in road ecology.

Our goal in this review is to bridge the gap between molecular ecology and road ecology by exploring the potential of genetic approaches to contribute to road ecology. Specifically, we highlight that current genetic studies often only assess local road effects on genetic population connectivity, even though many other road effects could be detected using genetic approaches. We begin by summarizing the general findings of road ecology research and then briefly review published studies that use genetic approaches to evaluate road effects. Finally, we outline molecular approaches for assessing additional road effects on all levels of biodiversity and provide recommendations for improved study designs in molecular road ecology.

### Road effects on wildlife

Roads impact individual organisms, populations, species, ecosystems and landscapes in various ways. In this article, we summarize the main road effects on wildlife and highlight information that might be particularly novel and interesting for molecular ecologists. We refer readers to other studies for more general reviews of

environmental road effects (e.g. Andrews 1990; Bennett 1993; Forman & Alexander 1998; Spellerberg 1998; Trombulak & Frissell 2000; Seiler 2001; Carr *et al.* 2002; Forman *et al.* 2003; Coffin 2007).

The effects of roads can be direct or indirect, short-term or permanent and apply to spatially restricted locations or affect extensive areas. First, roads lead to an immediate loss of suitable habitat. These losses are often much greater than the actual area covered by roads, because of road-zone effects that make areas close to roads less suitable for certain species (e.g. due to noise, Parris & Schneider 2009; Parris *et al.* 2009; or artificial lighting, Outen 2002). However, roads can also create and enhance habitat for some species, particularly small mammals. Similarly, roads can serve as movement and dispersal corridors and thus increase movement rates and gene flow over long distances. Roads can also increase movement rates of (feral) predators, and contribute to the spread of infectious diseases and exotic species. More commonly, roads serve as barriers to individual movements, for example, through behavioural road avoidance, or when roads and fences along roads present physical obstacles. These barrier effects can make certain resources (e.g. mates, food, breeding sites) inaccessible for animals, thus affecting individual fitness and overall habitat quality. Road mortality has a similar barrier effect, but can additionally reduce population sizes directly. Finally, the road network increases landscape fragmentation, resulting in small and more or less isolated habitat patches.

In combination, road-induced habitat loss, barrier effects, mortality and landscape fragmentation can lead to increased extinction risks for wildlife populations (Fig. 1, Fahrig 2002; Jaeger 2004). In addition, these road effects can also lead to increased genetic structure and decreased genetic diversity, which further reduce population viability. Of these two components, genetic structure (i.e. the distribution of genetic variation) will usually respond faster to habitat fragmentation than genetic diversity (i.e. the amount of genetic variation; Keyghobadi 2007; Lowe *et al.* 2004).

While most molecular ecologists are likely familiar with these general road effects, it is important to highlight that the different effects are highly intertwined and their relative magnitude depends on a multitude of factors, such as road-, species- and landscape-specific characteristics. Generally, wider roads with greater volumes of high-speed traffic affect wildlife populations more strongly than small, less travelled roads (e.g. Clevenger *et al.* 2001; Jaarsma *et al.* 2006). For example, traffic noise can substantially reduce breeding bird densities in proximity to busy highways (Reijnen & Foppen 2006; see also Parris & Schneider 2009; Slabbekoorn & Ripmeester 2008). However, McGregor *et al.* (2008) and

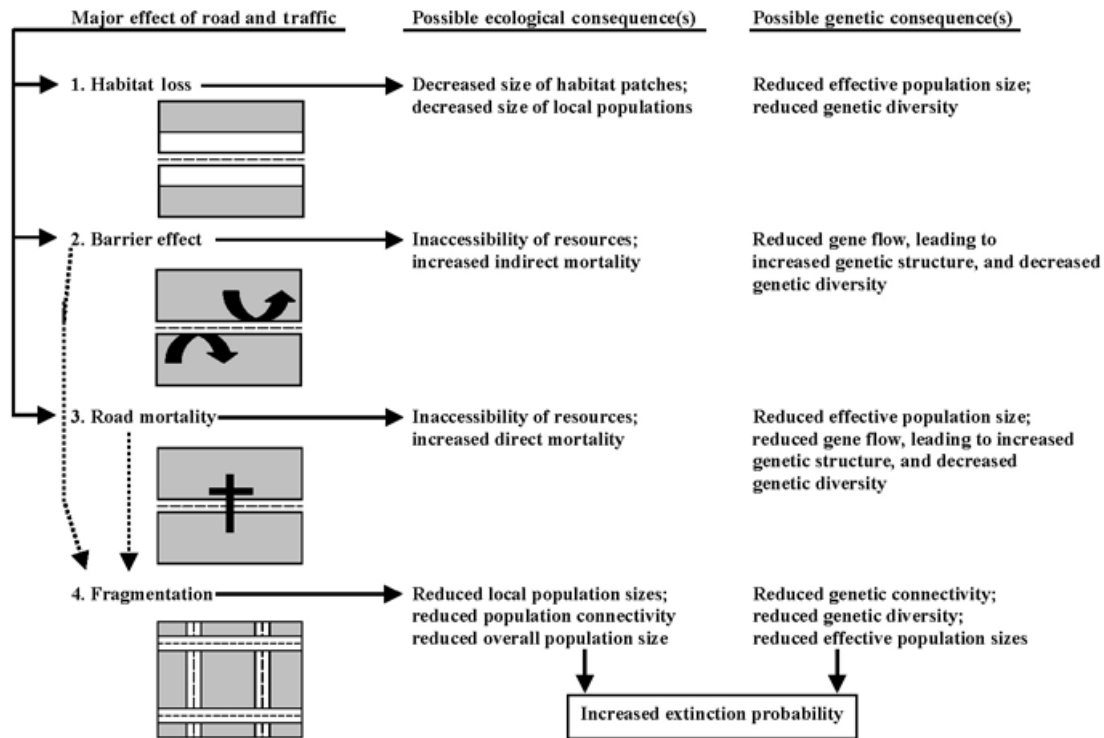


Fig. 1 Overview of major road impacts (left column) and their ecological (centre column) and genetic (right column) consequences. Adapted from Fahrig (2002) and Jaeger (2004).

Fahrig & Ford (2008) concluded that small mammals avoid crossing roads because of the road surface itself, and not because of associated traffic or noise. Also, Alexander *et al.* (2005) suggest that road mortalities increase up to a certain traffic threshold, beyond which only a few animals still attempt to cross the roads, so that road-kill frequencies decrease. While road mortalities and road avoidance can both lead to a decrease in (genetic) connectivity, distinguishing them is important for practical conservation. If connectivity is decreased because of road mortality, building exclusion fences is a suitable mitigation measure. However, fences can further exacerbate the situation if only a few animals attempt to cross the road in the first place (i.e. road avoidance; Jaeger *et al.* 2005; Jaeger & Fahrig 2004).

Clearly, species characteristics influence if and how road effects occur (Carr & Fahrig 2001; Aresco 2005; Ford & Fahrig 2007; Eigenbrod *et al.* 2008b). For example, Kerth & Melber (2009) found that roads are effective barriers for Bechstein's bats (*Myotis bechsteinii*), while barbastelle bats (*Barbastella barbastellus*) frequently cross motorways. These findings can be explained by differences in foraging behaviour and wing morphology between these two species. Such species-specific road effects are also important for conservation planning, because different crossing structures are required to mitigate road effects on different species (Clevenger &

Waltho 2005; Ascensao & Mira 2007; Grilo *et al.* 2008; Mata *et al.* 2008).

Characteristics of the surrounding landscape also influence the response of wildlife to roads (van der Grift & Pouwels 2006; Huijser & Clevenger 2006; Ng *et al.* 2008; Langen *et al.* 2009). For example, Grilo *et al.* (2009) found that road-kill rates for a variety of species were highest in areas with high quality habitat that experienced little human disturbance, because animals tend to cross roads more frequently if habitat on both sides is favourable. This also means that crossing structures imbedded in a suitable habitat usually receive greatest use by wildlife species (e.g. Ng *et al.* 2004; Clevenger & Waltho 2005).

Understanding secondary road effects can be particularly challenging. For example, Rytwinski & Fahrig (2007) found a positive relationship between abundance of white-footed mice (*Peromyscus leucopus*) and road densities, even though movement of the species is inhibited by roads. Rytwinski & Fahrig (2007) attribute this to negative effects of high road densities on the abundance of predator species, or possibly to positive (but unknown) road effects on habitat quality for mice. Similarly, Bissonette & Rosa (2009) did not detect any clear relationships between distance-to-roads and abundance or diversity of small mammal communities, probably because roads not only act as movement

barriers, but also create suitable micro-habitats for the studied species (see also Fahrig & Rytwinski 2009).

Many road effects are also only detectable after a certain time lag. Generally, effects of habitat loss and degradation are detectable earliest, followed by the effects of road-kills and landscape fragmentation (Forman *et al.* 2003). However, it is also possible that the magnitude of different road effects varies over time. For example, Shepard *et al.* (2008) suggest that snake densities could initially be decreased by road mortalities, because snakes attempt to cross newly constructed roads. Over time, behavioural adaptation should lead to fewer road crossing attempts and stable densities, and reduced connectivity across roads. Road effects can also be dependent on seasonal variations in animal behaviour, for example, when movement rates increase during breeding seasons (e.g. van Wieren & Worm 2001; Bond & Jones 2008).

Accounting for these potentially confounding factors in (molecular) road ecology is crucial for improving our understanding of road effects and the effectiveness of mitigation measures. For example, Corlatti *et al.* (2009)

note that incongruent results have been obtained from studies that evaluated the ability of wildlife overpasses to provide genetic connectivity. This is mainly because of a lack of long-term studies that combine different data types and account for confounding variables (Corlatti *et al.* 2009). Overall, analysing and interpreting road effects is a complex task, and predicting the exact mechanistic response of wildlife to roads and road mitigation measures can be challenging.

While many of these challenges also apply to research on general habitat loss and fragmentation, roads have certain unique characteristics that influence research in road ecology (Table 1). Roads lead to relatively little direct habitat loss, but the modification of habitat is extreme and it leads to extraordinarily sharp edges. This means that assessing edge and road-zone effects on habitat quality is particularly important. Also, many types of habitat conversion only lead to direct mortality during the actual conversion phase (e.g. during clear-cutting), while road-kills impact mortality rates indefinitely. As road characteristics influence how certain species respond to roads, it is also necessary to measure,

**Table 1** Unique characteristics of road effects and road ecology research

<b>Unique characteristics of roads and road effects</b>	<b>Relevance for road ecology</b>	<b>Implication for studies in (molecular) road ecology</b>
Roads lead to relatively little direct habitat loss, but habitat modification is extreme.	Roads lead to extraordinarily sharp edges in the landscapes, and road-zone effects on habitat quality can potentially be more pronounced than edge effects caused by general habitat loss and fragmentation.	In addition to assessing local (barrier) effects of roads, studies should evaluate how (genetic) estimates of habitat quality (e.g. population sizes, reproductive rates) change with varying proximity to roads.
Roads are associated with wildlife-traffic mortalities.	In addition to the indirect mortality effects of general habitat loss and fragmentation, roads can directly impact mortality rates.	Studies should attempt to determine the relative influence of road mortality vs. other road effects on observed (genetic) patterns.
Roads are associated with varying degrees of traffic, pollution, artificial lighting and noise.	Specific road characteristics will influence how certain species are affected by roads.	Studies should not treat roads as binary features that are either present or not. Instead, it is desirable to sample roads with a wide variety of characteristics and statistically evaluate the relative influence of different factors on observed (genetic) patterns.
Roads stretch over very large spatial distances.	Roads are more likely to have broad-scale (e.g. landscape-level) effects than many other causes of habitat loss and fragmentation.	Studies should specifically assess how roads/road densities affect wildlife species across broad spatial regions.
New roads enable humans and invasive species to access formerly remote areas.	The cumulative effect of roads and road-related activities may be even stronger than the overall effect of general habitat loss and fragmentation.	Studies should not only focus on direct, short-term and local road effects, but attempt to assess the virtual footprint of roads on population viability and species persistence.
From a policy and management perspective, there is an increased interest/need to mitigate ecological impacts of existing and future roads.	Studying road effects and the effectiveness of mitigation measures can potentially have a more direct influence on conservation planning than research on general habitat loss and fragmentation.	Studies should be designed and conducted in collaboration with transportation planners and focus on research questions of high relevance for practical transportation planning.

[Correction added after online publication 25 September 2009: the table had its headings put in bold text and periods added after each entry.]

report and analytically separate the different factors. Thus, simply distinguishing habitat from nonhabitat—as is commonly done in general fragmentation studies—seems particularly unsuitable for road ecology. As road networks cover large spatial areas, they are also more likely to have broad-scale effects than many other types of habitat loss and fragmentation. Furthermore, roads are likely to have indirect, long-term effects caused by road-related activities. Therefore, the overall virtual footprint of roads can be assessed only through long-term studies that focus on more than just local and direct road effects. Finally, road ecology can potentially have a more direct impact on practical conservation planning, because of increased agency efforts to include environmental considerations into road construction and mitigation (TRB 2002; Donaldson & Bennett 2004; Dolan *et al.* 2006; Karani 2008; Thorne *et al.* 2009). Overall, while theories and concepts derived from general research on habitat loss and fragmentation can apply to road effects, road ecology is unique in certain aspects (Table 1). This uniqueness of road ecology also offers many opportunities to address important questions with novel research approaches. In the next section, we briefly review studies that have used genetic data to assess road effects, and point out limitations of current studies in molecular road ecology.

### Review of studies using genetic data to assess roads effects

Our review of the literature identified 33 studies that used genetic data to detect effects of roads or urbanization (i.e. including road effects; see Table S1). We refer readers to Keyghobadi (2007) for a review of studies investigating the genetic effects of habitat fragmentation in general. The identified studies were mostly conducted on mammals ( $N = 15$ ) and amphibians ( $N = 10$ ), but also include one study on a reptile, and seven studies on different invertebrates. Sample sizes varied greatly across studies, ranging from 18 individuals (Zachos *et al.* 2007) to 1456 individuals (Johansson *et al.* 2005). Of the 33 studies, 25 used nuclear DNA microsatellite data, five used allozyme markers, five used mitochondrial DNA (mtDNA) sequences and one study used random amplified polymorphic DNA markers. Three studies combined multiple marker systems in their analyses.

The studies used a wide variety of analytical approaches, reflecting the great diversity of available analysis options for genetic data. Twenty studies conducted traditional (i.e. population-level) analyses (e.g.  $F_{ST}$ ); eight studies used individual-based analysis approaches (e.g. clustering and spatial autocorrelation approaches) and five studies combined population- and individual-based analyses. Despite these differences,

several general conclusions can be drawn from the reviewed studies.

First, the review illustrates that modern molecular approaches have high power to detect genetic and ecological road impacts, with all but four studies reporting significant road effects. Investigated roads had been constructed as little as *c.* 20 years ago, demonstrating that genetic analyses can address road-related research questions over relatively fine temporal scales. The relatively high proportion of studies reporting significant road effects may be a result of a simple publication bias. However, most studies did not solely investigate road effects on genetic variation, but instead included roads as one of several landscape features that could potentially affect genetic diversity and structure. Thus, most of the studies probably would have been published even if a greater percentage of them had not found significant effects of roads.

The reviewed studies also show that genetic data can be used to obtain detailed information about road effects. For example, various studies reported scale-, species- and sex-specific responses to roads (e.g. Reh & Seitz 1990; Mills & Conrey 2003; Proctor *et al.* 2005). Also, the studies demonstrate that genetic data can be used to evaluate the relative importance of road characteristics (size, age, traffic volume) in gene flow and resulting genetic structures (e.g. Gerlach & Musolf 2000; Vos *et al.* 2001a; Keller & Largiadere 2003). Furthermore, it is possible to distinguish genetic road effects from other, confounding landscape influences, such as spatial, elevation or habitat gradients. Accounting for such confounding factors is possible through study design (e.g. Marsh *et al.* 2008) and sophisticated statistical analyses (e.g. Cushman *et al.* 2006). Also, several studies provide examples for distinguishing influences of historic and recent landscape patterns on observed genetic structures (Holzhauer *et al.* 2006; Ficetola *et al.* 2007; Vandergast *et al.* 2007). Finally, empirical studies also demonstrate that genetic effective population size influences the magnitude and detectability of genetic road effects. For example, Gauffre *et al.* (2008) demonstrated through simulations that genetic barrier effects are difficult to detect in species with large effective population sizes. On the other hand, Epps *et al.* (2005) used simulations to demonstrate that human infrastructures led to a rapid increase of genetic differentiation in desert big-horn sheep (*Ovis canadensis nelsoni*) because local populations had small effective population sizes. Thus, genetic diversity and structure are not only influenced by gene flow across roads, but also by the effective size of local populations within the road network.

All of these examples illustrate that genetic data can be used to assess population genetic consequences of roads, and more importantly, can increase our



understanding of the underlying mechanisms. This is particularly true for studies that combine genetic analyses with nongenetic (i.e. field-based) approaches. For example, Riley *et al.* (2006) used radio-telemetry and genetic assignment methods to evaluate movement and gene flow of bobcats (*Lynx rufus*) and coyotes (*Canis latrans*) across a California freeway. Even though radio-telemetry suggested that individuals of both species were able to move across the road, the genetic data showed that the freeway had become a considerable impediment to gene flow. Riley *et al.* (2006) attribute this to road effects on home-range boundaries, and territory pile-up along the freeway, which made it difficult for crossing individuals to reproduce successfully. Thus, combining various data sources and research approaches can help clarify the exact response of individuals and populations to roads.

#### *Limitations of reviewed studies*

Overall, molecular approaches have been successfully applied to investigate certain road effects, and they are highly flexible with respect to study questions, sampling designs and analytical techniques. However, the review also identified several limitations that currently restrict the utility of molecular approaches in road ecology (Table S2). While 28 of the studies assessed barrier effects on genetic structure, only less than half of the reviewed studies ( $N = 16$ ) also evaluated genetic diversity in relation to roads, and only four studies assessed road effects on effective population size. Furthermore, only six studies evaluated the relative importance of various road characteristics for observed genetic patterns, or accounted for potentially confounding (landscape or historical) factors. Also, only 12 studies used a study design in which results obtained in roadless areas could be compared with those obtained in areas with roads ('Control-Impact' study design). No study compared genetic variation before and after road construction, and only one study conducted multi-year monitoring to investigate long-term effects of roads (Tamura & Hayashi 2007). Only a single study explicitly addressed road-zone effects (Lesbarreres *et al.* 2003). Less than a quarter of the studies ( $N = 6$ ) combined genetic and nongenetic data for their analyses. Finally, with the exception of two studies (Mills & Conrey 2003; Riley *et al.* 2006), all studies focused on a single species.

In sum, current studies address only a few of the many road effects described earlier and they often use suboptimal study and sampling designs. In the next section, we outline genetic approaches for assessing additional road effects on all levels of biodiversity, and give recommendations for future studies in molecular road ecology.

### **Towards molecular road ecology**

Our review clearly demonstrates that molecular genetics can substantially contribute to road ecology research. However, instead of focusing primarily on *barrier* effects of roads on local genetic connectivity, molecular approaches should target a greater variety of road effects.

#### *Road effects on different levels of biodiversity*

Biodiversity can be measured at multiple levels of organization, for example, at the level of genes, population-species, community-ecosystems and landscapes (Noss 1990). At each level, compositional, structural and functional components can be distinguished. Compositional components describe the identity and variation of measured elements, for example, allelic or species richness. Structural components describe the spatial or temporal patterns of biodiversity, for example, species distributions or genetic structures. Finally, functional components include the ecological and evolutionary processes through which the different levels of organization interact with each other, and with their environment.

Modern laboratory techniques can be used to quantify genetic diversity and structure, or to identify species, sexes and individuals. This information can help investigate a large variety of road effects on biodiversity (Table 2):

(a) *Genetic level road effects*: As reviewed in the previous section, molecular approaches are well-suited to study road effects on compositional and structural components at the genetic level (i.e. road effects on genetic diversity and structure, respectively). In addition, genetic data can also be used to assess road effects on functional components of genetic variation, for example, by estimating genetic effective population sizes, or number of effective migrants (e.g. Wang & Whitlock 2003; Palstra & Ruzzante 2008).

(b) *Species-population level applications*: Noninvasive genetic sampling (i.e. sampling genetic material without directly capturing or observing animals; Long *et al.* 2008; Taberlet *et al.* 1999; Waits & Paetkau 2005; Waits 2004) can be used to assess species presence/absence, and can thus help to evaluate road and road-zone effects on species-specific habitat suitability. For certain species (e.g. many carnivores), noninvasive genetic sampling can greatly increase detection rates and thus lead to more accurate inferences about species distribution and abundance (Solberg *et al.* 2006; Long *et al.* 2008). Genetic data can also be analysed within a mark-recapture framework, to identify individual animals and estimate population sizes and densities (e.g. Bellemain *et al.* 2005; Leberg 2005; Piggott *et al.* 2006; Kendall

**Table 2** Overview of potential molecular applications for assessing road effects on different levels and components of biodiversity

<b>Level of organization</b>	<b>Biodiversity component</b>	<b>Attribute(s) of biodiversity component</b>	<b>Molecular application(s) to assess road effect</b>
a) Genetic	Compositional	Population-specific genetic diversity	Estimates of genetic diversity (e.g. allelic richness, heterozygosity)
a) Genetic	Structural	Genetic structure	Population-specific $F_{ST}$ , assignment and clustering analyses; analyses based on genetic distances among individuals, incl. spatial autocorrelation statistics
a) Genetic	Functional	Gene flow; genetic effective population size	Estimates of gene flow, genetic differentiation (e.g. $F_{ST}$ ), number of effective migrants ( $N_m$ ), genetic effective population size ( $N_e$ )
b) Population-species	Compositional	Species presence/absence	Species identification via (noninvasive) genetic sampling (single species);
b) Population-species	Structural	Abundance/population size; sex-ratio	Noninvasive genetic mark-recapture for identification of individuals; gender identification via (noninvasive) genetic sampling
b) Population-species	Functional	Population growth; survival, reproduction; (sex-specific) movement/dispersal	Noninvasive genetic mark-recapture for identification and temporal tracking of individuals and genetic effective and census population sizes; parentage and kinship analysis; endocrine analyses to determine pregnancy rates; assignment and clustering analyses; analyses based on genetic distances among individuals, incl. spatial autocorrelation statistics
c) Community-ecosystem	Compositional	Species composition/communities	Species identification via (noninvasive) genetic sampling (multiple species)
c) Community-ecosystem	Structural/functional	(Distribution of) Functional groups	Species identification via (noninvasive) genetic sampling (multiple species)
d) Landscape	Structural/functional	Species-specific landscape characteristics (e.g. habitat patches and fragmentation)	Landscape genetics; genetically-scaled indices of landscape fragmentation
d) Landscape	Functional	(Meta-)Population connectivity	Assignment, clustering, and admixture analyses; 'traditional' estimates of genetic differentiation such as $N_m$ , $F_{ST}$ , $F_{IS}$ , etc.

[Correction added after online publication 25 September 2009: the table had its headings put in bold text.]

*et al.* 2008). Furthermore, such genetic mark-recapture analyses can track individual movements and provide estimates of survival rates (e.g. Flagstad *et al.* 2004; Piggott *et al.* 2006; Squires *et al.* 2007). Sex-determination from genetic data can be used to evaluate sex-specific barrier effects and also to detect changes in sex-ratios caused by roads (Aresco 2005). Genetic parentage and kinship analyses can provide insights into road-induced changes of reproductive rates and mating schemes (e.g. Wilson *et al.* 2002; Sorin 2004). Such road effects on reproduction could also be assessed via protein or hormone analyses that determine pregnancy rates (e.g. Czekala *et al.* 1994; Drew *et al.* 2001). Hormone analyses could also help to determine stress levels (e.g. Koren *et al.* 2002; Schwartz & Monfort 2008), which may be increased near roads because of noise and lighting (Outen 2002). Molecular approaches can also illuminate the spread of infectious diseases and invasive

species that can be facilitated by roads (Pauchard & Alaback 2004; Hansen & Cleveenger 2005; Miura 2007; Archie *et al.* 2009). Stable isotopes could also help to assess such road effects by tracking wildlife movement and migration patterns (Hobson & Wassenaar 2008). Finally, analysing genetic patterns of pathogens could help reveal road effects on animal movement and social interactions at very fine temporal scales (e.g. Biek *et al.* 2006).

The suggested molecular applications for species-population level analyses go far beyond most current applications for analysing road-effects on genetic variation. Instead, the applications use molecular genetic methods to address specific ecological research questions and hypotheses. Thus, they can lead to the same kind of information that can be obtained via traditional population assessment, but can also address questions that are very difficult or impossible to address without

molecular genetic data. Addressing these questions can also yield the kind of information that is needed for practical transportation planning and conservation. As pointed out by Roedenbeck *et al.* (2007), road ecology currently has comparatively little impact on transportation planning and should therefore target research questions of high practical relevance, for example, with respect to road effects on population persistence or regarding the functionality of mitigation measures. Genetic data can be used to address these questions, because they are closely tied to successful reproduction and population viability and because the relative ease of data collection for certain species allows researchers to compare the effectiveness of different mitigation measures at many locations within a single study (e.g. Kuehn *et al.* 2007).

(c) *Community–ecosystem level applications:* Genetic identification of multiple target species can also assess road-related changes in species composition. This seems particularly important for achieving the goals of multi-species conservation approaches (e.g. Barrows *et al.* 2005; Early & Thomas 2007; Noon *et al.* 2008). Such multi-species approaches could potentially target entire guilds or functional groups (Blondel 2003; Bishop & Myers 2005) and thus allow inferences about road effects on ecosystem functions. Multi-species studies could also be conducted with nongenetic data, but molecular approaches may greatly facilitate them, at least for certain species (Waits 2004; Long *et al.* 2008). The emerging field of ecosystem and community genetics and genomics may also provide novel opportunities to study the effects of road-induced changes in genetic variation on species interactions and community structures (Whitham *et al.* 2006, 2008).

(d) *Landscape-level applications:* At the landscape level, biodiversity indicators include measures of patch type and distribution, fragmentation indices or rates of transfer among communities and patches in the landscape (Noss 2006). Ideally, many of these indicators should be scaled to the specific characteristics of different species. For example, a certain landscape may be highly fragmented from the perspective of one species, yet be entirely connected for another. Similarly, defining an ecologically meaningful patch depends on species-specific habitat relationships and area requirements. Molecular approaches can help derive meaningful indicators of landscape-level biodiversity, for example, by determining levels of functional (i.e. genetic) connectivity for various species inhabiting a certain landscape. Landscape genetics provide novel and improved statistical methods for analysing and interpreting genetic data over large spatial scales and for linking observed genetic patterns to landscape features (Manel *et al.* 2003; Storfer *et al.* 2007; Holderegger & Wagner 2008).

Thus, landscape genetic methods are especially well-suited for landscape-level applications in molecular road ecology. For instance, genetic data can be used to estimate dispersal distances, which are needed to parameterize ecologically scaled landscape indices (e.g. Vos *et al.* 2001b; Moilanen & Nieminen 2002). Such indices are often used to determine whether specific patches of suitable habitat within a landscape are isolated from other patches because of matrix effects, and to evaluate the importance of specific patches to (meta-)population connectivity and viability (e.g. James *et al.* 2005; Pascual-Hortal & Saura 2006; Minor & Urban 2007). Finally, genetic data can be collected over large spatial extents, and sample sizes are often in the hundreds or even thousands (Table S1). This makes genetic approaches especially interesting for broad-scale analyses that are necessary for assessing landscape-level road effects on biodiversity. Such broad-scale, landscape-level applications of molecular road ecology are also very valuable for practical transportation planning. Indeed, landscape genetic approaches have already been used to identify optimal placement for wildlife movement corridors to mitigate (road-induced) landscape fragmentation (Epps *et al.* 2007; Beier *et al.* 2008; Cushman *et al.* 2009).

#### *Inferential strength and study design in molecular road ecology*

The inferential strength of genetic studies generally depends on optimal choice of markers and loci, sound laboratory protocols, sufficient sample sizes and adequate data analysis. While these issues have been discussed in detail in the molecular ecology literature (e.g. Taberlet *et al.* 1999; Kalinowski 2002, 2005; McKelvey & Schwartz 2004; Paetkau 2004; Balkenhol *et al.* in press), other considerations have received far less attention.

First, inferential strength of a study increases with the number of competing hypotheses tested because data obtained from a certain study always could have resulted from the influences of multiple factors, each reflecting a different hypothesis. As demonstrated by some of the reviewed studies, molecular approaches are well-suited for teasing apart the complex and inter-related impacts of multiple landscape characteristics on genetic patterns (e.g. Reh & Seitz 1990; Cushman *et al.* 2006; Arens *et al.* 2007). Understanding the relative importance of these various road characteristics will require such research that specifically targets a wide range of road characteristics within multi-factorial study designs. Genetic data also provide opportunities to test for historical influences, for example, through the combined use of molecular markers with varying temporal resolutions (e.g. mtDNA for assessing more historic



broad-scale influences; nuclear microsatellites for assessing more recent fine-scale influences).

Second, data in road ecology should be gathered and analysed within a before-after-control-impact (BACI) study design, whenever possible. In this study design, data are gathered *before* and *after* roads or road-mitigation measures are constructed. Also, data from areas with roads or mitigation measures (*impact*) are compared with data obtained from areas without such structures (*control*). This study design may be difficult to implement in practice, but it greatly increases the inferential strength of road ecological research. When a full BACI study is not feasible, before-after (BA) or control-impact (CI) study designs are often possible. While not as optimal as the full BACI design, studies following a BA or CI design are still suitable for addressing research questions in road ecology, and Roedenbeck *et al.* (2007) discuss their respective advantages and limitations in detail.

Finally, the inferential strength of molecular applications in road ecology will be particularly high in combination with other field-based research approaches. Combining different approaches and techniques to address the same research questions can substantially increase the certainty of inferences and yield an in-depth understanding of affected processes (e.g. Tallmon *et al.* 2002; Cullingham *et al.* 2008). For example, two populations that have recently become isolated from another by a road may counterbalance decreased immigration rates with increased reproduction rates. In this case, a conventional field census may not detect any road effects, because local population sizes seem unaffected. However, a genetic study could detect a change in the number of effective breeders and also estimate the number of effective migrants via assignment tests. Thus, conducting a traditional population census in conjunction with genetic applications can identify cryptic population responses to roads, which may be important for long-term conservation. Similarly, genetics can determine if individuals move and reproduce across roads, but it does not necessarily provide the resolution to identify exact locations or timings of crossings. Furthermore, because of lack of spatio-temporal detail, genetic data do not improve our understanding of individual behavioural responses to roads (e.g. Graves *et al.* 2007; Lewis 2007). Thus, genetic and tracking data (e.g. obtained from GPS-collars) should be combined more often to understand and mitigate road effects better.

#### *Recommendations for future research*

Overall, we suggest the following research strategies for future efforts in molecular road ecology.

- 1 *Assess road effects on all levels of biodiversity*—Future studies in molecular road ecology should target road effects at all levels and components of biodiversity. Also, researchers should design studies that specifically evaluate road impacts on multiple species, or even on entire guilds and functional groups.
- 2 *Focus on research questions with direct relevance to transportation planning*—Future studies should also attempt to provide planners with practical advice on how to avoid or mitigate negative road effects on biodiversity. More specifically, researchers should correspond with relevant decision makers and discuss what kind of information would be most useful from a practical planning standpoint.
- 3 *Account for confounding factors*—To separate true road effects from other (e.g. landscape and historical) influences, future studies should pay greater attention to choosing appropriate control areas, multi-factorial study designs and multivariate analytical approaches.
- 4 *Assess landscape-scale road effects*—In addition to analysing local road impacts, increased efforts for evaluating broad-scale road effects are needed. Such studies would ideally use landscapes as the sampling unit, and should quantify road-induced fragmentation within each sampled landscape, for example, by relating estimates of genetic structure and (effective) population sizes to road densities, effective mesh sizes (Jaeger 2000; Moser *et al.* 2007) or accessible habitat (Eigenbrod *et al.* 2008a).
- 5 *Measure road characteristics*—Broad-scale sampling will also enable researchers to include roads with various characteristics (e.g. various widths, ages, traffic volumes) in their studies. Future research should actively attempt to sample these different road attributes and statistically evaluate their relative importance.
- 6 *Use landscape genetic approaches*—Landscape genetic approaches are particularly well-suited for accomplishing recommendations 3–5 and should therefore receive increased attention in molecular road ecology.
- 7 *Assess both within- and between-population road effects*—Local population viability is influenced by within- and between-patch processes, but current studies seldom assess patch-specific influences and instead focus on barrier effects of roads. Future genetic studies should quantify population-specific responses to roads (e.g. census and effective population sizes; number of effective breeders) in addition to estimating connectivity.
- 8 *Combine molecular and field-based approaches*—Utilizing multiple research approaches (e.g. genetics, hormone analyses, telemetry, mark-recapture) will increase our understanding of the consequences and underlying (behavioural) mechanisms of road impacts. This will also lead to more appropriate mitigation measures.

9 *Conduct long-term studies*—Ideally, the effects of roads and mitigation measures should be evaluated over extensive time periods, in which population parameters are repeatedly measured and analysed with the same (i.e. genetic and nongenetic) techniques (Schwartz *et al.* 2007). Such monitoring is necessary to understand fully the effects of roads on long-term population persistence.

We acknowledge that it would be very challenging to incorporate all of these suggestions in a single project and several of the recommendations may even be mutually exclusive for practical reasons. Nevertheless, many of our recommendations could be realized through collaborative research efforts in which multiple scientists work together under a common, road-related research theme. These projects could either use different research approaches for analysing road effects in the same area or use the same research approaches in different areas, and for different species. Clearly, such meta-projects would require a substantial amount of communication and coordination among all involved parties and a comparison and synthesis of derived information. However, many of the most interesting and most important research questions related to road effects warrant such a synthesis of projects that encompass a wide variety of circumstances and species (Roedenbeck *et al.* 2007).

## Conclusions

In sum, molecular approaches can be highly informative for road ecological research, but their full potential is yet to be realized. We do not argue that genetics is suitable for all research questions in road ecology or that it should replace other scientific approaches. Instead, we propose that genetics can fill in some pieces of the larger puzzle and that it should be combined with the many other research approaches for investigating road effects. The potential contributions of genetic approaches to road ecology research and transportation planning should be carefully evaluated and discussed among road ecologists, geneticists and transportation planners *before* designing road ecological studies for each particular project.

Vernesi *et al.* (2008) recently called for a greater use of genetic approaches in practical conservation and highlighted the need for applying molecular techniques to real-life problems. As illustrated throughout this review, molecular road ecology provides an exciting opportunity for conducting genetic research with high relevance for practical biodiversity conservation. We believe that creative and innovative thinking, combined with a sound understanding of road ecology relationships and their genetic signatures, will be vital to the

future development of molecular road ecology. Interdisciplinary collaborations are necessary to ensure that genetic techniques are used correctly and efficiently in road ecology, and to improve current study designs and analytical approaches. We hope that this review will stimulate discussion among road ecologists, transportation planners and geneticists, and that future developments in molecular road ecology will help find feasible and effective solutions for environmentally sustainable transportation planning.

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[Correction added after online publication 25 September 2009: the URLs of *Ecology and Society* articles were corrected to ‘iss1’ rather than ‘iss’.]

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Lisette Waits leads a research group that focuses on the molecular ecology, landscape genetics and conservation genetics of a variety of vertebrate taxa. Niko Balkenhol is a Ph.D. student of Lisette Waits with a general interest in (spatial molecular) wild life ecology. This paper is part of his dissertation, which focuses on evaluating and improving landscape genetics approaches for wildlife research.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Summary of studies that use genetic data to assess road effects on animals

**Table S2** Summary of characteristics of studies using genetic data to assess road effects on animals

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