

REVIEW

## The genetic effects of roads: A review of empirical evidence

Rolf Holderegger\*, Manuela Di Giulio

*WSL Swiss Federal Research Institute, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland*

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### Abstract

Roads exert various effects of conservation concern. They cause road mortality of wildlife, change the behaviour of animals and lead to habitat fragmentation. Roads also have genetic effects, as they restrict animal movement and increase the functional isolation of populations. We first formulate theoretical expectations on the genetic effects of roads with respect to a decrease in genetic diversity and an increase in genetic differentiation or distance of populations or individuals. We then review the empirical evidence on the genetic effects of roads based on the available literature. We found that roads often, but not always, decrease the genetic diversity of affected populations due to reduced population size and genetic drift. Whether the reduction in genetic diversity influences the long-term fitness of affected populations is, however, not yet clear. Roads, especially fenced highways, also act as barriers to movement, migration and gene flow. Roads therefore often decrease functional connectivity and increase the genetic differentiation of populations or the genetic distance among individuals. Nevertheless, roads and highways rarely act as complete barriers as shown by genetic studies assessing contemporary migration across roads (by using assignment tests). Some studies also showed that road verges act as dispersal corridors for native and exotic plants and animals. Genetic methods are well suited to retrospectively trace such migration pathways. Most roads and highways have only recently been built. Although only few generations might thus have passed since road construction, our literature survey showed that many studies found negative effects of roads on genetic diversity and genetic differentiation in animal species, especially for larger mammals and amphibians. Roads may thus rapidly cause genetic effects. This result stresses the importance of defragmentation measures such as over- and underpasses or wildlife bridges across roads.

### Zusammenfassung

Straßen üben verschiedene Effekte aus, die für den Naturschutz von Bedeutung sind. Straßen verursachen den Straßentod von Tieren, verändern deren Verhalten und führen zu Habitatfragmentierung. Da Straßen die Bewegung von Tieren einschränken und die funktionale Zerschneidung von Populationen hervorrufen, verursachen Straßen auch genetische Effekte. Wir formulieren zuerst theoretische Erwartungen zu den genetischen Effekten von Straßen in Bezug auf eine Verminderung der genetischen Diversität und eine Erhöhung der genetischen Differenzierung oder Distanz zwischen Populationen oder Individuen. Wir geben dann einen Überblick über die empirische Literatur zu den genetischen Effekten von Straßen. Wir zeigen, dass Straßen oft, aber nicht immer, die genetische Diversität von Populationen infolge kleinerer Populationsgröße und erhöhter genetischer Drift vermindern. Ob diese Reduktion der genetischen Diversität längerfristig die Fitness der betroffenen Populationen beeinträchtigt ist allerdings unklar. Straßen, und insbesondere eingezäunte Autobahnen, sind auch Barrieren für Tierbewegungen, Wanderung und Genfluss. Straßen reduzieren oft die funktionale Vernetztheit und erhöhen die genetische Differenzierung von Populationen oder die genetischen Distanz zwischen Individuen. Allerdings wirken Straßen und Autobahnen nur selten als vollständige Barrieren, wie dies genetische Studien zeigen, die die aktuelle Wanderung über Straßen hinweg nachweisen (mittels assignment tests). Einige Studien zeigen zudem, dass Straßenränder als Ausbreitungskorridore für einheimische und exotische Tiere und

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\*Corresponding author. Tel.: +41 44 739 25 27; fax: +41 44 739 22 15.  
E-mail address: rolf.holderegger@wsl.ch (R. Holderegger).

Pflanzen dienen. Genetische Methoden sind gut geeignet, entsprechende Ausbreitungswege retrospektiv zu erfassen. Die meisten Straßen wurden erst vor kurzer Zeit gebaut. Obwohl deshalb meist nur wenige Generationen seit der Erstellung der Straßen verflossen sind, zeigt unsere Literaturübersicht, dass viele Studien negative Auswirkungen von Straßen auf die genetische Diversität und Differenzierung von Tierarten fanden, insbesondere bei größeren Säugetieren und Amphibien. Straßen führen somit schnell zu genetischen Effekten. Dieses Resultat unterstreicht die Bedeutung von Defragmentierungsmaßnahmen an Straßen wie etwa Durchlässen, Überführungen oder Grünbrücken.

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## Introduction

The ecological effects of roads, ranging from unpaved paths on agricultural and forest land to fenced highways, have long been identified (e.g. Forman & Alexander, 1998; Forman et al., 2003). The ecological effects of roads include the destruction of habitat during road construction, habitat alteration due to changes in the physical and chemical environment, mortality of individuals from collision with vehicles or modification of animal behaviour (Coffin, 2007). However, roads may also foster migration along road verges, which serve as dispersal corridors for native and exotic species. Two of the above effects are thought to be of special relevance for the genetic composition of populations affected by roads. First, road mortality influences the genetic diversity of populations through its negative influence on abundance and population size (e.g. Fahrig & Rytwinski, 2009), and, second, fragmentation by roads causes lower rates of exchange of individuals and genes among populations. In this case, roads act as barriers to movement, migration and dispersal (e.g. Kostrzewa, 2006).

Until recently, theoretical expectations on the genetic effects of roads had little empirical support. This led Forman et al. (2003: 130) to note that “little is known about the long-lasting ecological effects of roads on animal populations in terms of reduced mobility, increased isolation, and/or the splitting of gene pools”. The last decade, however, has seen a growth of interest in how roads affect the genetic composition of populations, and, accordingly, the picture given by Forman et al. (2003) has changed substantially. Two recent overview articles dealt with the genetic effects of roads. While Balkenhol and Waits (2009) mainly explored the potential of genetics for the investigation of the impact of transportation infrastructure on wildlife, Corlatti, Hackländer, and Frey-Roos (2009) evaluated whether wildlife overpasses served to minimize the genetic isolation effect of roads. It is the aim of the present article to generally summarize the empirical evidence of the genetic effects of roads based on the available literature. To this aim: (1) we review the theoretically expected influences of roads on the genetic composition of populations and (2) present the results of a literature survey focusing on the effects of roads on genetic diversity, genetic differentiation and genetically detectable contemporary movement across roads. We performed an ISI Web

of Knowledge (<http://apps.isiknowledge.com/>) search, using the search words *genetic\** AND *road\** OR *highway\** and the like (last assessed on August 1, 2009). In addition, we consulted the relevant references given in the identified articles in order to locate other articles on the effects of roads. Only those studies which either specifically dealt with the genetic effects of roads or which compared landscapes with different road densities (e.g. urban vs. rural areas) were considered (38 studies in total). (3) We then introduce the issue of road verges as corridors for the movement of native and exotic species and searched for genetic studies investigating whether road verges serve as dispersal corridors (5 studies in total). (4) Finally, we identify some limitations of the available literature and end with some brief remarks on future research needs in the evaluation of the genetic effects of roads.

## Theoretical expectations

Roads cause animal mortality due to collision with vehicles. Road mortality often results in reduced local abundances and population sizes (e.g. Fahrig & Rytwinski, 2009) and decreased rates of individual exchange among populations on different sides of roads (e.g. Ree, Van der Grift, Mata, & Suarez, 2007). Roads also change the behaviour of animals: animals may learn to avoid roads (e.g. Reijnen & Foppen, 2006; Roedenbeck & Voser, 2008). While road avoidance behaviour has the positive effect of decreasing road mortality, it also comes with the negative effect of decreasing individual and gene exchange across roads, leading to an increased barrier effect of roads.

Reduced population size may lead to adverse genetic effects such as reduced genetic diversity and/or increased inbreeding (e.g. Frankham, 2005). (1) Genetic drift is increased in small populations. Under genetic drift, the frequencies of alleles within populations alter stochastically. The frequencies of rare alleles should especially be affected, and rare alleles can completely be lost from local gene pools. In the long run, the genetic diversity of populations may decrease because of genetic drift (e.g. Amos & Balmford, 2001). (2) In small populations, individuals are often genetically related, which could lead to inbreeding when genetically related individuals mate. Inbreeding increases the number of loci in homozygous state and causes the expression of

recessive detrimental alleles, which are not expressed when in heterozygous state. The expression of detrimental alleles may then cause inbreeding depression, which reduces the fitness of affected individuals (Keller & Waller, 2002; Höglund et al., 2001; Armbruster & Reed, 2005). If individuals and genes are regularly exchanged among populations (i.e. if gene flow occurs), the negative effects of genetic drift and inbreeding are counteracted (e.g. Vila et al., 2003). If individual exchange and gene flow is hampered, genetic drift, which will differently affect the allele frequencies in different populations, should slowly drive apart the populations on the two sides of a road. Lacking gene flow therefore should lead to increased genetic differentiation of populations (Wright, 1943) isolated by roads.

The negative genetic effects of small population size and genetic isolation need time to become detectable, i.e. it needs several generations for spatial genetic structure to build up (Barton & Wilson, 1995; Murphy, Evans, Cushman, & Storfer 2008). As many roads have recently been constructed, being younger than 50 years, few animal generations might have passed since road construction, especially in long-lived mammals. In consequence, only weak genetic effects are to be expected. This asks for a high resolution power of the genetic markers used (e.g. 10–20 microsatellite loci; for a general description of genetic methods, parameters and statistical tools see Lowe, Harris, & Ashton 2004) to identify the genetic effects of recently built roads (e.g. Marsh et al., 2008).

Animals most vulnerable to the barrier effect of roads are flightless, avoid roads, have high habitat specificity or need multiple resources requiring them to have large home ranges (e.g. Forman et al., 2003). Even if two populations are completely isolated by a road, each of them could still be functionally connected with populations in its hinterland (Kuehn et al., 2007). In such a situation, inbreeding, loss of genetic diversity and population differentiation may be prevented due to large effective population sizes (Waples, 2002; Gauffre, Estoup, Bretanolle, & Cosson, 2008). It would be difficult to detect any effects of roads by using standard population genetic approaches in such situations. Only genetic methods inferring contemporary migration across roads are then able to detect a barrier effect of roads. For instance, genetic assignment tests are able to identify first generation migrants in real time (Manel, Gaggiotti, & Waples, 2005). However, assignment tests also rely on significant population differentiation. As population differentiation needs time to form (see above), many populations separated by roads can be assumed to be only weakly differentiated, thus potentially impairing the statistical power of assignment tests to identify contemporary migrants (Berry, Tocher, & Sarre, 2004).

## Road effects on genetic diversity

We detected 19 species-specific studies that reported on the effects of roads on genetic diversity (Table 1). These studies mainly investigated animals, ranging from crickets and

ground beetles to amphibians and mammals such as bank voles, bighorn sheep, roe deer, bobcat or bears. Only a single study investigated the effects of roads on the genetic diversity of a plant species and found no negative effects (Hogbin, Ayre, & Whelan, 1998; Table 1).

Fourteen studies reported negative effects on various measurements of genetic diversity such as allelic richness, effective number of alleles, number of fragments (for minisatellites), observed heterozygosity, gene diversity, percentage of polymorphic loci (for isozymes) or haplotype diversity (for mitochondrial DNA). In contrast, five studies reported no effects of roads on genetic diversity. Table 1 shows that roads have often, but not always, negative effects on the genetic diversity of animal populations.

Studies investigating road effects by comparing study regions with different road densities should replicate the treatment, i.e. regions with high and low road densities, in order to attain statistical significance (Holderegger & Wagner, 2008). This is exemplified by the study of Johansson, Primmer, Sahlsten, and Merilä (2005), which determined the genetic diversity at microsatellite loci in the common frog (*Rana temporaria*) in three Swedish regions. The authors applied a stratified sampling design, in which each region contained several subregions. Nine 1-km<sup>2</sup> sampling plots were assessed per subregion. The regions and subregions differed in their landscape composition, including road density. By applying principal component analysis, Johansson et al. (2005) found that roads have a negative effect on the number of alleles ( $A$ ) and observed or expected heterozygosity ( $H_o$ ,  $H_e$ ) of the common frog populations from that study region where agriculture was most intense (Table 1).

As the genetic studies reported in Table 1 determined genetic diversity at neutral loci such as microsatellites, the observed decrease in genetic diversity caused by roads does not directly exert negative effects on the fitness of populations (Reed & Frankham, 2001; Chapman, Nakagawa, Coltman, Slates, & Sheldon, 2009). In this context, it is noteworthy that two studies found negative road effects on effective population size ( $N_e$ ). Effective population size is a measure of genetic stochasticity or, in other words, evaluates how prone a population is to inbreeding and genetic drift (e.g. Waples, 2002). Both Keller, Excoffier, and Largiadèr (2005) for the ground beetle *Carabus violaceus* and Zachos, Althoff, von Steynitz, Eckert, and Hartl (2007) for red deer (*Cerphus elaphus*) found that the effective population size of populations affected by roads was significantly lowered (Table 1). However, there are no studies available so far that directly evaluated the influence of roads on fitness-relevant genetic variation.

## Road effects on genetic differentiation

Thirty-two studies reported on potential effects of roads on the genetic differentiation of populations using a broad variety of different measures of genetic differentiation or distance

**Table 1.** Empirical studies on the genetic effects of roads. Given are the species studied, the genetic markers used, road effects on genetic diversity and genetic differentiation, contemporary migration across roads (based on assignment tests), the country origin of the study and the corresponding reference.

Species	Genetic marker <sup>a</sup>	Road effect on genetic diversity	Road effect on genetic differentiation	Contemporary migration <sup>b</sup>	Country	Reference
Cricket ( <i>Metrioptera roeseli</i> )	RAPDs	–	Decrease in genetic similarity	–	Germany	Holzhauser, Ekschmitt, Sander, Dauber, and Wolters (2006)
Cricket ( <i>Stenopelmatus</i> n. sp.)	ISSRs, mtDNA	Decrease in haplotype diversity	Increase in genetic differentiation	–	USA	Vandergast et al. (2009)
Seed borer ( <i>Coccytrypes dactyliperda</i> )	SSRs	–	Increase in genetic differentiation	–	USA	Holzman et al. (2009)
Ground beetle ( <i>Carabus violaceus</i> )	SSRs	Decrease in effective population size	–	Some movement across roads (GENECLASS)	Switzerland	Keller et al. (2005)
Ground beetle ( <i>Carabus violaceus</i> )	SSRs	Decrease in allelic richness	Highway increases genetic differentiation	–	Switzerland	Keller and Largiadèr (2003)
Ground beetle ( <i>Abax parallelepipedus</i> )	SSRs	No effect	Increase in genetic differentiation	–	Switzerland	Keller, Nentwig, and Largiadèr (2004)
Salamander ( <i>Plethodon cinereus</i> )	SSRs	Decrease in allelic richness and heterozygosity	Increase in genetic differentiation	–	Canada	Noël, Ouellet, Galois, and Lapointe (2007)
Salamander ( <i>Plethodon cinereus</i> )	SSRs	–	Highway, but not smaller roads, increases genetic distance	–	USA	Marsh et al. (2008)
Salamander ( <i>Ambystoma maculatum</i> )	SSRs	–	No effect	–	USA	Purrenhage, Niewiarowski, and Moore (2009)
Frog ( <i>Rana temporaria</i> )	Isozymes	Decrease in heterozygosity and polymorphic loci	Increase in genetic distance	–	Germany	Reh and Seitz (1990)
Frog ( <i>Rana temporaria</i> )	SSRs	Decrease in number of alleles and heterozygosity	Increase in genetic differentiation	–	Sweden	Johansson et al. (2005)
Frog ( <i>Rana temporaria</i> )	Isozymes	No effect	Increase in genetic differentiation	–	UK	Hitchings and Beebee (1997)
Frog ( <i>Rana arvalis</i> )	SSRs	Decrease in effective number of alleles and heterozygosity	Increase in genetic differentiation	–	Netherlands	Arens et al. (2007)
Frog ( <i>Rana arvalis</i> )	SSRs	–	Increase in genetic differentiation	–	Netherlands	Vos, Antonisse-deJong, Goedhart, and Smulders (2001)
Frog ( <i>Rana dalmatina</i> )	Isozymes	Decrease in allelic richness and heterozygosity	Increase in genetic differentiation	–	France	Lesbarrères, Primmer, Lodé, and Merilä (2006)
Frog ( <i>Rana latastei</i> )	SSRs	Decrease in multivariate genetic diversity	–	–	Italy	Ficeleto, Garner, and De Bernardi (2007)
Toad ( <i>Bufo bufo</i> )	Isozymes, VNTR	Decrease in number of alleles and heterozygosity	Increase in genetic differentiation	–	UK	Hitchings and Beebee (1998)
Carnivorous marsupial ( <i>Antechinus flavipes</i> )	SSRs, mtDNA	–	No effect	–	Australia	Lada, Thomson, Mac Nally, and Taylor (2008)
Vole ( <i>Microtus arvalis</i> )	SSRs	–	No effect	–	France	Gauffre et al. (2008)
Bank vole ( <i>Clethrionomys glareolus</i> )	SSRs	–	Highway increases genetic subdivision	–	Germany, Switzerland	Gerlach and Musolf (2000)
Bank vole ( <i>Clethrionomys glareolus</i> )	SSRs	–	No effect	Few movements across road (GENECLASS)	Denmark	Redeker et al. (2005)

**Table 1** (Continued).

Species	Genetic marker <sup>a</sup>	Road effect on genetic diversity	Road effect on genetic differentiation	Contemporary migration <sup>b</sup>	Country	Reference
Squirrel ( <i>Sciurus lis</i> )	mtDNA	Decrease in haplotype diversity	–	Immigration of new haplotypes across road in sequential sampling	Japan	Tamura and Hayashi (2007)
Bighorn sheep ( <i>Ovis canadensis</i> )	SSRs, mtDNA	Decrease in number of alleles	Fenced highway strongly increases genetic differentiation	–	USA	Epps et al. (2005)
Bighorn sheep ( <i>Ovis canadensis</i> )	SSRs	–	Increase in genetic differentiation and decrease in migration rate	–	USA	Epps, Wehausen, Bleich, Torres, and Brashares (2007)
Roe deer ( <i>Capreolus capreolus</i> )	SSRs	No effect	Increase in genetic differentiation	–	Switzerland	Kuehn et al. (2007)
Roe deer ( <i>Capreolus capreolus</i> )	SSRs	–	Population boundaries coincide with highway	–	France	Coulon et al. (2006)
Red deer ( <i>Cerphus elaphus</i> )	SSRs, mtDNA	Decrease in number of alleles and effective population size	–	–	Germany	Zachos et al. (2007)
Red deer ( <i>Cerphus elaphus</i> )	SSRs	–	Increase in genetic differentiation	–	UK	Pérez-Espona et al. (2008)
Bobcat ( <i>Lynx rufus</i> )	SSRs	–	Highway increases genetic differentiation	Some movement across highway (STRUCTURE)	USA	Riley et al. (2006)
Bobcat ( <i>Lynx rufus</i> )	SSRs	–	No effect	–	USA	Millions and Swanson (2007)
Cougar ( <i>Puma concolor</i> )	SSRs	–	No effect	–	USA	Sinclair et al. (2001)
Marten ( <i>Martes americana atrata</i> )	SSRs	–	No effect (at large scale)	–	Canada	Kyle and Strobeck (2003)
Badger ( <i>Meles meles</i> )	VNTR	Decrease in number of fragments	–	–	Denmark	Pertoldi, Loeschke, Madsen, Randi, and Mucci (2001)
Badger ( <i>Meles meles</i> )	SSRs	No effect	Road underpasses counteract population subdivision	–	Denmark, Netherlands	Van de Zande et al. (2007)
Coyote ( <i>Canis latrans</i> )	SSRs	–	Highway increases genetic differentiation	Many movements across highway (STRUCTURE)	USA	Riley et al. (2006)
Bear ( <i>Ursus americanus</i> )	SSRs	–	Equivocal effects of highway and roads on genetic distance	–	USA	Cushman et al. (2006)
Bear ( <i>Ursus arctos</i> )	SSRs	–	Equivocal effects of highways	Few movements across highways (GENECLASS)	USA Canada	Proctor et al. (2005)
Shrub ( <i>Grevillea barklyana</i> )	RAPDs	No effect	No effect	–	Australia	Hogbin et al. (1998)

<sup>a</sup>ISSRs: inter-simple sequence repeats; mtDNA: mitochondrial DNA sequences; RAPDs: random amplified polymorphic DNAs; SSRs: nuclear microsatellites; VNTR: minisatellites.

<sup>b</sup>GENECLASS: Piry et al. (2004); STRUCTURE: Pritchard et al. (2000).

(e.g.  $F_{ST}$ ,  $G_{ST}$ , genetic similarity, genetic distance, genetic clustering, etc.; Table 1). Only one study dealt with a plant species (Hogbin et al., 1998; Table 1). Twenty-one studies found that roads increase the genetic differentiation of populations, increase the genetic distance among individuals or enhance genetic subdivision. Eight studies did not find negative effects of roads on population differentiation (Table 1). One of these studies (Van de Zande et al., 2007) explicitly related the lack of road effects on genetic differentiation to the positive effect of underpasses (Glista, DeVault, & DeWoody, 2009). Two studies on bears detected equivocal effects of roads, which were difficult to interpret (Proctor, McLellan, Strobeck, & Barclay, 2005; Cushman, McKelvey, Hayden, & Schwartz, 2006). One can conclude that many studies found that roads and especially fenced highways often, but not always, increase the genetic differentiation of animal populations (Table 1).

In the absence of gene flow, genetic population differentiation builds up over several generations. Genetic differentiation and distance may thus reflect historical rather than present landscape composition, especially, if human land use has recently changed. A direct relationship between present genetic structure and present landscape structure does therefore not necessarily exist, resulting in a time lag between causal process and biological response (Balkenhol et al., 2009; Anderson et al., in press). It is thus surprising that many studies found negative genetic effects of roads.

Two papers that are representative of the literature on the genetic effects of roads on genetic differentiation are exemplified in the following. Arens et al. (2007) evaluated the causes of genetic population differentiation among populations of moor frog (*Rana arvalis*) in two regions of the Netherlands. They found that populations in the more fragmented region had a higher genetic differentiation at microsatellite loci than populations in the less fragmented region. The single landscape element, besides geographical distance, that significantly explained this pattern in multiple Mantel tests was the cumulative length of roads in 200 m wide strips between pairs of studied populations (Table 1). Arens et al. (2007) concluded that roads thus form an impediment to the movement of moor frogs. Using a map overlay approach and microsatellite data from individual roe deer (*Capreolus capreolus*) in a region of about 2200 km<sup>2</sup> in France, Coulon et al. (2006) applied genetic clustering techniques (STRUCTURE; Pritchard et al., 2000; GENELAND, Guillot, Mortier, & Estoup, 2005) and found that the boundaries of the genetic groups inferred were in parallel to a highway. This result suggested that this highway acted as a barrier to gene flow.

In contrast to the majority of studies, some studies investigated the effects of roads on genetic differentiation by applying specialised and replicated sampling designs. Following the sampling design of Marsh et al. (2008), four populations are ideally sampled at equidistance along and across a road. Genetic differentiation is then compared among populations on the same side of a road or across the road.

The differences between these comparisons reflect the barrier effect of the road. The sampling scheme has to be replicated for proper statistical analysis, and resampling tests should be used in statistical analysis because of non-independence of the data points. If populations cannot be sampled at equidistance, geographic distance should be included as a co-variable in the analysis. Using such a design, Marsh et al. (2008) found a significant barrier effect of a highway on genetic differentiation ( $F_{ST}$ ) in red-backed salamanders (*Plethodon cinereus*), but no such barrier effect of smaller roads. In contrast to this study, which discriminated between highways and other road types, most published studies did not consider different road types. While it is evident that highways, especially when fenced (Table 1), act as strong barriers to animal movement, the barrier effect of other road types cannot yet be evaluated, as there are few studies available that report on the effects of different road types.

## Contemporary movement across roads

There are several genetic methods available that allow the determination of current or recent migration across roads. In particular, assignment tests (Manel et al., 2005) detect immigrants into populations as well as their source populations based on multilocus genotypes. Most useful are first generation migrant assignment tests, as they restrict immigration to direct migrants (e.g. GENECLASS, Cornuet, Piry, Luikart, Estoup, & Solignac, 2005; BAYESASS, Wilson & Rannala, 2003).

We found six investigations on contemporary migration of animals across roads or highways (Table 1). All of them reported at least some migration across roads. These results indicate that roads and even highways are rarely complete barriers to animal movement (Table 1). In a widely cited study, Riley et al. (2006) found (using STRUCTURE; Pritchard, Stephens, & Donnelly, 2000) that bobcats (*Lynx rufus*) and coyotes (*Canis latrans*) cross a major highway in California. The genetic results on migration across the highway for these two species were congruent with, but higher than migration estimates based on radio-telemetry. As the populations on different sides of the highway were significantly differentiated from each other, the highway acted as a barrier to gene flow in bobcats and coyotes despite some contemporary migration across the highway. Riley et al. (2006) concluded that individuals crossing the highway did not necessarily mate with individuals on the other side; a behaviour resulting in weak effective gene flow across the highway (Strasburg, 2006; Table 1). Roads and highways also affect contemporary movement of invertebrates. For instance, Keller et al. (2005) investigated current migration of the ground beetle *Carabus violaceus* in a forested area of Switzerland (using GENECLASS; Piry et al., 2004). They studied two sampling sites completely isolated from each other and from other populations by main roads and a highway. In three consecutive years, one population received five,

**Table 2.** Empirical evidence on the corridor effect of road verges as inferred by genetic studies. Given are the species studied, the genetic markers used, the corridor effects of road verges, the country origin of the study and the corresponding reference.

Species	Genetic marker <sup>a</sup>	Corridor effect of road verges	Country	Reference
Earthworm ( <i>Dendrobaena octaedra</i> )	mtDNA	Passive invasion along roads	Canada	Cameron et al. (2008)
Snail ( <i>Helix aspera</i> )	Isozymes, SSRs	Migration along road verges	France	Arnaud (2003)
Bush-cricket ( <i>Metrioptera roeseli</i> )	RAPDs	Migration along road verges	Germany	Holzhauser et al. (2006)
Reed ( <i>Phragmites australis</i> )	cpDNA	Invasion along highway verges	Canada	Lelong et al. (2007)
Shrub ( <i>Calothamnus quadrifolia</i> )	SSRs	Movement of pollinators along road verges	Australia	Byrne, Elliott, Yates, and Coates (2007)

<sup>a</sup>cpDNA: chloroplast DNA sequences; mtDNA: mitochondrial DNA sequences; RAPDs: random amplified polymorphic DNAs; SSRs: nuclear microsatellite.

six and six immigrants, while the other population obtained one, two and three immigrants, respectively.

Although studies on contemporary gene flow are appealing, the interpretation of corresponding results is not easy. The basic problem lies in the question of how much migration is enough to counteract fragmentation or genetic drift. Are one, 10 or 20 migrants per generation across a road sufficient to secure the genetic coherence of populations on different sides of a road? Unfortunately, this question cannot be answered for wild populations in a generally applicable way (e.g. Whitlock & McCauley, 1999).

### Road verges as dispersal corridors

While roads act as barriers to movement, road verges provide dispersal corridors for animals and plants. Road verges often offer linear strips of suitable habitat within an otherwise hostile landscape (e.g. Forman et al., 2003). This positive effect of road verges also comes with a trade-off: road verges act as immigration corridors for exotic and invasive species all over the world (e.g. Coffin, 2007). When investigating the corridor effect of road verges, genetic methods offer an ideal tool to retrospectively monitor the respective migration pathways (Lelong, Lavoie, Jodoin, & Belzile, 2007). In the absence of detailed historical records, it would be difficult to get similar evidence for the corridor effect of road verges by using non-genetic methods.

Five genetic studies, using a diverse set of genetic methods, reported that road verges act as dispersal corridors for several animal and plant species, both native and exotic (Table 2). For instance, Cameron, Bayne, and Coltman (2008) found, by investigating mitochondrial DNA sequences, that roads favour the dispersal of invasive earthworms (*Dendrobaena octaedra*) in Canada (Table 2). Passive earthworm dispersal along roads was human-mediated. Roads and road verges also serve as dispersal corridors in plants. By simply plotting haplotypes of chloroplast DNA onto a topographical map, Lelong et al. (2007) showed that road verges favour the expansion of exotic common reed (*Phragmites australis*) in Canada (Table 2). By using genetic paternity analysis of open pollinated seed-progeny (Sork, Nason, Campell, & Fernandez, 1999). Byrne, Elliott, Yates, and Coates (2007)

found that spatially isolated groups of the native bird-pollinated shrub *Calothamnus quadrifolius* along Australian road verges were functionally connected by contemporary, pollinator-mediated gene flow through pollen (Table 2).

### Conclusions on the genetic effects of roads

From our literature survey, we draw the following general conclusions, which are largely in agreement with other overviews on the genetic effects of roads (Corlatti et al., 2009; Balkenhol & Waits, 2009):

- (1) Roads often, but not always, decrease the genetic diversity of affected populations due to reduced population size and genetic drift. Whether this influences the long-term fitness of populations is, however, not yet understood.
- (2) Roads, especially fenced highways, often act as barriers to animal movement, migration and gene flow. Roads therefore decrease the functional connectivity and increase the genetic differentiation of populations or the genetic distance among individuals. Nevertheless, roads and highways rarely act as complete barriers to animal movement as shown by studies assessing contemporary migration.
- (3) Road verges can act as dispersal corridors for native and exotic plant and animal species. Genetic methods are well suited to retrospectively trace migration pathways.

Although substantial progress has recently been made in the assessment of the genetic effects of roads (Balkenhol & Waits, 2009), the available literature (Tables 1 and 2) has some relevant limitations. Corresponding studies mostly originate from industrialized countries, which are characterized by high road densities, i.e. North America, Europe and Japan (Table 1). There are almost no genetic studies on the effects of roads from less heavily populated and less disturbed regions (Giulio, Holderegger, & Tobias, 2009). In addition, only few animal groups have been studied in a thorough way so far (Table 1): large mammals received more attention than small mammals, and there are also several studies on amphibians, probably because roads hinder the annual amphibian migration to breeding ponds and because of the generally low

dispersal capacity of amphibians (Beebee, 2005; Cushman, 2006). In contrast, only few insect and snail species have been assessed. To our knowledge, no study on the genetic effects of roads on reptiles has been carried out. Plants have also been scarcely studied, although one can foresee indirect genetic effects of roads on plants, if animals serve as pollinators or diaspore vectors. We therefore urge investigators to broaden the taxonomic basis of studies dealing with the genetic effects of roads. Where possible, we advocate the use of estimates on contemporary migration (e.g. assignment tests) in parallel with the evaluation of long-term population differentiation. The former yields estimates on the exchange of individuals or genes in real time, which can be correlated to current census population sizes (Riley et al., 2006), while the latter integrates the genetic effects of roads over the time period elapsed since road construction or since the implementation of defragmentation measures such as under- and overpasses or green bridges (Glista et al., 2009).

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