Review

Ability of Wildlife Overpasses to Provide Connectivity and Prevent Genetic Isolation

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Abstract: *We reviewed research on wildlife overpasses in the context of their genetic effectiveness to provide connectivity between population patches that have been isolated by road construction. The potential ecological consequences of such habitat fragmentation include reduction of gene flow between subpopulations and hence an increase in genetic differentiation and a decrease in genetic diversity. Among the solutions to provide connectivity between patches isolated by roads, wildlife overpasses are one of the most expensive alternatives. Despite the high costs associated with their construction, most of the studies assessing their use by wildlife remain observational, reporting evidence for passage use but few data on the number of individual crossings. Moreover, the use itself of wildlife overpasses does not appear sufficient to assess their effectiveness from a genetic viewpoint because a minimum number of individuals is required to assure gene flow between population patches and because the spatiotemporal dimension of individual movements and demographic parameters of subpopulations must be considered. So far, there is no evidence that wildlife overpasses do or do not efficiently address genetic issues. This lack of data is probably due to the fact that few mitigation efforts have implemented monitoring programs that incorporate sufficient experimental designs into pre- and postconstruction evaluation. To assess the genetic effectiveness of wildlife overpasses, long-term monitoring programs, including fieldwork and genetic analyses, are needed.*

Keywords: connectivity, fragmentation, genetic isolation, vertebrates, wildlife overpasses

Capacidad de los Pasos Elevados para Vida Silvestre para Proporcionar Conectividad y Prevenir el Aislamiento Genético

Resumen: *Revisamos la investigación sobre pasos elevados para vida silvestre en el contexto de su efectividad genética para proporcionar conectividad entre poblaciones que han sido aisladas por la construcción de caminos. Las potenciales consecuencias ecológicas de tal fragmentación de hábitat incluyen la reducción del flujo genético entre poblaciones y, por lo tanto, un incremento en la diferenciación genética y un decremento en la diversidad genética. Entre las soluciones para proporcionar conectividad entre fragmentos aislados por caminos, los pasos elevados para vida silvestre son una de las alternativas más costosas. No obstante los altos costos asociados con su construcción, la mayoría de los estudios que evalúan su uso por vida silvestre son de observación, registrando evidencia del uso de los pasos pero con datos escasos sobre el número de cruces individuales. Más aun, el uso mismo de los pasos elevados para vida silvestre parece insuficiente para evaluar su efectividad desde un punto de vista genético porque se requiere de un número mínimo de individuos para asegurar el flujo génico entre poblaciones y porque se deben considerar la dimensión espaciotemporal de los movimientos de individuos y los parámetros demográficos de las subpoblaciones. Hasta el momento, no hay evidencia de que los pasos elevados para vida silvestres atienden o no atienden los aspectos genéticos eficientemente. Esta carencia de datos se debe probablemente al hecho de que pocos esfuerzos de mitigación han implementado programas de monitoreo que incorporen suficientes diseños experimentales en la evaluación pre y post construcción. Para evaluar la efectividad genética de los pasos elevados para*

vida silvestre, se requieren programas de monitoreo a largo plazo, que incluyan trabajo de campo y análisis genéticos.

Palabras Clave: aislamiento genético, conectividad, fragmentación, pasos elevados para vida silvestre, vertebrados

Introduction

Roads are widely accepted to be a source of habitat fragmentation (Reed et al. 1996; Fleury & Brown 1997; Forman & Alexander 1998). The evaluation of direct and indirect consequences of habitat fragmentation on wildlife is profoundly complex and depends on both the intrinsic eco-ethological characteristics of target species and the features of the matrix surrounding fragments, which may act as a source area for species adapted to the matrix itself (Janzen 1986).

Conservation biology theory suggests that construction of linkage structures for wildlife between isolated habitat patches may increase or at least maintain levels of interpatch dispersal, thus maintaining gene flow and sustaining population viability of target species (Kozakiewicz 1993; Forman & Alexander 1998). For this purpose, crossing structures for wildlife have been incorporated into road-construction and -improvement projects (Clevenger & Waltho 2005). Wildlife overpasses are expected to provide habitat connectivity, generally defined as "the degree to which the landscape facilitates or impedes movement among resource patches" (Taylor et al. 1993). The idea of such wildlife passages is relatively new, and a variety of different terms are used to define them (see Keller & Pfister 1997). Iuell et al. (2003) propose a distinction between wildlife overpasses and landscape overpasses, mainly based on width of the structure, which reflects different levels of connection (population and landscape, respectively).

Because the aim of these structures, however, is to provide wildlife crossing, herein we define *wildlife overpasses* (hereafter overpasses) as all bridge-like structures of whatever size, designed for use by fauna or, at the most, for dual use by farm vehicles and wildlife, and planted with grass, shrubs, or trees. Overpasses are far more common on roads than other linear infrastructures (e.g., railway lines), and although information about them is available (especially concerning construction technique, see Putman et al. [2004] for a review), the literature lacks information about their genetic effectiveness. From a genetic viewpoint, effectiveness may be defined as the ability of overpasses to prevent isolation between otherwise disjunct subpopulations.

We sought to understand whether overpasses might effectively provide connectivity and ensure genetic exchange within vertebrate populations that have been fragmented by road construction. (We define *population*

as a group of interbreeding individuals living in the same area at the same time; subpopulations occur after the geographical subdivision of a population as a consequence of environmental or anthropogenic constraints.) We posed the following questions: Are wildlife passages necessary to provide genetic connectivity between subpopulations (namely, do roads act as genetic barriers for wildlife)? If roads do act as barriers, can overpasses be used to improve connectivity between subpopulations (i.e., Are overpasses used by wildlife)? Provided that overpasses are used by wildlife, can they effectively ensure genetic exchange? To address these questions, we posed the following hypotheses: if roads act as genetic barriers, they affect the genetic structure of disjunct wildlife subpopulations; if overpasses provide subpopulation connection through individual crossings, overpasses increase or at least maintain interpatch dispersal rate; the use of overpasses is insufficient to prevent the genetic decline and isolation of subpopulations because a minimum number of individuals is required to move between patches to assure gene flow.

Roads as Genetic Barriers for Wildlife

Roads have a broad spectrum of ecological effects on wildlife populations, both direct and indirect (Forman & Alexander 1998; Trombulak & Frissel 2000; Iuell et al. 2003). They facilitate animal–vehicle collisions; decrease reproductive success (e.g., litter failure; Bjurlin & Cypher 2003); cause disturbance and pollution (Iuell et al. 2003); constrain movement and distribution of species (Clarke et al. 1998; Alexander & Waters 2000; Lodé 2000; Yale Conrey & Mills 2001; Proctor 2003; Jędrzejewski et al. 2004; McDonald & St.Clair 2004; Whittington et al. 2005; Olsson & Widen 2008; Shepard et al. 2008); decrease colonization rate; increase extinction rate (Mader 1984); and change population density (Bjurlin & Cypher 2003), biodiversity (Gutzwiller & Barrow 2003; Chen & Roberts 2008), and prey availability (Bjurlin & Cypher 2003).

Genetic Consequences of Road Construction

The evidence that roads cause detrimental effects on habitat persistence and animal life-history traits, ranging movements, and density variations appears overwhelming. Nevertheless, one of the most severe consequences of habitat loss due to road construction is thought to be

the creation of isolated pockets of habitat that cannot support viable populations in the long term (Fahrig 2003). Reductions in the range of species may decrease probability of their successful movement between habitat patches, which affects gene flow, a complex process influenced by several intrinsic (e.g., dispersal ability) and extrinsic (e.g., landscape) factors (Burgman & Lindenmayer 1998). Genetic theory suggests that the reduction of gene flow between subpopulations may lead to greater inbreeding and loss of genetic diversity within fragments, the raw material that allows populations to evolve in response to environmental changes (Wright 1943; Frankham et al. 2002).

Although biological extinctions result from the combination of deterministic (e.g., habitat loss, overexploitation) and stochastic (demographic, environmental, and catastrophic) factors, loss of genetic diversity is thought to contribute to extinction risk (Bascompte & Solé 1996; Forman & Alexander 1998). Following genetic bottlenecks, populations may experience loss of rare alleles and a diminution of heterozygosity through genetic drift and inbreeding. These processes contribute to the erosion of genetic diversity and can lead to fitness reduction (Reed & Frankham 2003). The risk of genetic erosion is highest in small, isolated populations because of increased random genetic drift, elevated inbreeding, and reduced interpopulation gene exchange (Randi 1993; Hedrick 2001).

Several examples illustrate the link between genetic diversity and the fitness of individuals or populations (e.g., Frankham et al. 2002; Zachos et al. 2007 for red deer [*Cervus elaphus*]). Therefore, "continued habitat fragmentation can be expected to fuel the ongoing global extinction" (Reed 2004). Nevertheless, the link between genetic connectivity and extinction in nature remains largely a matter of debate and conjecture, rather than an empirical truth. As a result the investigation of genetic consequences due to road construction is of great importance in the light of conservation strategies to avoid inbreeding and maintain genetic diversity especially in threatened species (O'Grady et al. 2006).

Evidence of Roads as Genetic Barriers

The effects of fragmentation are influenced by dispersal ability of the species and associated migration rates between remaining habitat fragments (Frankham et al. 2002). Less vagile species are expected to be more subject to genetic issues. Research on the common frog (*Rana temporaria*) and desert tortoise (*Gopherus agassizii*) shows the role of barriers to gene flow played by motorways (Reh & Seitz 1990; Edwards et al. 2004). In the Netherlands, roads appear to play a major role as gene-flow barriers for moor frog (*Rana arvalis*) populations (Arens et al. 2007). Noël et al. (2007) indicate that allelic richness and heterozygosity are lower in urban populations of red-backed salamander (*Plethodon cinereus*). Exact differentiation tests and pairwise F_{ST} show that populations of the salamander in fragmented habitats are genetically differentiated, whereas populations in continuous habitats are genetically homogeneous.

The effect of major roads on population genetic structure has been studied for small- to large-sized mammals as well. Bank vole (*Myodes glareolus*) populations separated by a motorway show clear genetic subdivision (Gerlach & Musolf 2000). Pertoldi et al. (2001) used hypervariable minisatellite DNA to screen the genetic variation in 5 populations of the Eurasian badger (*Meles meles*) in Denmark. They found low genetic variability within populations, likely related to fragmentation of the Danish landscape, which reduces effective population size of local populations and gene flow between different subpopulations. This assumption has been upheld by van de Zande et al. (2007).

In a study of roe deer (*Capreolus capreolus*), Coulon et al. (2004)—using a direct quantitative approach—show that female gene flow is linked to forested areas. This seems to suggest that fragmentation of woodlands (e.g., due to road construction) may alter landscape connectivity, resulting in some change in gene flow. Later observations of genetic structure of a roe deer population inhabiting a fragmented landscape provide empirical evidence of landscape features (including a highway) acting as moderators of gene flow (Coulon et al. 2006). Kuehn et al. (2007) found that transportation infrastructures influence genetic divergence of roe deer, but not genetic diversity. The development of road networks in southern Kantoh (Honshu, Japan) may represent a cofactor in interruption of gene flow in Japanese sika deer (*Cervus nippon*), which is leading to different spatial genetic structures (Yuasa et al. 2007). Hartl (1998) reports examples that suggest genetic separation in red deer populations due to fenced roads in Germany and France. In Scottish Highland red deer, roads represent a cofactor for gene-flow alteration, even though their role as a genetic barrier is secondary to that of sea lochs and mountain slopes (Pérez-Espona et al. 2008). Gehle and Herzog (2003) in Germany found no significant difference in the genetic structure of 2 red deer populations before and after construction of a highway between the populations. Incorporating effects of human-made barriers in isolation-by-distance regressions, Epps et al. (2005) found evidence that fenced highways cause a rapid decrease of gene flow between populations of desert bighorn sheep (*Ovis canadensis nelsonii*) in southern California.

Dispersal and gene flow among bobcat (*Lynx rufus*) and coyote (*Canis latrans*) populations inhabiting habitats fragmented by the Ventura Highway (Southern California) have been measured by Riley et al. (2006). The authors found that migration rates for each species were 1.3% and 3.3% per generation, respectively.

Nevertheless, the genetic variation at the microsatellite loci showed significant differentiation between subpopulations on either side of the highway. They conclude that relatively high rates of dispersal across the fenced freeway do not translate into genetic connectivity (Strasburg 2006). Dixon et al. (2007) confirm lower heterozygosity in smaller and less connected populations of Florida black bears (*Ursus americanus floridanus*), a possible consequence of habitat fragmentation and anthropogenic barriers such as major roads that appear to limit their dispersal capabilities, reducing gene flow among populations. Onorato et al. (2007) suggest that uncommon events such as long-range dispersal are key events in delineating the genetic structure and maintaining variation within the black bear (Mexico-Texas metapopulation). Nevertheless, Millions and Swanson (2007) found no evidence of detrimental effects on genetic structure of bobcat populations (Michigan, U.S.A.) despite a high density of roads.

On the whole, these studies suggest that anthropogenic barriers may constitute a severe threat to persistence of naturally fragmented populations and that long-distance dispersal events, although typically rare, are crucial to population spread and to maintenance of genetic connectivity (Trakhtenbrot et al. 2005). Nevertheless, some controversial results persist. We suggest that the role of roads as genetic barriers for several vertebrate taxa may be particularly relevant in the presence of exclusion fences, which effectively reduce animal–vehicle collisions and have been adopted widely, for example, in several European countries.

Use of Overpasses by Wildlife

Overpasses are largely a European phenomenon (Evink 2002), but they are also present in the United States, Canada, and Australia. In Europe, as early as the 1960s, overpasses were built in France, Luxembourg, and the Netherlands, especially to look after the interests of hunters who were concerned that roads might prevent deer from moving from one area to another (Bekker 1998; Folkeson 2004). France was the first country to adopt such crossing structures, and 125 small overpasses have been constructed so far. (Results of research suggest that use of multiple, smaller overpasses is more effective than use of one large overpass [Bank et al. 2002].) Germany has more than 30 overpasses (most are dual use) and almost the same number are being constructed or are planned. Switzerland has more than 20 overpasses (Bank et al. 2002). In the Netherlands 4 hourglass and straight-shaped overpasses have been built and some others have been modified by covering one lane with vegetation and using fencing to direct animals toward it (Bank et al. 2002). There are also overpasses in Spain, Italy, Croatia,

Austria, Hungary, Czech Republic, Luxembourg, Sweden, and Norway (Santolini et al. 1997; Stahan 1998; Keller et al. 2003; Iuell et al. 2003; Mata et al. 2003; Folkeson 2004; Kusak et al. 2008). The first overpass constructed in the United States was completed in 2000 in Florida. Other overpasses have been constructed in Hawaii, New Jersey, and Utah and are being planned in Montana and Connecticut (Bank et al. 2002). In Canada there are 2 overpasses in Banff National Park (Alberta) (Evink 2002). Australia also has overpasses (Bond & Jones 2008).

Evidence of Overpasses Use

The use of overpasses by wildlife appears to be affected by several factors such as locations in relation to natural paths, size, design, visual appearance, and woody cover at the entrances (Putman 1997; Bekker 1998; Ng et al. 2004). Despite the role of overpasses in assisting the movement of species being widely accepted from a theoretical viewpoint, such assertions are seldom supported by empirical evidence. Evidence of the effectiveness of wildlife crossings derived from long-term monitoring programs is currently limited for most species (Clevenger & Waltho 2005; Mata et al. 2005; Bond & Jones 2008). Moreover, the use of underpasses (and other crossing structures such as culverts) appears to be more studied than that of overpasses (e.g., Ng et al. 2004; Ascensão & Mira 2007; Bond & Jones 2008; Braden et al. 2008).

In France Ballon (1985, in van Wieren & Worm 2001) found evidence that roe deer, wild boar (*Sus scrofa*), Eurasian badger, and red fox (*Vulpes vulpes*) use 2 overpasses, even though the crossing frequency was limited because of joint use by humans and small size of the passage. Again in France Vassant and Brandt (1998) found evidence of use of a wildlife overpass by roe deer, red deer, and wild boar.

In Germany overpasses are used by the red fox, European hare (*Lepus europaeus*), and domestic cat (*Felis catus*), whereas Swiss overpasses are well used by mammals such as the roe deer, Eurasian badger, wild boar, stone marten (*Martes martes*), red fox, and European hare (De Vries 1994, in van Wieren & Worm 2001).

Other studies, conducted in Switzerland through use of infrared video camera technology, show that overpasses are effective for a wide variety of animals including invertebrates. Specifically, structures at least 60 m wide are more effective than overpasses narrower than 50 m, especially for larger mammals (Evink 2002). In Switzerland viaducts and overpasses are the most effective structures for the widest range of species (Bank et al. 2002).

In the Netherlands an overpass is well used by the European hedgehog (*Erinaceus europaeus*), red squirrel (*Sciurus vulgaris*), European rabbit (*Oryctolagus cuniculus*), European hare, red fox, stone marten, and roe deer (Nieuwenhuizen & van Apeldoorn 1994, in van Wieren

& Worm 2001). Van Wieren and Worm (2001) investigated the use of an overpass across a motorway in central Netherlands, comparing data from 1989 and 1994/1995. In 1989, shortly after being built, the crossing structure was frequently used by the red deer, roe deer, wild boar, and red fox. In 1994/1995, 3 other large species were recorded: fallow deer (*Dama dama*), Eurasian badger, and Highland cattle (*Bos taurus*). The authors suggest a close relation between the frequency of passages and mating season. Small mammals including the wood mouse (*Apodemus sylvaticus*), common vole (*Microtus arvalis*), and common shrew (*Sorex araneus*) also used the structure.

In northwestern Spain underpasses and overpasses appear to be the most used structures, although with different levels of use according to species. Anurans and ophidians tend to avoid overpasses; lacertids, small mammals, and red foxes generally use all passage types; red deer use wide passages both under or above the road; roe deer only use underpasses; and wild boar mainly use overpasses (Mata et al. 2003, 2005, 2007).

Moose (*Alces alces*) and roe deer in Sweden use an overpass mostly during nocturnal hours, and the frequency with which ungulates use this particular overpass declines with increased traffic volume (Olsson et al. 2008). Despite construction of crossing structures, exclusion fencing along highways may have a negative impact on gene flow between moose populations (Olsson & Widen 2008).

Kusak et al. (2008) showed that the ratio of large mammals crossing the highway in Gorski Kotar (Croatia) via wide overpasses (100 m and wider) is 3 to 6 times higher than crossings through narrow underpasses.

In the United States there is little information about the use of overpasses because crossing structures are relatively new (Evink 2002).

In Canada a research project launched in 1996 showed that crossing structures not including overpasses are effective for elk, deer (*Odocoileus* spp.), and coyotes, but not for large carnivores, such as wolves (*Canis lupus*), cougars (*Puma concolor*), black bears (*Ursus americanus*), and grizzly bears (*Ursus arctos*). This led to the construction of 2 overpasses (Clevenger 1999) and subsequent monitoring actions. So far, deer use the wildlife overpasses 12 times more than the underpasses, elk use the overpasses 3 times more than underpasses, and moose use overpasses 6 times more (and only once used the underpass). All carnivores except cougars use both overpasses. Wolves used the 2 overpasses as a group (2–7 individuals) 5 times. In the first 3 years after being built, the 2 overpasses showed an increase in use, particularly by large carnivores such as grizzly bears, wolves, and cougars (Evink 2002). Although the results of some studies suggest that ungulates seem to prefer overpasses, where overpasses and underpasses are available close to each other, results of other studies suggest that the use

of overpasses by red deer, roe deer, and fallow deer is lower than that of underpasses (Staines et al. 2001).

Overpass Effectiveness

Despite the abundance of literature assessing the use of overpasses by wildlife, there seems to be a lack of evaluation of dispersal rates before and after construction. This makes their effectiveness at providing interpatch movements difficult to assess. Some evidence for lessening of animal–vehicle collisions after the construction of overpasses suggests they may be capable of increasing dispersal rates and providing genetic connectivity. Nevertheless, the diminution of animal–vehicle collisions alone is not enough to prove the increase of dispersal rates. Olsson and Widen (2008) show that rather than favoring dispersal rates, overpasses seem to mitigate the negative effect of exclusion fencing along highways. Fences, on the other hand, represent a tool "sculpted" to funnel individuals belonging to certain taxa (e.g., deer) toward overpasses (Putman 1997). Sometimes overpasses may also represent ecological traps for some taxa because individuals may face dim prospects as they arrive in unsuitable habitats. Consequently, overpasses may reduce gene flow and lower migration rates, relative to what would happen in their absence.

Overpasses and Genetic Connectivity

Animal use of an overpass in itself may not be sufficient to determine that an overpass will guarantee the survival of an entire population (Bekker 1998) because we expect that a species-specific minimum number of individuals is required to move between patches to assure gene flow. Nevertheless, most studies have been observational, reporting evidence for passage use. Few data on the number of individual crossings, before or after construction, are available. Most claims about the genetic effectiveness of overpasses are based on indirect assumptions. Given that habitat connectivity is vital for maintaining genetic flow between populations, results suggests that cross-highway structures facilitate wildlife attempting to cross major roads and thus may represent a useful tool to avoid genetic issues. For example, Olsson et al. (2008) suggest that 5–7 moose/year using an overpass is enough to maintain gene flow between subpopulations.

Modeling Gene Flow

An indirect (deterministic) approach to assess whether overpasses prevent genetic isolation is to compare the observed rate of crossings and the number of individuals theoretically required to maintain genetic diversity. One migrant individual per generation might be enough to prevent inbreeding depression (Wright 1931; Wang 2004a). The "one migrant per generation" rule, however,

may be sufficient in many scenarios, and for fluctuating populations or those violating the assumptions of Hardy–Weinberg equilibrium, up to 10 animals may be needed to maintain current levels of genetic diversity (Mills & Allendorf 1996; Vucetich & Waite 2000).

Nevertheless, the minimum number of individuals required to assure the maintenance of gene flow is expected to depend on the species (Whitlock & McCauley 1999), the size of the recipient subpopulation (Vucetich & Waite 2001), and, therefore, on census size of subpopulations (Toro & Caballero 2005). Law and Linklater (2007) contend that the sex ratio within populations affects immediate survival and reproductive performance of individuals, and the rate at which their alleles are transferred and persist in the target subpopulation. Moreover, age structure of populations, survival of migrants, and level of gene flow within the subpopulation where migrants originate (Couvet 2002; Wang 2004*a*) must be taken into account. In addition, the spatial and temporal dimension of individual movements should be considered. That is, the effectiveness of an overpass to ensure genetic connectivity would be compromised if individuals from different subpopulations did not meet in the same area during the same mating season (e.g., overpass could be used only for reaching feeding sites outside the breeding period). Hence, an integrated evaluation of crossing rates and spatiotemporal behavior of the study species and estimates of population parameters (e.g., size, sex–age structure), intrinsic migratory rates, and survival of migrants are needed to understand the potential of the overpass to avoid genetic issues. "For a given population, different models may be developed and used for its genetic management, depending on the details of the information being observed and recorded for the population" (Wang 2004*b*).

Conclusions

Even though anthropogenic infrastructures such as roads are commonly thought to limit genetic exchange between fragmented patches, few studies have demonstrated clearly that major roads are acting as barriers to gene flow among subpopulations (Strasburg 2006). Even fewer studies have investigated the extent to which overpasses are effectively used by wildlife, and no study has investigated the genetic effectiveness of overpasses. This situation is probably due to the fact that few mitigation programs have implemented monitoring programs that incorporate sufficient experimental design into pre- and postconstruction evaluation. "Although limited movement across roads may be sufficient to insure genetic mixing, it may not prevent population isolation and decline. Only subsequent monitoring will determine if the mitigation was effective. If not, reconsideration of additional crossings is warranted" (Bissonette & Adair 2008).

Moreover, the success of monitoring programs aimed at single target species may fail to evaluate the barrier effects on other nontarget species (Clevenger & Waltho 2005).

Considering the high costs associated with construction of wildlife overpasses and the lack of information on their effectiveness, there is an urgent need to study the ability of such structures to prevent genetic isolation. Rather than adopting a deterministic approach, it would be best to adopt an adaptive approach with which to compare the situation before and after overpass construction. For this task, long-term monitoring projects, including fieldwork and genetic analyses, are needed.

Whenever possible we recommend the following 3 types of investigations. First, investigate the genetic diversity of disjunct subpopulations and the dispersal rate of individuals before overpass construction (when no effect on genetic structure of populations can be detected, overpasses might not even be needed in the first place). Details on analysis of molecular data can be found in Pritchard et al. (2000), Corander et al. (2003), and Mank and Avise (2004).

Second, investigate the use of overpasses by different wildlife species from a quantitative point of view, taking into consideration the spatiotemporal scale (e.g., distribution and dispersal rate before, during, and after the mating season). We recommend global positioning satellite tracking, photographic monitoring, pellet-transect monitoring, and hair sampling. (For an extensive review of these techniques, see Goosem [2005].) These studies should start after the habituation period, which may take several years depending on the species, and be carried out on an annual basis.

Third, investigate genetic diversity of the reconnected subpopulations a few years after construction of an overpass. This adaptive approach requires taking into consideration variables such as characteristics of the bridge itself (e.g., size, access to the structure, type of surface, presence of farm tracks, and vegetation distribution, density, and structure) and of the matrix surrounding the structure (e.g., size of the road, traffic load, presence and size of exclusion fences, human disturbance, presence of other roads nearby, density and sex-age structure of animal populations, and habitat characteristics on either side of the bridge because overpasses could be ecological traps for some taxa). To our knowledge no study has evaluated the use of overpasses in relation to all the above variables, a lack that might lead to uncertain results about their effectiveness from a genetic viewpoint (Clevenger & Waltho 2005).

We suggest that the effectiveness of overpasses as tools to provide connectivity and prevent genetic isolation of subpopulations of several vertebrate taxa may be linked to the presence of fences, which simultaneously act as barriers to direct access to roads and facilitate access to crossing structures.

Finally, the choice to build overpasses across roads (or modifying already existing structures) does not entirely rely on genetic considerations (population level). Considering benefits to the individual, overpasses should be used whenever possible because "they are less confining, quieter, maintain ambient conditions of rainfall, light and temperature, and can serve both as passageways for wildlife and intermediate habitat for small animals such as reptiles, amphibians and small animals" (Jackson & Curtice 1998). Furthermore, overpasses can act as tools to prevent wildlife–vehicle collisions and could reduce potential stress when crossing traffic barriers. Improving the quality of life of individuals could result overall in higher population viability, although the effect may be difficult to quantify. The definition of overpass effectiveness therefore changes depending on the goal (Clevenger 1999). From a genetic perspective there is still much to be done.

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