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# An Assessment Of The Role Of Roads In Population Demography And Genetic Structuring In Two Species Of Lizards In The Northern Chihuahuan Desert

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AN ASSESSMENT OF THE ROLE OF ROADS IN POPULATION  
DEMOGRAPHY AND GENETIC STRUCTURING IN TWO SPECIES OF  
LIZARDS IN THE NORTHERN CHIHUAHUAN DESERT

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Charles Ambler, Ph.D.  
Dean of the Graduate School

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by

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## **Dedication**

This work is dedicated to my wife, who supported me throughout this journey and always made challenging days better. This is also dedicated to my parents and sister, whose love and support has made me the person I am today.

AN ASSESSMENT OF THE ROLE OF ROADS IN POPULATION  
DEMOGRAPHY AND GENETIC STRUCTURING IN TWO SPECIES OF  
LIZARDS IN THE NORTHERN CHIHUAHUAN DESERT

by

KEVIN WESLEY FLOYD, M.S.

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## Abstract

Roads can fragment habitat and increase mortality rates of wildlife, potentially reducing population sizes and gene flow, which in turn can reduce genetic diversity through genetic drift. Although negative road impacts have been found in a variety of taxa, not all species are impacted. For instance, species that move less and rarely encounter roads and those that avoid the roads are predicted to not be negatively affected. Yet tests of these predictions are uncommon, especially for reptiles and particularly lizards. Side-blotched lizards (*Uta stansburiana*) are small sit-and-wait predators, while the larger marbled whiptails (*Aspidoscelis marmorata*) are active foragers. Their responses to roads were tested in populations inhabiting areas near a large high volume road and a smaller local road. Whiptails were hypothesized to encounter roads frequently, decreasing survival rates and abundance near roads. Conversely, side-blotched lizards should encounter roads less often and have less mortality. Potentially lower abundance of whiptails near roads should also lead to decreased genetic diversity, and the lack of movement across roads should lead to increased genetic differentiation.

Neither the abundance nor survival of either species was decreased near the roads. Both species crossed the smaller road less often than expected, and no individuals were found to have crossed the larger road. Genetic diversity was not correlated with distance to road and no divergence was detected. Both species were abundant at all sites, and some lizards crossed the small road, maintaining genetic connectivity. Although unlikely that lizards cross the larger road, the lack of differentiation is likely due to large populations where genetic drift acts slowly. Furthermore, the roads are relatively new, perhaps allowing insufficient time for detectable differentiation. Despite biological differences, these two lizard species do not appear to experience negative demographic or genetic effects of roads. Future studies should examine how lizard species with divergent life histories and behaviors respond to increasing road density. This study is the first on lizard road ecology in the desert Southwest, and one of the few studies that integrates demographic and genetic data to gain a better understanding of animal responses to roads.



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# **Chapter 1: General Introduction**

## **1.1 ROAD ECOLOGY**

Habitat loss and habitat fragmentation are two of the main threats to terrestrial biodiversity (Fahrig 1997, Pimm and Raven 2000, Sala et al. 2000, Fahrig 2003, Haddad et al. 2015). One major cause of habitat fragmentation are roads. In 2013, the road network of the United States was longer than 6.6 million km with >4.8 trillion vehicle km traveled (US Department of Transportation 2014). Roads can directly impact population vital rates, such as an increase of death rates caused by road-related mortality (Forman and Alexander 1998, Trombulak and Frissell 2000). Roads can also fragment contiguous habitats and potentially serve as barriers to dispersal and gene flow among populations (Trombulak and Frissell 2000, Keller et al. 2004, Boarman and Sazaki 2006). Some impacts spread beyond the road itself, particularly for animals that are sensitive to light or traffic noise (Eigenbrod et al. 2009, Parris and Schneider 2009, Jack et al. 2015). The total area impacted directly and indirectly by roads, the “road-effect zone,” was estimated as 20% of the United States (Forman 2000, Forman and Deblinger 2000). Approximately 78% of all land area is within 1000 m of a road (Riitters and Wickham 2003). Given the ubiquitous nature of roads in the landscape, it is critical to understand how they affect the short- and long-term persistence of wildlife.

Roads can both reduce the amount of available and suitable habitat and increase the isolation of local wildlife populations. The consequences of these negative impacts can affect populations both demographically and genetically. Once a habitat patch becomes small and isolated, stochastic and deterministic factors can lead to a decline in vital rates, such as birth and death rates (Lande 1993). Even if populations persist, severe declines in population size can result in genetic bottlenecks and loss of genetic diversity (Hoelzel 1999, Frankham et al. 2004, Kuo and

Janzen 2004), and reduction of gene flow further enhances these negative effects (Frankham et al. 2004). As genetic diversity is required for adaptive evolution, the loss of genetic diversity strongly decreases the long-term survival probability of a population (Frankham et al. 2004).

Much of the early work in road ecology focused on documenting road mortality: which species, how many deaths, and where mortality is likely (e.g., Dodd et al. 1989, Rosen and Lowe 1994, Ashley and Robinson 1996). While this work, and that which followed, has been important in helping to assess species and landscape characteristics that seem to increase mortality rates, by itself such work does not address the impact that roads have on the persistence of the populations. A species that is killed in large numbers on roads might not be impacted if the population is highly abundant, while for rare species even a few additional mortalities might have serious consequences for population persistence (Taylor et al. 2002). Indeed, the most pressing question for road ecology identified by Roedenbeck et al. (2007) was “Under what circumstances do roads affect population persistence?”

A variety of taxa have been negatively impacted by roads, either in terms of increased mortality, isolation, or reduced reproduction (reviewed in Trombulak and Frissell 2000, Taylor and Goldingay 2010). These include amphibians and reptiles (Carr and Fahrig 2001, Marsh and Beckman 2004, Row et al. 2007, Delaney et al. 2010, Baxter-Gilbert et al. 2015, Souza et al. 2015), birds (Bujoczek et al. 2011, Loss et al. 2014, Gagné et al. *in press*), and mammals (Clevenger et al. 2003, Riley et al. 2006, Rytwinski and Fahrig 2011). Of the studies that measured impacts on population abundance (addressing the question about persistence from above), the negative impacts of roads outnumbered the positive aspects by a factor of five (Fahrig and Rytwinski 2009). There are two general categories of hypotheses for which types of species should experience negative impacts: life history traits and behavioral responses (Rytwinski and Fahrig 2012). Life



history traits such as higher mobility are expected to increase the negative impacts, as individuals should encounter roads more often than less mobile species (Carr and Fahrig 2001, Gibbs and Shriver 2002, Forman et al. 2003, Cushman 2006). Negative impacts should also be seen more frequently in species with lower fecundity and longer generation times, as they will not be able to compensate for increased road mortality (Gibbs and Shriver 2002, Rytwinski and Fahrig 2011). Behavioral responses were summarized by Jaeger et al. (2005) as 1) avoidance of traffic disturbance (e.g., noise, vehicle emissions), 2) avoidance of the road surface, and 3) avoidance of vehicles (either waiting to enter roads when there are no vehicles or actively moving out of the vehicle path). Population abundances would decrease at some distance from the road (the road effect zone from above) for animals that avoid traffic disturbance (Eigenbrod et al. 2009), while there would be no change for species that avoid either the road surface or vehicles. Avoidance of either traffic disturbance or the road surface will reduce mortality rates, but the road will still act as a barrier to gene flow, potentially leading to long-term population persistence problems. Animals that do not avoid the traffic disturbance or the road surface, but can avoid vehicles should experience minimal negative impacts. A fourth behavioral response is attraction to roads (Rytwinski and Fahrig 2012). Scavengers might be attracted to road-killed animals (e.g., Meunier et al. 2000, Hubbard and Chalfoun 2012), females to nesting sites (e.g., Steen et al. 2006), or ectotherms for thermoregulation (e.g., Rosen and Lowe 1994). A meta-analysis by Rytwinski and Fahrig (2012) found that large mammal species with greater mobility and lower reproductive rates, more mobile birds, and amphibians and reptiles in general were more likely to experience negative road effects. They recommended that efforts to mitigate the negative effects of roads be focused on these types of taxa. However, they also noted that amphibians and reptiles were understudied relative to other groups, and warranted further investigation.

Studies on the genetic effects of roads have focused on measurements of genetic diversity and differentiation (Holderegger and Di Giulio 2010). In situations where roads decrease animal abundance and reduce gene flow, the resulting small isolated populations are expected to lose diversity and become more differentiated through genetic drift and/or increased inbreeding (Holderegger and Di Giulio 2010). Both situations decrease long-term viability of populations (Allendorf et al. 2013). Although changes in genetic diversity and differentiation require time to become detectable, many studies have found road effects in a variety of taxa (e.g., Keller et al. 2004, Epps et al. 2005, Riley et al. 2006, Kuehn et al. 2007, Clark et al. 2010, Delaney et al. 2010, Holderegger and Di Giulio 2010). Although decreases in fitness have been caused by low genetic diversity and inbreeding in some natural populations (reviewed in Frankham 2005), to my knowledge no studies have related loss of genetic diversity caused by roads with decreased fitness (but see Brady 2012 for an example of local adaption to road-related environmental conditions). A related use of molecular methods in road ecology is assessment of movement across roads in a cost-effective and often more logistically feasible manner than radio-tracking or mark-resight studies (Simmons et al. 2010). Individual-based measures of genetic relatedness and population of origins can be used to identify individuals who dispersed across roads when traditional techniques such as mark-recapture or radio-tracking are not effective (e.g., Riley et al. 2006, Simmons et al. 2010, Sackett et al. 2012). Thus studies of genetic diversity and structure can inform researchers on short- and long-term potential for population persistence.

## **1.2 ROAD ECOLOGY AND REPTILES**

Both reptiles and amphibians have been experiencing global declines, with habitat alteration implicated as a critical factor (Gibbons et al. 2000, Cushman 2006, Gardner et al. 2007). Although reptiles and amphibians are widely considered to be negatively impacted by roads, they

have been understudied relative to other taxonomic groups (Andrews et al. 2008, Rytwinski and Fahrig 2012). Most studies of road impacts on reptiles have focused on turtles and snakes, with only limited research on lizards (Andrews et al. 2008, Holderegger and Di Giulio 2010).

Lizards are a diverse and abundant group, but are rarely detected in surveys of road mortality (Andrews et al. 2008). Dodd et al. (1989) found only 17 lizards out of 303 reptiles (5.6%) on highways in Alabama. They also did not find many of the smaller snakes in the region on the roads, likely because small carcasses have low detectability or are rapidly scavenged. Aresco (2005) documented >10,000 herpetofauna trying to cross a highway in Florida, dead on the highway, or in the vegetated shoulders. Of the 152 lizards, only one was reported as roadkill, while 42% of the 363 snakes and 55% of the 838 anurans were found as roadkill. The lizards appeared to remain near the shrubs outside of the mowed road verge, which likely reduced the likelihood of attempting to cross the highway (M. Aresco, pers. comm.). The large number of dead anurans found and high frequency of surveys suggests that the survey methods would have detected more road-killed lizards if they had indeed been present. Despite being more common than snakes in the surrounding forests, far fewer lizards were found as road-kills (15% of 73 reptile road-kills) during walking surveys in the Western Ghats of India (Vijayakumar et al. 2001). An intensive survey in Indiana recorded >10,000 road-kills, 93% of which were amphibians (Glista et al. 2008). Only 141 reptiles were detected, and no lizards were found despite the survey finding large numbers of other small animals. Meek (2009) found 47 dead lizards along with 152 dead snakes during four years of surveying in France. He also searched for live animals in the adjoining habitat to estimate the ratio of roadkill to live animals, and found that the two lizard species had much lower ratios than the four snake species. He found greater mortality in the larger western green lizards (*Lacerta bilineata*) than in common wall lizards (*Podarcis muralis*), a difference

Meek (2009) attributed to increased road basking by western green lizards. An extension of that study over nine years (including the four years reported in Meek 2009) found 369 lizards of those two species, 272 of which were found dead (Meek 2014). Observations revealed that western green lizards did spend more time basking on roads, while common wall lizards spent more time foraging. Carcasses of western green lizards were found closer to the road edges, a pattern expected if they primarily bask at the roadside while common wall lizards foraged on the road surface. Few individuals of either species were observed successfully crossing roads. Those findings support those of Lebboroni and Corti (2006), who found more dead western green lizards ( $N = 10$ ) than either common wall lizards ( $N = 1$ ) or Italian wall lizards (*P. sicula*,  $N = 5$ ). In Turkey, Tok et al. (2011) found fewer road-killed lizards than other reptiles (14% of 183 road-kills). In South Korea, snakes were the most commonly detected road-killed reptile, with only two lizards detected out of 863 total reptiles (Seo et al. 2015). Wotherspoon and Burgin (2011) found more road-killed lizard individuals and species than snakes in suburban Australia. However, the road-killed lizard species found were only 38% of the total number of species in the region, while for snakes they found 56% of the possible species (Wotherspoon and Burgin 2011). This might reflect more species of lizards avoiding roads, or the generally lower detectability of lizards. The relatively small size of most lizards reduces their detectability during road-kill surveys (Langen et al. 2007, Gerow et al. 2010, Teixeira et al. 2013). A comparison between walking and driving surveys in Saguaro National Park in Arizona found that only 11 of 294 (3.7%) reptile road-kills found by walking were also detected while driving (Gerow et al. 2010). The larger animals like snakes were more likely to be detected by people in vehicles. That same study estimated more than 12,000 reptiles were killed annually within the park boundaries (Gerow et al. 2010). Road-killed small animals also have low persistence on roads, with most carcasses only remaining  $\leq 3$

days prior to removal by scavengers or being completely destroyed by passing vehicles (Santos et al. 2011, Teixeira et al. 2013, Beckmann and Shine 2015). Thus it is possible that lizards are experiencing road mortality at rates much higher than commonly reported.

Alternatively, many lizard species have life history traits and behaviors that minimize their risks of negative road effects. Road mortality risks generally increase with increased movement across the landscape, as the number of road encounters increases, or with use of the road surface for foraging or thermoregulation. Species that suffer large amounts of road mortality generally have seasonal migrations, such as to and from breeding habitats, dispersal of particular life stages, such as juveniles or males, or are attracted to roads (Rytwinski and Fahrig 2012, Beebee 2013). If seasonal migrations occur in many lizard species, they are not well documented. For example, northern alligator lizards (*Elgaria coerulea*) and western skinks (*Plestiodon skiltonianus*) had high site-fidelity and did not move large distances (Rutherford and Gregory 2003). Juvenile dispersal has been observed in several lizard species, but average dispersal distances are relatively short, usually <200 m (e.g., Doughty and Sinervo 1994, Olsson et al. 1996, Massot et al. 2003, Sinervo et al. 2006, Warner and Shine 2008, Dubey and Shine 2010). Movement distances of sexes can vary, causing differential road mortality risks. More male green iguanas (*Iguana iguana*) than females were found dead on roads during breeding season, when smaller males were dispersing to seek territories (Rodda 1990). More male Texas horned lizards (*Phrynosoma cornutum*) than females were found on roads during the spring, possibly due to increased mate-seeking movements (Sherbrooke 2002). There is mixed evidence regarding lizard attraction to roads for thermoregulation. Tanner and Perry (2007) found 71 road-killed Galapagos lava lizards (*Microlophus albemarlensis*) during weekly surveys over one month, and the majority of the carcasses were found in areas with dense vegetation in the surrounding habitat. They suggested

that lizards were attracted to the road for thermoregulation, and the dense vegetation created fewer opportunities to do so away from the road (Tanner and Perry 2007). Similar results were found with land mullets (*Egernia major*) and a Canary Island endemic lizard (*Gallotia galloti*), with increased abundances near roads likely due to increased basking opportunities in forested habitats (Klingensböck et al. 2000, Delgado Garcia et al. 2007). Overall, the life history traits and behaviors are mixed in regards to road mortality risks. Further, there have been very few studies that investigated the effects of roads on the abundance and/or survival of lizards (Rutherford and Gregory 2003, Delgado Garcia et al. 2007), which are critical for assessing population persistence.

To my knowledge, only three studies have addressed how roads impact the genetics of lizards. Three co-occurring species (side-blotched lizards (*Uta stansburiana*), western skinks (*Plestiodon skiltonianus*), and western fence lizards (*Sceloporus occidentalis*)) separated by major highways in southern California were genetically divergent, but had similar levels of genetic diversity (Delaney et al. 2010). Black-tailed brush lizards (*Urosaurus nigricaudus*) in Baja California had lower levels of gene flow across open areas, including highways (Munguia-Vega et al. 2013). There was only weak evidence that Florida scrub lizards (*Sceloporus woodi*) could not disperse across a highway, but lack of gene flow in this species across the entire landscape increased overall differentiation, and decreased the researchers' to detect any particular feature as a barrier (Tucker et al. 2014). These studies indicate that increasing our understanding of the impacts of roads on the genetic structure of lizards is critical for conservation efforts of individual species; lizards are understudied and decreased genetic diversity can decrease long-term population persistence (Frankham 2005).

### 1.3 STUDY QUESTION AND HYPOTHESES

Herein, I investigate how roads affect the abundance, survival, and genetic diversity and structure of two species of co-occurring lizards, the side-blotched lizard (*Uta stansburiana*) and the marbled whiptail (*Aspidoscelis marmorata*), in southern New Mexico. Side-blotched lizards are small, sit-and-wait predators with small home ranges, females can produce up to three clutches with an average of four eggs, and typically live one year (Spoecker 1967, Tinkle 1967, Peterson and Whitford 1987). Marbled whiptails are medium sized, active foragers with larger home ranges, females produce 1–2 clutches of 2–3 eggs annually, and typically live 3–4 years (Parker 1972, Anderson 1993, McElroy et al. 2011). The contrasting life histories and movement behaviors of these two lizards allow testing of predictions of susceptibility to road effects while helping to reduce the gaps in our current knowledge about lizard road ecology.

I also compared how these two lizard species respond to roads of different sizes and traffic volumes. Large, wide roads with high traffic volume and speeds are expected to have stronger effects on animal populations (Oxley et al. 1974, Trombulak and Frissell 2000, Clevenger et al. 2003, Farmer and Brooks 2012, Rhodes et al. 2014). However, even small roads can be barriers. For example, Marsh et al. (2005) found that even lightly traveled dirt roads are movement barriers to red-backed salamanders (*Plethodon cinereus*). I compared a wide, heavily traveled interstate highway (Interstate 10, hereafter I-10) with a smaller, lightly traveled rural state highway (New Mexico State Highway 9 [NM-9], also called Dona Ana County Road A003).

In the first data chapter (Chapter 2), I measured the impacts that these roads had on the population demography of both species using mark-recapture techniques. Specifically, I asked 1) is the abundance and survival of lizards living near roads lower than those away from roads, 2) are larger, more vagile, and less fecund lizards more sensitive to road effects, and 3) are the effects of living near a large, highly traveled road more severe than living near a small, lightly traveled road?

I hypothesized that both abundance and survival would be lower near the roads, but that the specific effects would depend on the species and road characteristics. Marbled whiptails move larger distances and live longer than side-blotched lizards, so have greater cumulative risks of road mortality. Thus the differences between the near-road and away-from-road abundance and survival should be greater for whiptails than for side-blotched lizards. The increased traffic volume of the interstate reduces the likelihood that an individual can successfully cross (Hels and Buchwald 2001), so the negative impacts should be greater near the interstate than the rural highway.

In the second data chapter (Chapter 3), I measured the impacts of the roads on the genetic diversity and differentiation of both species using microsatellite markers. I asked 1) do populations near roads have lower genetic diversity than those away from roads, 2) do roads act as barriers to movement, 3) do different life history and movement behaviors influence the impacts of roads on population genetics, and 4) are the effects of a large, highly-traveled road stronger than those of a small, lightly-traveled road? Again, I hypothesized that the effects would differ for the two species. The increased mortality risks for the marbled whiptails should reduce their populations near the roads, and smaller population sizes should lose genetic diversity more rapidly. Side-blotched lizards are less likely to encounter the roads due to their shorter movement distances, so their abundance near the road should only be minimally effected at most, and they will not show any decrease in genetic diversity. If a road is not a complete barrier, a sufficient number of individuals should be able to successfully cross and maintain genetic connectivity (Mills and Allendorf 1996). I hypothesized that some proportion of whiptails would be able to successfully cross the smaller road, preventing genetic differentiation from occurring. The large road is expected to be a complete barrier, and the populations of whiptails on opposite sides of the road



should be differentiated. It is possible that side-blotched lizards could successfully cross the smaller road, but with lower frequency than the whiptails, while the larger road would act as a complete barrier to them as well. Thus the populations on opposite sides of the larger road should be more differentiated than those on opposite sides of the smaller road.

I synthesize the major findings of this project and make recommendations for future studies that will broaden the scope of this project to include a wider variety of roads, degrees of habitat fragmentation, and lizard species in the final chapter (Chapter 4).

The two data chapters are formatted for planned submission to peer-reviewed journals for publication. Chapter 2 will be submitted to *Conservation Biology*, and the co-authors are Carl Lieb and Elizabeth Walsh. Chapter 3 will be submitted to *Conservation Genetics*, and the co-author is Elizabeth Walsh.

## **Chapter 2: The Effects of Roads on the Population Demography of Two Species of Lizards in Southern New Mexico**

### **2.1 ABSTRACT**

Decreased animal abundance is likely the greatest short-term threat to wildlife populations near roads. Species with longer movements, lower reproductive rates, and no behavioral road avoidance are expected to experience great reductions in abundance. Among the taxa studied to date, reptiles and amphibians show the strongest negative impacts. However, very few studies have examined how roads impact abundance and survival of lizard populations. We compared the effects that two roads, a large interstate highway and a small rural state highway, had on two species of lizards with different life histories and movement behaviors, side-blotched lizards (*Uta stansburiana*) and marbled whiptails (*Aspidoscelis marmorata*). We hypothesized that the widely-foraging whiptails would experience higher mortality rates than the sit-and-wait side-blotched lizards, and the lower reproductive rates of the whiptails would make them less able to rebound from that higher mortality. We captured, marked, and released lizards from eight sites along the two roads during 2009–2011. Generalized linear models were used to determine the effects of proximity to the roads on abundance, survival, and body condition, including potentially confounding habitat structure metrics as additional explanatory variables. Neither species had decreased abundance nor survival near either road. Side-blotched lizard and juvenile whiptail abundance was slightly higher near the roads. Survival was most affected by the different years, likely reflecting differences in annual precipitation during the study. Male side-blotched lizards were slightly larger near the roads, and there were no road effects for female side-blotched or either sex of whiptails. Both species crossed the smaller road less often than expected, and we did not detect any successful movements across the large road. Despite expected differences in the impacts that the roads would have on the different species, both were largely unaffected. Both

species generally stay under plant cover, with whiptails foraging under many plants while side-blotched lizards remain under a few plants. The lack of cover on the pavement likely prevents many lizards from attempting to cross the roads. Even if the whiptails encounter roads more often due to their increased vagility, if they avoid attempting to cross the road they will not suffer any decreased abundance or survival. Although many reptiles do suffer negative road effects, it appears that small lizards are not affected.

## **2.2 INTRODUCTION**

### **2.2.1 Road effects and life history traits**

Roads and traffic can have many negative impacts on wildlife populations, including decreased abundances, reduced movement across landscapes, and increased exposures to pollution and other stressors (Trombulak and Frissell 2000, Forman et al. 2003, Fahrig and Rytwinski 2009, Taylor and Goldingay 2010). Decreased abundance is likely the greatest near-term threat to animal population persistence near roads. A review by Fahrig and Rytwinski (2009) showed that 59% of populations studied had decreased abundances near roads while some species show neutral (29%) or positive (12%) responses to roads (Fahrig and Rytwinski 2009). Hypotheses explaining which species experience negative, positive, or no effects of roads have focused on two main categories: differences in life history, including size, vagility, and reproductive rate (Gibbs and Shriver 2002, Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012), and differences in behavioral responses to roads (Jaeger et al. 2005). Negative impacts are expected in highly mobile animals with large home ranges due to increased frequency of road encounters and those with lower reproductive rates and longer generation times because they are less able to compensate for increased road mortality (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012, 2013). Negative impacts are also expected in species that do not avoid entering roads but are not able to avoid vehicle collisions, or species that avoid disturbances associated with roads, such as increased traffic noise or pollution (Jaeger et al. 2005). Neutral or positive impacts are predicted for species without the above traits and behavioral responses, along with species that experience predator release. If the abundance of predators decrease because of negative road effects, it could release the prey populations, which are generally smaller animals, to increase (Rytwinski and Fahrig 2007, Bissonette and Rosa 2009, Rytwinski and Fahrig 2012, 2013). Large mammals, most birds, and reptiles and amphibians generally show negative responses to roads, while small mammals generally show neutral or

positive responses (Fahrig and Rytwinski 2009). Although reptiles and amphibians were the taxonomic groups with the most consistently negative response, they were also the least studied (Rytwinski and Fahrig 2012). Recent reviews found that most studies have focused on turtles and snakes, with only one study on lizards (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). While many turtles and snakes share the life history characteristics of negatively impacted species (more mobile, long generation times), there are a wide range of traits among lizard species, including those more commonly associated with species not negatively affected by roads. Given that mitigation efforts are often expensive (van der Grift et al. 2012), and that other studies recommend focusing mitigation on reptiles and amphibians as a broad taxonomic group (e.g., Rytwinski and Fahrig 2012), it is important to understand how lizards respond to roads.

### **2.2.2 Lizard road mortality**

Lizards make up approximately 60% of all reptile species (<http://www.reptile-database.org>, accessed 6 May 2015), and are particularly abundant in arid and semi-arid regions worldwide (Pianka 1966, 1967, 1969). They can act a major components in energy flow through a community (Turner et al. 1976). Lizards are rarely detected in surveys of road mortality, including those focused on herpetofauna (Dodd et al. 1989, Aresco 2005, Andrews et al. 2008, Glista et al. 2008, Seo et al. 2015), or are found at rates less than expected based on their relative abundance in the surrounding habitat (Vijayakumar et al. 2001, Meek 2009, Wotherspoon and Burgin 2011). Road-killed lizards, and small animals in general, have lower persistence on roads, generally lasting only 1–3 days before the carcasses are removed by scavengers or completely destroyed by passing vehicles (Santos et al. 2011, Teixeira et al. 2013, Beckmann and Shine 2015). In addition, the small carcasses are difficult to detect in surveys, particularly those done by vehicle. Comparisons of driving versus walking surveys have found that driving surveys can miss >90%

of the smaller vertebrate carcasses (Langen et al. 2007, Gerow et al. 2010, Teixeira et al. 2013). Thus it is possible that lizards are experiencing road mortality at rates much higher than commonly reported.

However, many lizard species have life history traits that could minimize their chances of being negatively impacted. Road mortality risks are generally correlated to increased movement across landscapes and use of roads or road verges for foraging or thermoregulation, both behaviors that increase the likelihood of encountering roads. Movements can be seasonal migrations from breeding/nesting sites to hibernation sites, or dispersal of juveniles. Rutherford and Gregory (2003) found that both northern alligator lizards (*Elgaria coerulea*) and western skinks (*Plestiodon skiltonianus*) had high site-fidelity and did not move large distances, both likely reasons for the lack of road mortality documented in their study. Aresco (2005) found lizards primarily under shrubs outside of the highway right-of-way, which likely contributed to his only finding one road-killed lizard during his extensive walking surveys in Florida. It is thus unclear whether the lack of reported lizard road-kills are due to difficulties in finding the carcasses because of low persistence and detectability, or if lizards have certain life history or behavioral traits that make them less susceptible to road mortality.

Basking on roads has been proposed as an explanation for the negative road effects seen in snakes and turtles (Andrews et al. 2008, Rytwinski and Fahrig 2012). Many species of lizards are heliothermic, and roads can provide enhanced basking opportunities, particularly when in habitats with dense vegetation. This attraction may increase mortality risks. The majority of road-killed Galapagos lava lizards (*Microlophus albemarlensis*) were found in areas with dense vegetation in the surrounding habitat, which created fewer opportunities for basking and may have caused the lizards to be attracted to the road (Tanner and Perry 2007). Although the mortality rates were not

addressed, Delgado Garcia et al. (2007) found greater abundances of a Canary Island endemic lizard (*Gallotia galloti*) near roads than in the interior of forests, which they attributed to improved basking opportunities. In Australia, lizard species with greater ability to thermoregulate were more abundant near a road, while those that were less tolerant to higher temperatures were less abundant (Lee and Croft 2008). Given the variety of population-level responses to roads, and the uncertainty about the level of road mortality actually experienced by lizards, we studied the impacts of roads on the abundance and survival of two species of lizards in the southwestern United States.

### **2.2.3 Study system**

Side-blotched lizards (*Uta stansburiana*) and marbled whiptail lizards (*Aspidoscelis marmorata*) are locally abundant lizards in the deserts of the southwestern United States (Pianka 1967, Tinkle 1967, Parker and Pianka 1975). The ecology and life history of both species have been well-studied (e.g., Tinkle 1967, Whitford and Creusere 1977, Baltosser and Best 1990, Anderson 1993), making them ideal study species. They also represent the different life history strategies that may influence how they are affected by roads. Side-blotched lizards are small (snout-vent length [SVL] of males rarely exceeds 60 mm; Tinkle 1967) sit-and-wait predators, and generally do not move large distances (Spoecker 1967, Tinkle 1967, Peterson and Whitford 1987). Females are sexually mature during their first spring (about 8–10 months old) producing up to three clutches per season (about four eggs/ clutch) and typically live one year (Tinkle 1967). Marbled whiptails are larger lizards (male SVL 90–100 mm; Turner et al. 1969). They are active foragers, and move over greater distances than do side-blotched lizards (Parker 1972, Peterson and Whitford 1987, Anderson 1993, McElroy et al. 2011). Females that hatch early in the summer can become sexually mature the following spring, with an average clutch size of 2–3 eggs, and females can produce two clutches per year if there are abundant resources (Turner et al. 1969, Pianka 1970,

Parker 1972). Neither species has seasonal migrations between hibernation, feeding, or nesting sites (Castañeda et al. 2007, Scoular et al. 2011). Although there are conflicting reports about the dispersal distance of juvenile side-blotched lizards (Tinkle et al. 1962, Doughty and Sinervo 1994, Doughty et al. 1994), maximum distances are on the order of 100s of meters, not 1000s of meters. To the best of our knowledge, dispersal behavior of juvenile marbled whiptails has not been reported. Given the long history of studies with marked whiptail lizards, it seems unlikely that juvenile dispersal is common. Average life span is 3–4 years, although individuals >6 years old have been reported (Turner et al. 1969). The contrasting life histories and foraging behaviors of these syntopic species allow testing of the predictions of susceptibility to road effects.

Size, traffic volume and speed, and road width are all expected to impact crossing behavior and mortality risks (Oxley et al. 1974, Trombulak and Frissell 2000, Clevenger et al. 2003, Farmer and Brooks 2012, Rhodes et al. 2014). Dirt roads with small traffic volumes have been shown to be barriers for salamanders (Marsh et al. 2005), while some species of lizards would cross lightly traveled roads, but not two-lane highways (Brehme et al. 2013). Further, animal behavior might change from occasional crossing attempts when traffic volumes are low to complete avoidance of crossing as traffic volumes increase (Clevenger et al. 2003, Lebboroni and Corti 2006). Road width may deter animals that avoid open spaces from attempting to cross (Oxley et al. 1974, Tremblay and St. Clair 2009, Brehme et al. 2013).

Thus, we compared the effects of two roads in southern New Mexico, a wider and more heavily traveled interstate highway and a smaller lightly traveled rural state highway. In this area, land adjacent to roads is largely used for cattle grazing, and there is little other human impact. This system allowed us to isolate the potential road effects without confounding other aspects of urbanization, such as a high density of roads correlated with small habitat fragments.



#### 2.2.4 Study objectives

Our objective was to assess how these two roads impact the abundance and individual survival of the two lizard species with different life histories and foraging behaviors. Because of their greater vagility and longer life span, we predicted that whiptails would experience greater mortality from vehicles than side-blotched lizards. This impact would be reflected in fewer individuals and/or lower survival near roads than away from the roads for whiptails, while side-blotched lizards would have similar abundances and survival at all distances from the roads. In addition, we predicted that the interstate highway would cause greater impacts on these parameters than the rural road.

### 2.3 METHODS

#### 2.3.1 Study area

We conducted this study in typical Chihuahuan desert shrublands in south-central New Mexico, USA from 2009 to 2011. The vegetation is dominated by honey mesquite (*Prosopis glandulosa*), with four-wing saltbush (*Atriplex canescens*), Mormon tea (*Ephedra trifurca*), soaptree yucca (*Yucca elata*), broom dalea (*Psoralea scoparius*), and snakeweed (*Gutierrezia* spp.) also present. Some of the sites located near the interstate highway (sites 1 north and 4 north, see below for site designations) had areas with perennial grasses. Individual sites vary from mostly flat with small mesquite coppices to large mesquite coppices >2 m high.

We used climate data recorded at the relatively nearby Santa Teresa, NM, National Weather Service office. The average annual precipitation from 1997 to 2013 (the span of available data) is  $22.6 \pm 2.0$  cm (mean  $\pm$  SE), with  $55 \pm 6\%$  falling during the summer monsoon season, from July 1 to September 30 (National Weather Service, [http://www.srh.noaa.gov/epz/?n=santateresa\\_monthly\\_precip](http://www.srh.noaa.gov/epz/?n=santateresa_monthly_precip), accessed 31 March 2014). Average monthly temperatures range from 6.5°C in January to 27.7°C in July (Western Regional

Climate Center: Cooperative climatological data summaries, <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?nm8127>, accessed 29 September 2014).

The two roads compared were Interstate 10 (I-10) west of Las Cruces, NM, and New Mexico State Highway 9 (NM-9, also called Doña Ana County Road A-003), west of El Paso, TX (Fig. 2.1). The roads are approximately 50 km apart, with little development between them, except for a rail line that runs NW-SE through the region, about 7 km south of the sites along I-10 and about 12 km north of the sites along NM-9, and lightly traveled dirt roads. Interstate 10 is a four-lane divided highway with an average annual daily traffic volume (AADT) of 15,187 vehicles in 2010 and 19,731 in 2011, with about 55% of the traffic composed of heavy commercial vehicles (New Mexico Department of Transportation [NMDOT], <http://www.dot.state.nm.us/en/Planning.html>, accessed 16 March 2014). The pavement width of each side of the highway is  $11.8 \pm 0.5$  m (mean  $\pm$  SE), and in the study region the median is vegetated and  $28.9 \pm 0.6$  m wide. New Mexico State Highway 9 is a two-lane road. We measured the traffic volume using a TRAX Apollyon (JAMAR Technologies, Inc., Hatfield, PA, USA) because there was no recent information on traffic volume in the study area. We measured the traffic volume for one week (9–15 August 2010), and the AADT was  $508 \pm 11.8$  (mean  $\pm$  SE) vehicles,  $28 \pm 2\%$  of which were heavy commercial vehicles. The pavement width was  $8.7 \pm 0.2$  m.

### **2.3.2 Field methods**

There were three trapping sites along each road, with two remote control sites (1000 m from road; Fig. 2.1). Sites 1, 3, 4, and control site 2 (CN2) are located adjacent to I-10, while sites 6, 8, 9, and control site 3 (CN3) are adjacent to NM-9. Sites 2, 5, 7, 10 and control sites 1 and 4 were initially surveyed for suitability, but not used. We retained the original numbering to

maintain consistency with those surveys. All sites were located on land managed by the U.S. Bureau of Land Management (BLM). Because we were not able to trap in the road right-of-way, trapping distances are reported in meters from the BLM fence line (“fence”), not the actual pavement. For sites 3 and 4 on I-10, the fence was about 22 m from the pavement. There was a frontage road between the fence and the pavement at site 1. The frontage road was a two-lane paved road about 7 m wide. The fence for the north side of site 1 was 19 m from the frontage road and 50 m from I-10, while for the south side the fence was 4 m from the frontage road and 29 m from I-10. Both frontage roads had an AADT of less than 300 vehicles per day when measured by NM-DOT in 2008 (NM-DOT traffic data accessed via New Mexico Resource Geographic Information System, <http://rgis.unm.edu/>, accessed 4 June 2013). For the sites along NM-9, the northern fence was about 27 m from the pavement while the southern fence was about 11 m from the pavement. There was little vegetation in the road right-of-way south of NM-9, while the rest of the road right-of-ways had vegetation similar to the surrounding habitat for at least 10 m from the fence towards the pavement.

At each site pitfall trap arrays were placed at four distances in relation to the fence: close, opposite side of the road from the other traps (close, opposite); close, same side as the other traps (close, same); 50 m (mid); and 125 m (far) (Fig. 2.2). The traps in the close arrays were  $5.9 \pm 2.1$  m from the fence line. The distances between the trapping groups on the same side of the road represent the distances required to cross each road: about 50 m between traps on opposite sides of NM-9 and  $\geq 100$  m for those on I-10. This arrangement allowed us to compare the frequency of lizard movements across the road with movements of the same distance across the desert. We selected which side of the road, north or south, would have the three trapping distances based on avoidance of landscape features such as nearby dirt roads. On I-10, sites 1 and 4 had the three

trapping distances on the northern side, while on NM-9 only site 6 had the three trapping distances on the northern side. The remaining sites had the three trapping distances on the southern side of the respective road. The control sites had three trapping arrays at the same three distances, arranged with the close traps approximately 1000 m from the fence line.

We installed two pitfall trap arrays at each of the distances (Fig. 2.2). Each array consisted of four 19 L buckets buried in the ground, arranged in a “Y” pattern (Fisher et al. 2008). Traps were about 5 m apart, with 0.3 m high drift-fences connecting the outer traps to the central trap. Center traps of the two arrays at each distance were an average of 21.7 m apart (range 17.4–30.5 m).

We trapped lizards from 2009 to 2011. In 2009, we trapped from July through October (4 months), and in 2010 and 2011 we trapped from May through September (5 months). Each site was trapped for 1–3 consecutive days in 2009, and 3 consecutive days in 2010 and 2011 (see supplementary table S2.1 for trapping effort at each site). We stopped trapping at the northern side of site 4 after August 2010 because of a nearby road expansion project. Traps were open 10–11 hr/day and opened early each morning and closed in the evening to reduce unwanted captures of small mammals. We checked traps in the afternoon or early evening, and removed all animals prior to closing the traps for the night.

All lizards captured were measured, marked, and released near the original capture location within 24 h. We determined the sex of each individual and measured its snout-vent length (SVL), vent-tail length, and mass. All whiptails were given unique toe-clips (Tinkle 1967), while only side-blotched lizards >25 mm SVL were given unique toe-clips on first capture. Most side-blotched lizards  $\leq$ 25 mm SVL were given a batch toe-clip based on which side of the road they were captured. Batch-marked individuals >25 mm SVL were given a unique toe-clip number upon

recapture. If we could not determine the identity of the recaptured individual, it was treated as a new individual in the data analyses. There were some exceptions in the marking scheme due primarily to changes in the marking protocol early in the study: four individuals  $>25$  mm SVL given batch marks and 137 individuals  $\leq 25$  mm SVL given unique marks. These individuals were all treated as hatchlings in the data analyses (see below).

### **2.3.3 Site-specific habitat structure**

Although we chose locations for our study sites that had generally similar plant communities and habitat structure, there were still potential differences we were unable to control that could potentially influence the abundance and survival of lizards. For example, both species tend to forage under plants, with side-blotched lizards generally waiting under the same plant while whiptails move among plants (Tinkle 1967, Vitt and Ohmart 1977, Peterson and Whitford 1987). Vegetation near desert roads tends to be denser with different species composition due to increased soil moisture from pavement runoff (Johnson et al. 1975, Lightfoot and Whitford 1991), which could potentially attract lizards to the roadside habitat for increased prey or shelter availability.

To address the potentially confounding effects of habitat structure, we created shapefiles of all objects near each trapping array using Google Earth imagery (map data from April 2013 from Google and the Mexican National Institute of Statistics and Geography [INEGI]) in ArcMap 10.2 (ESRI, Redlands, CA, USA). Additional details on the creation and editing of the vegetation features and example figures are available in the supplementary documents. We did not distinguish among plant species, but did create three groups based on their area covered: all plants  $\geq 0.5$  m<sup>2</sup> (“AllSizes”), plants covering  $\geq 2$  m<sup>2</sup> (“MediumAndLarge”), and plants covering  $\geq 10$  m<sup>2</sup> (“Large”). Plants smaller than 0.5 m<sup>2</sup> were not reliably identified in the satellite imagery, so were not included. The 0.5–2 m<sup>2</sup> range includes small perennials (e.g., snakeweed and small shrubs),

the 2–10 m<sup>2</sup> range includes larger shrubs (e.g., yucca, four-winged saltbush, broom dalea, and mesquite), and the  $\geq 10$  m<sup>2</sup> range includes the larger mesquite shrubs, many of which form coppice dunes. We did not measure the annuals, but observed much lower densities in 2011 when precipitation was below normal.

Side-blotched lizards and marbled whiptails are expected to interact with their environment at different scales because of their specific movement behaviors. Based on the distances lizards moved between captures in this study, we used a buffer distance of 10 m around the trapping distances for the side-blotched lizards and one of 25 m for the whiptail lizards for calculating habitat structure metrics. We used FRAGSTATS v. 4.2 (McGarigal et al. 2012) to calculate the percentage of area covered per hectare (PercentageLandscape), the number of individual plants per 1000 m<sup>2</sup> (PatchDensity), and the median and range of the Euclidean nearest neighbor distances (ENN\_MD and ENN\_RA, respectively). Because the size and distribution of different size classes of plants can potentially have different impacts on lizards (i.e., foraging, thermoregulation, protection from predators), these metrics were calculated for the three size cutoffs described above (AllSizes, MediumAndLarge, and Large). These metrics were used as explanatory variables in the generalized linear modeling analyses described below.

We calculated Pearson correlation coefficients of the different vegetation metrics with the log-transformed distance to the road individually to identify potentially confounding variables. We also used Welch's t-test to look for overall differences between the sites along I-10 and those along NM-9.

#### **2.3.4 Data analysis**

We were most interested in determining the effect of the distance from the road and size of the road on the abundance and survival of both species of lizards. Because the age of the individual

might affect its response to the road, and its overall survival, we ran some analyses for juveniles separately from those for sub-adults and adults (called “adults”). Thus we considered any side-blotched lizard with an SVL 25.1–30 mm to be a juvenile, and individuals with larger SVLs as adults. This reliably distinguished the young-of-the-year from the adults, and there were no individuals of juvenile size captured in the early summer (May and June) for any year. Individuals with an SVL  $\leq 25$  mm were classified as recent hatchlings. For the survival analyses, we only included individuals given unique toe clips at an SVL  $> 25$  mm. This restriction excluded 410 individuals who had received a batch mark toe clip and/or were smaller than 25 mm SVL initially. Hatchlings have lower survival rates than other age classes (Tinkle 1967), so our estimates of survival could be lower if the hatchlings were included in the analyses. We did calculate the proportions of hatchlings that survived and were recaptured as juveniles or adults as an estimate of hatchling survival (described below).

There is more overlap in the size distributions of whiptail lizards in the spring and early summer, complicating our ability to make a clear distinction in ages. We considered whiptail lizards with an SVL  $\leq 60$  mm to be juveniles, with individuals with larger SVLs as sub-adults or adults. Individuals smaller than 60 mm SVL were captured in the early summer (May or June) each year, before the emergence of hatchlings in July or August. These individuals were assigned to the previous year’s hatchling cohort.

We estimated abundance as the minimum number of individuals per 100 trap days to correct for differences in trapping effort between sites. We counted the minimum number of individuals per year to examine potential annual changes. We included all individuals trapped, including those not given unique toe-clip numbers.

We estimated individual survival rates in two ways: 1) the number of times captured per individual, and 2) the persistence of individuals (the number of days between the first and last capture). The data were highly skewed, with most individuals (62% of side-blotched and 53% of whiptails) captured once (persistence of 0 days). This data distribution prevented us from using the actual number of captures and days of persistence as dependent variables, so for the number of times captured, we used the proportion of individuals captured only one time as a metric for low survival rates (although we could not distinguish mortality from emigration or trap avoidance). For persistence, we used the proportion of individuals with persistence longer than the average across all locations. Because the maximum persistence depends on the year marked, with individuals marked in 2009 having potentially longer persistence than those marked in 2011, we calculated the average persistence for each year, and then calculated the proportion of individuals marked that year that had persistence longer than the average for that year. We did not analyze juveniles separately for persistence because of expected changes in survival rates as they aged. To estimate juvenile survival, we calculated the proportion of individuals marked as juveniles that were recaptured as sub-adults or adults. We estimated hatchling side-blotched lizard survival as the proportion recaptured as juveniles or adults.

We also calculated the finite annual survival rates for just adults at each trapping location. The finite annual survival is the number of marked individuals captured in year  $t+1$  divided by the number of individuals marked in year  $t$  (Krebs 1999). We compared differences in survival rates among distance groups during the two periods (2009–2010 and 2010–2011).

We used generalized linear modelling to examine the effects of distance from the road (measured from the pavement, not the BLM fenceline and  $\log_{10}$  transformed), the particular road (NM-9 or I-10, both to test effects of the size of the road and any broader landscape features not



included in the vegetation measurements), the year (primarily as a proxy for the different climatic conditions each year: 2009 and 2010 had average rainfall, 2011 was a drought year (National Weather Service, [http://www.srh.noaa.gov/epz/?n=santateresa\\_monthly\\_precip](http://www.srh.noaa.gov/epz/?n=santateresa_monthly_precip), accessed 31 March 2014)), and the vegetation structure metrics using a 25 m buffer for whiptails and a 10 m buffer for side-blotched lizards. I-10 and 2009 were set as the reference values for the models. We looked for potential collinearity among all pairs of predictor variables (excluding road and year) using Pearson correlation coefficients with a cutoff of  $r \geq 0.70$ . We excluded the percentage of the landscape covered by medium and large plants because it was highly correlated with the area covered by all plants (10 m buffer: Pearson's  $r = 0.94$ , 25 m buffer: Pearson's  $r = 0.93$ ) and just large plants (10 m buffer: Pearson's  $r = 0.97$ , 25 m buffer: Pearson's  $r = 0.97$ ). The percent cover for all plants and large plants only were also significantly correlated (10 m buffer: Pearson's  $r = 0.864$ , 25 m buffer: 0.852), but we included both metrics because we were interested in the potential differences in the effects of smaller plants on abundance and survival. We also addressed potential multicollinearity by examining the variance inflations factors (VIF) for the different regression models. The percentage of landscape covered and the patch density per 1000 m<sup>2</sup> for the three different plant size groups had VIFs of 8.6–26.8. We compared model results both including and excluding those two metrics for the medium and large and only large plant groups, and did not find any substantial changes to the results. We included all metrics except for the percentage landscape coverage for medium and large plants to facilitate comparisons of explanatory factors between the side-blotched and the whiptail lizards, which we anticipated may preferentially use plants of different sizes. The ranges of values for the vegetation structure metrics are available as supplementary data (Table S2.2).

We did not have any *a priori* hypothesis regarding the potential importance of the explanatory variables on abundance or survival, and to avoid potential problems with stepwise model selection (e.g., Whittingham et al. 2006, Burnham et al. 2011), we ran all possible models ( $N = 16384$ ) using package *glmulti* (Calcagno and de Mazancourt 2010) in R version 3.2.0 (R Core Team 2014), retaining the 1000 models with the lowest AICc scores. We identified candidate model sets in two ways: all models within 2 AICc of the “best” model (that with the lowest AICc), as models within this  $\Delta\text{AICc}$  are considered to have equal support, and within 6 AICc (equal to a probability of minimizing information loss of 5%; Burnham and Anderson 2002). We calculated model averages for both sets of candidate models to determine parameter estimates using *glmulti*, but did not find any substantial differences in the estimates, so we report the more conservative averages for all models  $\leq 6 \Delta\text{AICc}$ . To evaluate the importance of each explanatory variable in the candidate model set we used the relative evidence weight, which is the sum of the Akaike weights per model summed over all models in which that particular variable occurs (Burnham and Anderson 2002, Calcagno and de Mazancourt 2010). Variables that are reported as important are those with both importance values  $> 0.80$  (as recommended by Calcagno and de Mazancourt 2010) and 95% confidence intervals around the beta estimate that do not overlap 0. We used the negative binomial distribution for abundance estimates because of overdispersion of the data and the quasibinomial distribution for survival metrics because of underdispersion of the data.

We used two different metrics to assess the fit of the models. We ran the full model with all of the predictors and calculated the explained deviance (estimated as: (null deviance – residual deviance) / null deviance; Zuur 2009). Although the full model likely contains variables that are not important in explaining the variance in the data, the explained deviance is maximized when all variables are included. If the explained deviance of the full model is low, that suggests that the

overall model does not explain much of the variation. We calculated the Pearson's correlation between the observed and predicted dependent variable (e.g., number of individuals per 100 trap days or finite annual survival rate) using the model averaged coefficients of the models within 6 AICc of the best model. We examined plots of model residuals for all tests. Values are reported as mean  $\pm$  standard error unless otherwise noted. We used an alpha level of 0.05 for all statistical tests. Tests were performed in R version 3.2.0 (R Core Team 2014).

### **2.3.5 Body condition**

We examined the potential effect of living near roads on individual body condition using two metrics. Lizards might benefit from the increased vegetation found near roads in arid regions (e.g., Johnson et al. 1975, Lightfoot and Whitford 1991), or could suffer from increased stress created by traffic noise, vibration, or pollution (e.g., Crino et al. 2011, Navarro-Castilla et al. 2014). Two commonly used body condition indices are the residuals from ordinary least squares (OLS) regressions of the  $\log_{10}$  mass against the  $\log_{10}$  length (Jakob et al. 1996, Schulte-Hostedde et al. 2005) and the scaled mass index (Peig and Green 2009, 2010). Both indices attempt to standardize body size to more accurately reflect the underlying physiological condition of an individual. Because there is currently debate about which index performs best (Schulte-Hostedde et al. 2005, Peig and Green 2010, Bókonyi et al. 2012, Labocha et al. 2014, Cox and Calsbeek 2015), we calculated both.

Both the residuals ( $R_i$ ) from the  $\log_{10}$  regression of mass against length and the scaled mass index were calculated for each sex independently because males are both longer and heavier than females. The scaled mass index ( $M_i$ ) was calculated as the SVL of an individual divided by the arithmetic mean SVL of the population raised to the standardized major axis (SMA) slope ( $b_{SMA}$ ) from the regression of  $\ln$  mass on  $\ln$  SVL, times the mass of the individual. The SMA regression

was performed using the R package *lmodel2* version 1.7-2 (Legendre 2014). The  $b_{SMA}$  values for males and females were similar.

For side-blotched lizards, only individuals >25 mm were used because the precision of the spring scales used for weighting lizards ( $\pm 0.1$ g) was similar to the mass of the hatchling lizards (0.2–0.4 g) (Tinkle 1967). All marbled whiptail individuals were included. We calculated the average SVL and mass for individuals captured multiple times in the same trapping occasion to reduce bias. Because the data were approximately normally distributed with homogenously distributed errors, we used multiple linear regression modeling with the same predictor variables as detailed above. We again used *glmulti* to test all possible subsets of predictor variables, and used model averaging for all models  $\leq 6 \Delta AICc$ . Important predictor variables were defined as above. We first removed extreme outliers (<5 observations for each analysis), and calculated the critical values for males and females separately, and for each metric separately. Model fit was assessed by calculating Pearson's correlation coefficient between the observed and predicted values from the model averaged coefficients.

### **2.3.6 Movement**

We wanted to assess how widely each species actually moved in our study because increased movement is expected to increase the number of individuals that encounter a road. We calculated the distance that individual lizards moved between captures using the R package *adehabitatLT* (Calenge 2006). The distances are underestimates of the actual movement patterns because we only have data when an animal is trapped, and so movements away from a trapping array will not be detected. We compared the differences in the distances moved by sex and by road with nonparametric Mann-Whitney tests, both for all movements detected and the average

distance moved per individual. We also estimated the effect size with Cohen's  $D$  using the R package *compute.es* (Del Re 2013).

We also wanted to compare the number of times we detected an individual crossing a road with the number of times we detected an individual crossing a comparable distance across the desert habitat. The minimum distance between traps on opposite sides of the road was 45 m for the small road and 98 m for the large road. We used a Chi squared test to compare the number of movements  $\geq 45$  m that did not cross a road with those that did cross NM-9. We did not detect any individuals crossing I-10, so could not make that comparison.

## **2.4 RESULTS**

### **2.4.1 Habitat structure**

Our primary goal of the habitat structure analyses was to determine if any features were significantly correlated with the distance from the road, and could thus confound our ability to detect road effects. For the 10 m buffer, only the median nearest neighbor distance between all plants (ENN\_MD\_AllSizes) was significantly correlated with the distance to the road ( $r = 0.49$ ,  $P < 0.01$ ). None of the features were significantly different between the I-10 and NM-9 sites. For the 25 m buffer, the range of nearest neighbor distances (a measure of variability in the spatial arrangement of the plants) for all plants (ENN\_RA\_AllSizes,  $r = 0.37$ ,  $P = 0.04$ ) and the number of large plants per 1000 m<sup>2</sup> (PatchDensity\_Large,  $r = 0.41$ ,  $P = 0.03$ ) were correlated with the distance to the road. The percent cover for both all plants (PercentageLandscape\_AllSizes,  $t = -2.06$ ,  $P = 0.05$ ) and large plants (PercentageLandscape\_Large,  $t = -2.37$ ,  $P = 0.03$ ) were different between the roads, with the NM-9 sites having higher percentage of cover than the I-10 sites. Both the median (ENN\_MD\_Large,  $t = 2.92$ ,  $P < 0.01$ ) and range (ENN\_RA\_Large,  $t = 2.38$ ,  $P = 0.03$ ) of nearest neighbor distances between large plants were different between the two roads, with I-

10 sites having greater distance and more variation between large plants. Overall there was variation in the habitat structure metrics we measured, and they mostly did not show significant correlations with the distance to the roads. We thus are reasonably confident that any detected effects of roads on lizard abundance or survival will not be confounded by co-varying habitat features.

## **2.4.2 Abundance**

### ***Side-blotched lizards***

We captured 1954 side-blotched lizards a total of 3000 times during 9232 trap days. There were 873 initially marked in 2009, 498 in 2010, and 581 in 2011. There were also 65 individuals marked in 2009 that were captured in 2010 and 8 captured in 2011. Fifty-three individuals marked in 2010 were captured in 2011. There were 1897 captures of 1149 unique adults, defined as individuals  $>30$  mm SVL, and 1083 captures of 927 unique juveniles. Overall 122 individuals marked as juveniles were later recaptured as adults (discussed further in the survival section below).

When analyzing adults, year, road, and the distance to the pavement were important variables in determining abundance (Table 2.1). Contrary to our prediction, there were generally fewer adults away from the roads (beta = -0.15, 95% CI: -0.25– -0.04, Fig. 2.3a). There were also fewer adults in the NM-9 sites than the I-10 sites overall ( $12.1 \pm 1.8$  adults per location at NM-9 vs.  $17.6 \pm 2.7$  at I-10, beta = -0.31, 95% CI: -0.45– -0.17), and fewer in 2010 ( $10.0 \pm 1.8$  per location, beta = -0.86, 95% CI: -1.00– -0.72) and 2011 ( $10.3 \pm 2.0$  per location, beta = -0.82, 95% CI: -0.96– -0.68) than in 2009 ( $23.4 \pm 4.3$  per location). Much of the difference between the two roads was due to high abundances at the I-10 sites in 2009 (Fig. 2.3a). In 2010 and 2011 the abundances between the two roads were similar (Fig. 2.3a). The explained deviance of the full

model was 0.73, and the correlation between the observed and model-averaged predicted abundances was 0.87 (95% CI: 0.80–0.91).

For the juveniles, the important variables in determining abundance were year and the distance to the roads (Table 2.1). Similar to the adults, there were fewer juveniles away from the roads ( $\beta = -0.20$ , 95% CI:  $-0.36$ – $-0.05$ , Fig. 2.3b). There were fewer juveniles in 2010 ( $6.6 \pm 1.2$  juveniles per location,  $\beta = -0.77$ , 95% CI:  $-0.98$ – $-0.57$ ) and in 2011 ( $10.4 \pm 2.0$  per location,  $\beta = -0.29$ , 95% CI:  $-0.49$ – $-0.10$ ) than in 2009 ( $14.0 \pm 2.6$  per location, Fig. 2.3b). The explained deviance of the full model was 0.48, and the correlation between the observed and model-averaged predictions of abundance was 0.66 (95% CI: 0.52–0.77).

### ***Marbled whiptails***

We captured 1400 marbled whiptail lizards a total of 3147 times. There were 417 individuals initially marked in 2009, 553 in 2010, and 430 in 2011. There were also captures of 149 individuals in 2010 that had been initially marked in 2009, and 190 individuals in 2011 that had been initially marked in either 2009 or 2010. There were 1017 unique adults captured a total of 2451 times, and 515 juveniles captured a total of 690 times as juveniles. A total of 138 juveniles were recaptured at adult sizes during the study.

The important variables in determining the number of adults were year, road, and the median nearest neighbor distance between plants of all sizes (Table 2.1). There were more adults in 2010 ( $14.6 \pm 1.7$  per location,  $\beta = 0.58$ , 95% CI: 0.41–0.75) and 2011 ( $17.2 \pm 3.3$  per location,  $\beta = 0.75$ , 95% CI: 0.57–0.92) than in 2009 ( $8.1 \pm 1.5$  per location, Fig. 2.3c), and fewer adults at the NM-9 sites than the I-10 sites overall ( $12.0 \pm 1.8$  adults along NM-9 vs  $14.4 \pm 2.2$  adults along I-10,  $\beta = -0.20$ , 95% CI:  $-0.35$ – $-0.04$ ). There were more adults at locations with larger median distance between plants ( $\beta = 0.51$ , 95% CI: 0.17–0.85, Fig. 2.4a). The explained

deviance of the full model was 0.57, and the correlation between the observed and model-averaged predictions of abundance was 0.73 (95% CI: 0.61–0.81).

Year, road, and ENN\_MD\_AllSizes were also important variables in the abundance models for the juveniles, as were the distance to the pavement and the range of nearest neighbor distances for only large plants (Table 2.1). There were fewer juveniles in 2010 ( $6.4 \pm 1.2$  individuals,  $\beta = -0.25$ , 95% CI: -0.48– -0.02) and in 2011 ( $3.8 \pm 0.8$  individuals,  $\beta = -0.79$ , 95% CI: -1.06– -0.51) than in 2009 ( $8.3 \pm 1.5$  individuals, Fig. 2.3d), and in sites along NM-9 compared with I-10 sites ( $5.1 \pm 0.8$  juveniles along NM-9 vs.  $7.6 \pm 1.2$  along I-10,  $\beta = -0.48$ , 95% CI: -0.72– -0.23, Fig. 2.3d). The number of juveniles was lower away from the roads, contrary to our prediction ( $\beta = -0.36$ , 95% CI: -0.57– -0.15). The number of juveniles increased at locations with larger distances between plants of all sizes ( $\beta = 0.87$ , 95% CI: 0.35–1.39, Fig. 2.4b), but was slightly smaller at locations with more range in distances between only large plants ( $\beta = -0.05$ , 95% CI: -0.08– -0.01). The explained deviance of the full model was 0.53, and the correlation coefficient between the observed and model-averaged predictions was 0.68 (95% CI: 0.55–0.78).

### 2.4.3 Survival

#### *Side-blotched lizards*

Only animals that were given unique toe clips, were larger than 25 mm SVL, and were released alive were included in the survival analyses. These individuals were further divided into juveniles (25.1–30 mm SVL) and adults (>30 mm SVL). For side-blotched lizards, we had trap mortality of 77 individuals (4% of total individuals and 2.5% of capture events), 30 individuals that were not marked before release, and 410 individuals that were marked when  $\leq 25$  mm SVL. There were 2385 captures of 1451 individuals that were marked and released alive at least once during the study, 1869 captures of 1129 adults (including 133 individuals marked as hatchlings or



juveniles and recaptured as adults), and 516 captures of 438 juveniles (including 25 individuals first marked as hatchlings).

#### Number of times captured per individual

If an explanatory variable decreased survival rates, we expected it to be found as important in the GLM with the proportion of individuals captured once as the dependent variable. There were 691 (61.2%) adults captured once, 258 (22.9%) captured twice, and the remaining individuals (15.9%) captured 3–8 times, with a total of 1869 captures for all 1129 adults. The important variables were year and median nearest neighbor distance between medium and large plants. Shorter distances between plants were associated with more single captures ( $\beta = -0.08$ , 95% CI:  $-0.14$ – $-0.02$ ), while higher proportions of animals in both 2010 and 2011 had single captures relative to 2009 (Table 2.3, Fig. 2.5a). The explained deviance of the full model was 0.35, and the correlation between the observed and predicted proportions was 0.61 (95% CI: 0.46–0.73).

There were 437 individuals marked as juveniles, and these individuals were captured 757 times at some point, potentially while still juveniles or while adults. There were 250 individuals (57.2%) captured once, 117 (26.8%) captured twice, and 70 (16.0%) captured 3–9 times. Only year was an important variable in the model. As with the adults, both 2010 and 2011 had more single captures than 2009 (Table 2.3, Fig. 2.5b). The model fit was not as good as for the adult data set, with an explained deviance of 0.24 for the full model, and a correlation coefficient of 0.47 (95% CI: 0.28–0.62).

#### Persistence

Persistence measures the length of time between the first and last captures of an individual, and longer persistence reflects higher survival rates. The average persistence for the 1129 unique

adults was  $40.6 \pm 2.9$  days. The average persistence for the 573 individuals marked in 2009 was  $45.9 \pm 4.5$  days, and 119 individuals (20.8%) had longer than average persistence. The average persistence for the 286 adults marked in 2010 was  $55.6 \pm 6.6$  days, and 60 individuals (21.0%) had longer than average persistence. For the 270 individuals marked in 2011, the average persistence was  $13.3 \pm 1.6$  days, and 63 individuals (23.3%) had longer than average persistence. Only median nearest neighbor distance between large plants was important in determining persistence, and had a small positive association with longer than average persistence values (beta = 0.03, 95% CI: 0.01–0.05; Table 2.3). The distance from the road was not an important variable (Fig. 2.6a). The full model had an explained deviance of 0.22, and the correlation coefficient was 0.44 (95% CI: 0.25–0.59).

#### Juvenile survival

The majority of lizards captured as hatchlings ( $\leq 25$  mm SVL) were batch-marked, which limited our ability to determine their individual survival rates. We estimated survival rates as the number of individuals marked as hatchlings later recaptured as either juveniles or adults ( $>25$  mm SVL) divided by the total number of hatchlings marked. We did not consider the length of time between captures because there was no readily available method to identify a batch-marked individual to the day of its initial capture. We marked a total of 453 hatchlings, 155 in 2009, 110 in 2010, and 188 in 2011. We recaptured 39 as juveniles or adults in 2009, 23 in 2010, and 41 in 2011. A greater proportion of hatchlings marked at the NM-9 sites were later recaptured than those marked along I-10 (beta = 0.13, 95% CI: 0.01–0.25; Table 2.4), and none of the other variables were important (Fig. 2.7). The explained deviance of the full model was low, only 0.18, and the correlation coefficient was 0.39 (95% CI: 0.19–0.56).

Of the 412 lizards marked as juveniles, only 111 (26.9%) were recaptured as adults. The year an individual was marked was the only important variable in the models, and both 2010 and 2011 had lower proportions of juveniles recaptured as adults than 2009 (Table 2.4, Fig. 2.8a). The explained deviance of the full model was 0.23 and the correlation coefficient was 0.46 (95% CI: 0.27–0.61). The lower numbers of juveniles marked in 2011 getting recaptured as adults are partially explained by the end of our study that year. We would have likely recaptured some additional 2011 juveniles had we continued trapping in 2012.

#### Finite annual survival

The finite annual survival rate is an estimate of the proportion of individuals that survive from one year to the next. There were 573 adults marked in 2009 and 54 recaptured in 2010, an annual survival rate of 0.09. There were 346 adults marked in 2010 and 52 recaptured in 2011, for a survival rate of 0.15. The only important explanatory variable in the models was the time period, with 2010–2011 survival higher than 2009–2010 survival (beta = 0.07, 95% CI: 0.03–0.11, Table 2.5, Fig. 2.9a). The explained deviance of the full model was 0.25, and the correlation between model-averaged predicted and observed survival was 0.52 (95% CI: 0.29–0.68).

#### ***Marbled whiptails***

Trap mortality was 22 individuals (1.6% of all individuals; 0.7% of all captures) and five were released without marking, so survival analyses are based on 3120 captures of 1381 whiptails. There were 2442 captures of 1021 adults (including 146 individuals initially marked when juveniles), and 678 captures of 504 unique juveniles.

#### Number of times captured per individual

There were 448 (44.3%) adults captured once, 194 (19.2%) captured twice, and 370 (36.6%) captured >2 times. Year was the only important variable in determining the proportion of individuals only captured once, and 2010 and 2011 both had a greater proportion of individuals captured once than 2009 (Table 2.2), and distance to the roads was not significant (Fig. 2.5c). The explained deviance of the full model was 0.31, and the correlation between observed and predicted was 0.57 (95% CI: 0.40–0.69).

Fewer juveniles were recaptured more than once. Of the 504 individuals, 289 (57.3%) were captured once, 96 (19.1%) were captured twice, and 110 (23.6%) were captured >2 times. None of the explanatory variables were important in the models (Table 2.2, Fig. 2.5d), the explained deviance of the full model was 0.21, and the correlation coefficient was 0.43 (95% CI: 0.23–0.59).

#### Persistence

The average persistence of the 205 adults marked in 2009 was  $239.8 \pm 18.7$  days, and 103 (50.2%) had persistence values longer than average. For the 396 adults marked in 2010, 99 (25%) had persistence longer than the average of  $100.7 \pm 8.0$  days. For the 411 adults marked in 2011, 155 (37.7%) had persistence values longer than the average of  $20.0 \pm 1.5$  days. Only the year an individual was marked was an important variable in the model, with both 2010 and 2011 individuals having more individuals with shorter than average persistence values than in 2009 (Table 2.3). Neither the distance from the roads nor which road the populations were near were important (Fig. 2.6b). The explained deviance of the full model was 0.39, and the correlation between the observed and predicted proportions was 0.63 (95% CI: 0.48–0.74).

#### Juvenile survival

Hatch year 2009 included 236 individuals, 53 (22.5%) of which were later recaptured as adults. Hatch year 2010 had 236 individuals as well, 77 (29.0%) of which were later recaptured as adults. Only the time period was important in the models, with more individuals from hatch year 2010 recaptured as adults than hatch year 2009 ( $\beta = 0.15$ , 95% CI: 0.06–0.25, Table 2.4). There was no evidence that the distance to a road influenced the likelihood that a juvenile survived and was recaptured as an adult (Fig. 2.8b). The full model had an explained deviance of 0.31, and the correlation between the observed and predicted proportions was 0.53 (95% CI: 0.31–0.69, Table 2.4).

#### Finite annual survival

We marked 201 adults in 2009 and recaptured 99 in 2010 (survival = 0.49). We captured 444 adults in 2010 (345 newly marked) and recaptured 120 in 2011 (survival = 0.27, 37 from 2009 and 83 from 2010). Time period was the only important variable in the models, with finite survival rates lower from 2010–2011 than 2009–2010 ( $\beta = -0.16$ , 95% CI: -0.25– -0.08, Table 2.5, Fig. 2.9b). The explained deviance of the full model was 0.39, and the correlation between the observed and predicted rates was 0.59 (95% CI: 0.39–0.74).

### 2.4.4 Body condition

#### *Side-blotched lizards*

There were 1112 captures of 799 unique males and 995 captures of 683 unique females. Multiple linear regression analyses were qualitatively similar in terms of  $R^2$  and important predictor variables when using either the residuals from the  $\log_{10}$  regression of mass against length or the scaled mass index. We report the scaled mass index results because of the comparative ease of interpretation of values (SVL in mm vs unitless residuals). For males, the correlation coefficient between the observed and predicted index values was 0.16 (95% CI: 0.10–0.22). Several predictor

variables were important in determining body condition: Distance to Pavement (beta was positive), Year (2010 and 2011 both had positive betas relative to 2009), Road (NM-9 had a negative beta relative to I-10), PatchDensity\_MediumAndLarge and PercentageLandscape\_Large (both betas were positive), and PatchDensity\_Large (negative beta, Table 2.6, Fig. 2.10a). For females, the correlation coefficient was 0.21 (95% CI: 0.14–0.26). For Year, only 2010 was an important variable (positive beta relative to 2009, 2011 and 2009 beta estimates were similar). ENN\_RA\_AllSizes (positive beta), ENN\_RA\_MediumAndLarge (small negative beta), and road (NM-9 had a negative beta relative to I-10) were also important variables (Table 2.6, Fig. 2.10b). Overall the trends for both sexes were for smaller individuals in 2009 than 2010 or 2011 and smaller individuals living near NM-9. Fewer larger plants, which covered higher percentages of the landscape, were associated with larger males. More variation in the range of distances between plants of all sizes was associated with larger females. However, the large variation in body condition index values gives the models low explanatory ability overall, as reflected in the low  $r$  values.

### ***Marbled whiptails***

There were 1571 captures of 832 unique males and 1005 of 563 unique females. Similar to the results for the side-blotched lizards, the results using the regression residuals or the scaled mass index as the response variable in the multiple linear regression modeling yielded qualitatively similar results, and we report just the scaled mass index results. For males, the correlation coefficient between the observed and predicted index values was 0.30 (95% CI: 0.25–0.34). Only Year, Road, and ENN\_RA\_Large were important predictor variables for body condition (Table 2.6). Males in 2011 were smaller than those in 2009 (beta estimate negative), while those in 2010 tended to be larger than those in 2009, but the beta estimate overlapped 0 (Fig. 2.10c). Males

living near NM-9 were smaller than those living near I-10, and size was positively related to the range of nearest neighbor distances between large plants. For females, the correlation coefficient between observed and predicted index values was 0.22 (95% CI: 0.16–0.28). Year and Road were important predictor variables (Table 2.6). Females captured in 2011 were smaller than those captured in 2009 (negative beta estimate, Fig. 2.10d). Individuals living near NM-9 were smaller than those near I-10. For both sexes, the habitat structure and distance to roads were not important predictors of body size (Fig. 2.10c, d). As with the side-blotched lizards, the large variation in body condition index values gave the models low explanatory ability overall.

## **2.4.5 Movement**

### ***Side-blotched lizards***

We captured 559 individuals >25 mm SVL more than once, with a total of 946 movements detected. There were similar numbers of both individuals and movements for males and females (289 males with 486 movements, 270 females with 460 movements). The distances moved for both sexes were similar when analyzing each movement separately and when analyzing the average distance moved per individual. Because we were primarily interested in the frequency of movements long enough to cross a road, we only report the results for all movements, not the individual averages. Movements by males were significantly longer than those by females, but both were very short (males:  $8.1 \pm 0.5$  m, females:  $4.9 \pm 0.3$  m; Mann-Whitney  $W = 89572$ ,  $P < 0.0001$ ). The effect size was small (Cohen's  $D = -0.33$ , 95% CI: -0.46– -0.2). The movement lengths of individuals living near NM-9 ( $6.1 \pm 0.4$  m,  $N = 419$  movements by 254 individuals) were slightly shorter than those living near I-10 ( $6.8 \pm 0.3$  m,  $N = 527$  movements by 305 individuals), but the difference was only marginally significant (Mann-Whitney  $W = 118193$ ,  $P =$

0.058). The effect size was small (Cohen's  $D = 0.07$ , 95% CI:  $-0.06 - 0.2$ ). Thus, we combined the data across the roads, but analyzed males and females separately.

The vast majority of both female (94%) and male (82%) movements were  $\leq 10$  m, which are movements within the same trapping array. About 5% of female movements and 15% of male movements were 10–30 m, the distance between the two arrays in the same distance group. Only 0.7% of movements by females and 2.5% of movements by males were between different trapping distances,  $\geq 30$  m. There was one movement each by a female and a male across NM-9, and no detected movements across I-10. Males were significantly less likely to be detected crossing NM-9 (1 movement) than moving  $>45$  m across the desert (10 movements;  $\chi^2 = 7.36$ , d.f. = 1,  $P = 0.007$ ). There were not enough movements  $>45$  m by females ( $N = 3$ ) to statistically compare the likelihood of moving across NM-9 with the likelihood of moving across the desert. There was only one movement greater than 98 m, the minimum distance required to be detected crossing I-10. There were no batch-marked individuals detected to have crossed either road. The pavement itself is about 10 m wide, and 6% of female movements and 18% of male movements were greater than 10 m. It is therefore physically possible for males, and to some degree females, to cross the pavement with some regularity.

### ***Marbled whiptails***

We captured 644 individuals at least twice, with a total of 1747 movements detected. There were more males than females (396 individuals with 1169 captures for males: 59.6% of the total individuals and 66.9% of the total captures; 248 individuals with 578 captures for females), and male movements were slightly longer on average (males:  $13.2 \pm 0.4$  m, females:  $11.9 \pm 0.5$  m), but the differences were not statistically significant (Mann-Whitney  $W = 316764$ ,  $P = 0.07$ ) and the effect size was very low (Cohen's  $D = -0.09$ , 95% CI  $-0.19 - 0.01$ ). The average length of



movements near NM-9 ( $13.9 \pm 0.5$  m, 328 individuals with 883 movements) was slightly longer than near I-10 ( $11.6 \pm 0.4$  m, 316 individuals with 864 movements), but not statistically different (Mann-Whitney  $W = 370914$ ,  $P = 0.50$ ). The effect size was small (Cohen's  $D = -0.15$ , 95% CI: -0.24--0.06). Thus, we combined the data for both sexes and both roads for the remaining analyses.

Approximately 68% of the movement distances were  $\leq 10$  m (1188 of 1747 movements), with an additional 24% 10–30 m (427 movements). Only 122 movements (7%) were between different trapping locations on the same side of the road, and there were 10 movements (0.6%) across NM-9. There were significantly fewer movements across NM-9 than movements across a comparable distance ( $>45$  m) of desert habitat (86 movements;  $\chi^2 = 60.2$ , d.f. = 1,  $P < 0.0001$ ). There were only 7 movements  $>98$  m (0.4%), the minimum distance an individual needed to move to be captured on both sides of I-10, and no individuals were detected crossing I-10. I-10 has two paved sections about 10 m wide with a vegetated median, and there were 559 movements  $>10$  m detected (32%). Thus whiptails can readily move the distance required to cross the paved surface, and could potentially cross I-10 using the median as a stepping stone.

## 2.5 DISCUSSION

Reptiles are thought to suffer negative effects from roads more than other taxonomic groups, and mitigation measures are often recommended to increase reptile population persistence (Rytwinski and Fahrig 2012). However, only snakes and turtles have been reasonably well-studied (Fahrig and Rytwinski 2009), leaving the impacts of roads on lizard populations largely unknown. We found that roads had minimal impacts on the abundance, survival, or body condition of two lizard species in southern New Mexico. Despite expectations that the more vagile marbled whiptails would encounter the roads more often than side-blotched lizards, increasing mortality risks (Carr and Fahrig 2001, Roe et al. 2006, Rytwinski and Fahrig 2012), we found little difference

in how the species responded to the roads. We found that the proximity to the roads in our study had a small positive effect on the abundance of adult and juvenile side-blotched lizards and juvenile marbled whiptails, and no effect on adult whiptails. The roads had no detectable impact on any of the metrics we used for assessing survival. Only male side-blotched lizards had lower body condition near the road. Male side-blotched lizards and both sexes of whiptails crossed the small road less often than expected. Of the habitat features included, distance between plants was most often found to be important in models of abundance and survival.

### **2.5.1 Behavioral responses**

Several lines of evidence suggest that lizards are behaviorally avoiding roads. There were fewer movements across NM-9 than across similar distances of undisturbed habitat. We did not detect any movements across I-10, but we also only documented a small number of movements long enough for an individual to traverse this distance. It is possible that individuals could successfully cross I-10 by using the median as a stepping stone. However, the higher traffic volume on I-10 compared to NM-9, and the observed reduction in movements across NM-9, lead us to believe that successful crossing of I-10 is rare.

Jaeger et al. (2005) proposed three categories of behavioral road avoidance: noise, vehicle, and road surface. Noise avoidance includes avoidance of other traffic-related disturbances like light and pollution, and animals that show noise avoidance are predicted to have lower abundances at some distance from the roads, which we did not find. We also observed both species foraging near the roads, especially when plant cover was present. Vehicle avoidance requires animals to wait for gaps in traffic before attempting to cross, reducing road mortality. We have no evidence that lizards are able to make such decisions rapidly enough to avoid oncoming vehicles (speed limit is 105–120 km/hr for both roads). Road surface avoidance, which includes both the material

of the road and the lack of cover on the road, is the most likely explanation for our results. Although side-blotched lizards and whiptails have different foraging behaviors, both have been described as foraging under plants, with whiptails visiting more plants than side-blotched lizards, but both species moving rapidly across open areas (Tinkle et al. 1962, Asplund 1974, Vitt and Ohmart 1977, Peterson and Whitford 1987, García-De la Peña et al. 2007). Although both species are heliothermic, they are most often seen basking under or close to plants instead of the open (Tinkle 1967, Kay et al. 1973, Vitt and Ohmart 1977). This behavior reduces the likelihood of either species basking on road surfaces (Andrews et al. 2008). The median distance between plants in the study sites was about 0.5–1 m, so both species could move from plant to plant with minimal exposure to predators. The pavement width was 8.7 m and 11.8 m for NM-9 and I-10, respectively, with additional open area in the road verge of several meters for most study sites. This relatively large expanse of open area could represent increased exposure to predators, which both species would likely avoid. In addition, the black asphalt road surface in the study regions likely reaches greater temperatures than the surrounding sandy substrate, further reducing the ability of lizards to cross during the middle of the day (Asplund 1974).

The few studies that have examined lizard behavior near roads have found similar results. Koenig et al. (2001) radio-tracked blue-tongued lizards (*Tiliqua scincoides*) in suburban Australia and found that lizards avoided crossing roads, and mostly spent their time under vegetation or other cover. Radio-tracked land mullets (*Egernia major*) were found basking near roads in tropical rainforest remnants, but crossed roads less often than expected from random walk models (Klingensböck et al. 2000). Of the 17 Galapagos lava lizards that entered the roadway, two were killed and 15 turned around after a short distance, a behavior thought to be road avoidance by Tanner and Perry (2007). Brehme et al. (2013) used fluorescent powder tracking and found that

both western fence lizards (*Sceloporus occidentalis*) and orange-throated whiptails (*Aspidoscelis hyperythra*) avoided crossing a highway with large traffic volumes (7400–18,000 vehicles per day). The orange-throated whiptails crossed a lightly traveled secondary paved road (200–500 vehicles per day) while western fence lizards were only detected entering that road, never crossing it. Both species were described as foraging in open areas, unlike the side-blotched lizards and marbled whiptails. They concluded that both species demonstrated vehicle avoidance because the widths of the two paved roads were similar. Munguia-Vega et al. (2013) found that both roads and open areas increased genetic differentiation in the black-tailed brush lizard, while populations within continuous habitat showed little differentiation over >1000 m. The removal of vegetation cover in the open areas and roads reduced the availability of shaded microhabitats required by the lizard to prevent overheating.

Although some herpetofauna do not show any form of behavioral avoidance (e.g., Row et al. 2007, Bouchard et al. 2009), smaller snakes (Enge and Wood 2002, Andrews and Gibbons 2005), box turtles (Shepard et al. 2008), and Blanding's turtles (Proulx et al. 2014) do avoid crossing roads. Additional data on how behavioral responses relate to life history and foraging behavior can further our ability to generalize about potential road effects.

### **2.5.2 Increased abundance near roads**

We did find higher abundance of side-blotched lizards and juvenile whiptails near the roads, although the effects were not strong. Possible explanations for this pattern include better habitat near the road or predator release. In arid regions, the road verge often has increased vegetation and arthropod abundance from runoff from the pavement (Lightfoot and Whitford 1991). In our study sites, most of the increased vegetation cover consisted of smaller plants and grasses, features that we did not include in our habitat structure analyses. Because we trapped on

BLM land and not in the road verges, our traps were not located directly in the areas with increased plant cover. However, based on the movement data in our study, lizards trapped closest to the road could potentially utilize those resources, which could increase lizard abundance. Roads and roadside habitats can act as ecological traps if animals perceive them as suitable, but suffer increased road mortality or stress responses as a result (Mumme et al. 2000, Steen et al. 2006, Owen et al. 2014, Jack et al. 2015). Increased abundance in such situations could reflect continued migration of new individuals into the area to utilize increased food availability or territories left open when their occupant was killed. However, we found no evidence that individuals living near roads experienced lower survival rates. Based on the evidence that both species avoid crossing roads, it is possible that they were able to use the increased resources without suffering increased mortality.

If predators are negatively affected by roads, their prey species can increase in abundance. This phenomenon is called predator release, and has been suggested as a cause of increased small mammal populations near roads (e.g., Rytwinski and Fahrig 2007, Planillo and Malo 2013). Common predators of side-blotched and whiptail lizards include snakes (Anderson 1993), larger lizards (Wilson 1991), and birds (Vitt and Ohmart 1977), all species that could potentially be negatively affected by roads (Fahrig and Rytwinski 2009). However, we did not assess the relative abundances of potential lizard predators during the study, so we do not know how much of an effect potential predator release could have had on our study species.

### **2.5.3 Effect of habitat structure**

Greater habitat heterogeneity has been associated with greater lizard diversity (Pianka 1967), along with increased summer survival rates in juvenile side-blotched lizards (Fox 1978). A variety of locations for thermoregulation and refuges from predators increased growth and survival

in adult male side-blotched lizards (Calsbeek and Sinervo 2002). This heterogeneity can be provided by rock piles (Calsbeek and Sinervo 2002), pack rat nests (Tinkle et al. 1962), or a variety of shrub sizes (Asplund 1974, Fox et al. 1981). Side-blotched lizards and whiptails are less common in areas with higher density of grasses and other forbs (Fox et al. 1981, Germano and Hungerford 1981, Peterson and Whitford 1987, Doughty and Sinervo 1994). Although we did not include small plants (area covered  $<0.5 \text{ m}^2$ ) or annuals in our habitat metrics, we observed that they were not particularly common in most of the study sites, especially in the drought year of 2011. We found that median distance between plants was the habitat variable most often identified as an important predictor variable in our different analyses. Whiptail abundance was positively associated with larger distances between plants of all sizes, which represent habitats with more open space. None of the habitat variables examined here were identified as important in explaining the abundance of side-blotched lizards. The median distance between plants was associated with two survival metrics. Shorter distances between medium and large plants was associated with a greater proportion of adults captured only once, indicating lower survival in locations with plants closer to each other. Greater distances between large plants were associated with longer than average persistence. Thus our results generally conform to those of the previously mentioned studies in that more shrubs and less dense vegetation are associated with increased abundance and survival.

#### **2.5.4 Limitations**

While we were able to discern some road effects, there were some methodological issues that potentially decreased our ability to detect effects with greater certainty. Our study design could be improved by trapping lizards closer to the roads and using more intensive sampling efforts to increase the number of recaptures per individual. We only trapped lizards outside of the road

verge, which was 11–27 m wide. If the road effect zone (Forman and Deblinger 2000) was smaller than this, we would not have detected it. Animals in the road verge may have experienced lower survival rates than those trapped in our closest arrays. However, it seems unlikely that such a narrow road effect zone would affect population persistence in locations where the habitat away from the road is suitable for animals and extensive. In our study system the surrounding landscape is mostly natural desert shrubland, which can support large lizard populations.

The larger shortcoming in our study was the low number of recaptures of marked animals. We used passive trapping of lizards to increase the number of sites analyzed, as opposed to working fewer sites more intensively with a combination of trapping, visual encounters, and catching animals by hand (e.g., Tinkle 1967, Gadsden and Castañeda 2013). Although including more sites allowed us to incorporate a broader range of habitats and independent populations, we were limited in our analyses of the data, particularly the survival estimates. Around half of all lizards marked of both species were never recaptured and an additional 20% were only recaptured once, rates that are similar to other studies on lizards using only passive trapping methods (e.g., Hawlena et al. 2010). Because the data were highly skewed to individuals only captured once, we analyzed the survival data as proportions, which could have reduced the statistical power of our tests. Our data analysis did show that the roads affected population abundance, a metric that was less affected by lack of recaptures. It is possible that we would have detected a road effect had our capture data been more suitable to more robust analysis methods. The increased sampling effort (e.g., additional trapping, walking surveys to detect marked animals) would have reduced the number of sites we were able to survey. However, as the distance to the road was never found to be an important explanatory variable in our survival analyses, it appears that any effects that the roads had on survival were minor compared to other factors, such as habitat structure and

precipitation. Future studies that focused on increasing recapture rates would nonetheless be beneficial in addressing road effects on population persistence.

We did not directly measure road mortality rates because preliminary road-kill surveys did not detect any carcasses. We instead chose to focus our efforts on studying the consequences of potential mortality on population vital rates, which are ultimately more important in assessing population persistence (Roedenbeck et al. 2007). Although it appears that the populations in our study were not negatively affected by road mortality, future work to quantify lizard road mortality rates with surveys designed specifically for smaller animals (e.g., frequent and at slow speeds, Langen et al. 2007, Santos et al. 2011, Teixeira et al. 2013) would be useful, especially if done in concert with assessment of population densities near the roads. Ruiz-Capillas et al. (2015) performed just such a study on small mammals, and found that despite estimates of road mortality much larger than reported in the literature, the road-killed individuals were only about 6% of the entire population, not enough to threaten population persistence.

The amount of precipitation the previous year affects the abundance of side-blotched lizards (Parker and Pianka 1975), while whiptail abundance is more dependent on the total precipitation the previous five years (Pianka 1970). The annual precipitation from 2004 (five years before the study began) through 2010 was average to above average (range: 223–374 mm), while 2011 was a drought year with total precipitation of 104 mm (National Weather Service, [http://www.srh.noaa.gov/epz/?n=santateresa\\_monthly\\_precip](http://www.srh.noaa.gov/epz/?n=santateresa_monthly_precip), accessed 31 March 2014). This change in precipitation is the likely reason that year was identified as an important explanatory variable in many of the analyses of abundance and survival. The higher abundance of side-blotched lizards in 2009 than 2010 and 2011 (Figs. 2.3a, b) was likely associated with the much higher than average precipitation in 2008 (374 mm). Adult whiptail abundance was lowest in 2009



(Fig. 2.3c), which may reflect the later start to our trapping efforts (July in 2009 vs. May in 2010 and 2011). Adult whiptails can begin brumation in mid-July (Medica 1967), and if some of the populations did start brumation at that time we would not have captured them. Juvenile whiptail abundance was lowest in 2011 (Fig. 2.3d), which could be due to reduced or delayed reproduction in response to the drought conditions. Finite survival of adults was partially explained by year as well, although for side-blotched lizards survival was higher in 2010–2011 than 2009–2010 (Fig. 2.9a), while for whiptails the 2010–2011 survival was lower than 2009–2010 (Fig. 2.9b). The number of side-blotched lizard juveniles or hatchlings later recaptured as adults was highest for the individuals that hatched in 2009 (Figs. 2.7, 2.8a), while more juvenile whiptails that hatched in 2010 were recaptured as adults (Fig. 2.8b). The strong effect that precipitation has on lizard demography could have masked any road effects. However, any such road effects would likely be fairly weak, and less likely to have major effects on population persistence.

Finally, we expected to see more differences in the road effects on the two species given their different life histories and, particularly, the difference in foraging behavior. Several previous studies have found that more vagile amphibians (Carr and Fahrig 2001) and snakes (Bonnet et al. 1999, Roe et al. 2006, Jochimsen et al. 2014) had higher road mortality rates than more sedentary species. Although previous studies (e.g., Milstead 1957, Tinkle 1967, Kay et al. 1973) and our own data show that whiptails move longer distances than side-blotched lizards, only the abundance of adult whiptails showed any difference from side-blotched lizards in regards to road effects. The abundance of adult whiptails was not affected by roads, while for both age classes of side-blotched lizards and juvenile whiptails the abundance increased near the roads. This pattern could be seen if the roads provided some benefit to the lizards in general, but the increased movement distances of adult whiptails meant that they experienced the negative impacts of road mortality, in effect

countering the potential benefits. However, we did not see any evidence for lower survival rates of adult whiptails near the roads. It is also possible that both species have some degree of behavioral avoidance of crossing open spaces, so even though whiptails encounter the roads more often in their daily movements, they do not attempt to cross very often, so the overall impact is similar to that experienced by side-blotched lizards that encounter roads less frequently. Neither species performs seasonal migrations to different habitats, so are not biologically required to cross roads. Some amphibians and snakes do undergo seasonal migrations, and thus attempt to cross roads whenever they are encountered and experience increased mortality. The differences in road mortality explained by vagility are based on individuals actually entering the roads they encounter. However, if roads are not entered, then there will be little mortality risk, and thus abundance and survival for the different species will not differ, as seen in our study.

#### **2.5.5 Lizard road ecology**

Although lizards have been understudied in road ecology, it appears that at least some of them fit into the “not affected” categories of Fahrig and Rytwinski (2009), despite their general conclusions that amphibians and reptiles are the most negatively impacted groups. Namely, some smaller lizards are not attracted onto roads or affected by traffic disturbances, they have relatively small movement ranges, high reproductive rates, and high densities, and they behaviorally avoid open areas (e.g., Tinkle 1967, Asplund 1974, Vitt and Ohmart 1977, Rutherford and Gregory 2003). In this sense, many smaller lizard species seem to react to roads in a way more like that of small mammals. Small mammals show some degree of road avoidance (Mader 1984, Ford and Fahrig 2008, McGregor et al. 2008) and have life history characteristics and movement distances generally similar to at least some species of smaller lizards. Roads generally have neutral or

positive effects on small mammal abundance (Rytwinski and Fahrig 2007, Bissonette and Rosa 2009, Rytwinski and Fahrig 2011, 2012), similar to our results.

Future work on lizard road ecology should focus on those species that are more likely to be negatively impacted by roads, such as those attracted to roads or that have larger movement distances. Although several species of lizards appear avoid road surfaces, likely due to the lack of cover (e.g., Aresco 2005, this study), other species may be attracted to roads. Meek (2009, 2014) found greater mortality in the larger western green lizards (*Lacerta bilineata*) than in common wall lizards (*Podarcis muralis*), a difference attributed to increased road basking by western green lizards. Although Sherbrooke (2002) did not specifically collect data on road mortality of Texas horned lizards (*Phrynosoma cornutum*) during 18 years of road surveys, he estimated it to be about 25–50%. Differences in movement and thus potential road mortality by sex or age class also deserve further attention in lizard studies. For some species, the males move greater distances than females during mating seasons. More male green iguanas (*Iguana iguana*) were killed on roads during mating season, when individuals were dispersing to find territories, while the few female carcasses were mostly found during the nesting season (Rodda 1990). Sherbrooke (2002) found more male Texas horned lizards on the roads, possibly due to increased mate-seeking movements (although Moeller et al. (2005) consistently found more females on roads in south Texas). Differences in how males and females respond to roads warrants further study, especially as differential road mortality rates have shifted sex ratios of some freshwater turtle populations to become male-biased (Gibbs and Steen 2005). A variety of life histories and movement behaviors have been studied for mammals and birds, which allow for generalizations about what types of species are at greater risk for road mortality (Rytwinski and Fahrig 2011, 2012). Future studies

on a similar variety of lizard species will help to determine if lizards should be included with turtles and snakes in the category of “most impacted” taxa (Fahrig and Rytwinski 2009).

## **2.6 CONCLUSIONS**

In regions where roads are the main disturbance, outside of other human development, neither side-blotched lizards nor marbled whiptail lizards appear to be threatened by negative road impacts. The behavioral road avoidance observed in this study can reduce mortality rates, but at the cost of reduced gene flow. Genetic isolation can potentially cause inbreeding depression, threatening long-term population persistence (Frankham 2005, Balkenhol and Waits 2009). There is extensive desert shrub habitat surrounding both roads, likely supporting large enough lizard populations to reduce the risks of inbreeding. Thus it appears that the current conditions do not threaten population persistence. However, a future risk is increased traffic volume on the small road as the urbanization encroaches. While the current traffic volumes are low enough to present little mortality risk to individuals who do attempt to cross, increased traffic could result in greater mortality during crossing attempts. Eventually vehicle avoidance behavior may occur, reducing mortality risks. The greatest threats might be at intermediate traffic volumes. Indeed, higher numbers of road-killed lizards (Lebboroni and Corti 2006) and small and large mammals (Oxley et al. 1974, Clevenger et al. 2003, van Langevelde et al. 2009, Thurfjell et al. 2015) have been found on roads with lower traffic volumes. A better understanding of the interaction between traffic volume and mortality risks, and identification of possible risk thresholds, will allow us to focus mitigation on the roads where it can have the greatest benefit (Garcia-Gonzalez et al. 2012, Rhodes et al. 2014).

The tendency of some species to avoid open areas can guide management of road verges to reduce crossing attempts, and thus mortality. For these species, the greater the distance of an

open area an individual has to cross, the less likely it is to make the attempt (Trombulak and Frissell 2000). If vegetation or other structural features important to particular species (e.g., rock piles used by rattlesnakes, Jochimsen et al. 2014) are cleared away from the road, animals will not use the road verge as often, and the distance between useable habitat across roads will increase. This management strategy does potentially trade mortality risks for genetic risks, but it is possible that the benefit from the reduction in mortality outweighs the cost of increased genetic isolation (Jackson and Fahrig 2011). In our study system, removing shrubs and other forms of cover away from the roads may reduce the likelihood of either species attempting road-crossings, but it does not appear that any such mitigation efforts are currently required for side-blotched lizards or marbled whiptails.

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## 2.8 TABLES

Table 2.1. Model coefficients from the generalized linear models with abundance (number of unique individuals per 100 trap-days) as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data, and model fit (correlation between observed and predicted values, explained deviance of saturated model) is described in the results. For adult side-blotched lizards (N = 1264), the model with the lowest AICc had a score of 483.88 and there were 119 models within 6 AICc. For juvenile side-blotched lizards (N = 927), the AICc of the best model was 487.96 and there were 289 models within 6 AICc. For adult whiptails (N = 1252), the best model had an AICc of 495.20 and there were 233 models within 6 AICc. For juvenile whiptails (N = 545), the best model had an AICc of 426.32 and there were 121 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Adult side-blotched	Road:NM-9	-0.31	4.87E-03	[-0.45, -0.17]
	Year: 2011	-0.82	5.08E-03	[-0.96, -0.68]
	Year: 2010	-0.86	4.78E-03	[-1.00, -0.72]
	(Intercept)	3.56	6.20E-02	[3.07, 4.06]
	DistanceToRoad	-0.15	2.81E-03	[-0.25, -0.04]
Juvenile side-blotched	Year: 2011	-0.29	9.45E-03	[-0.49, -0.10]
	Year: 2010	-0.77	1.08E-02	[-0.98, -0.57]
	(Intercept)	3.16	7.21E-02	[2.63, 3.70]
	DistanceToRoad	-0.20	5.93E-03	[-0.36, -0.05]
Adult whiptail	ENN_MD_AllSizes	0.51	2.96E-02	[0.16, 0.85]
	Year: 2011	0.75	7.38E-03	[0.57, 0.92]
	Year: 2010	0.58	7.40E-03	[0.41, 0.75]
	(Intercept)	1.41	1.42E-01	[0.66, 2.15]
	Road:NM-9	-0.20	5.87E-03	[-0.35, -0.04]
Juvenile whiptail	ENN_MD_AllSizes	0.87	6.91E-02	[0.35, 1.39]
	DistanceToRoad	-0.36	1.06E-02	[-0.56, -0.15]
	Road:NM-9	-0.48	1.50E-02	[-0.72, -0.23]
	Year: 2011	-0.79	1.91E-02	[-1.06, -0.51]
	Year: 2010	-0.25	1.30E-02	[-0.48, -0.02]
	(Intercept)	2.12	3.74E-01	[0.90, 3.34]
	ENN_RA_Large	-0.05	3.38E-04	[-0.08, -0.01]

Table 2.2. Model coefficients from the generalized linear models with the proportion of individuals only captured once as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data and model fit (correlation between observed and predicted values, explained deviance of saturated model) is described in the results. Positive coefficients are associated with more individuals only captured once, so reflect decreased survival. For adult side-blotched lizards (N = 691), the model with the lowest AICc had a score of -102.2 and there were 153 models within 6 AICc. For juvenile side-blotched lizards (N = 250), the AICc of the best model was 16.70 and there were 229 models within 6 AICc. For adult whiptails (N = 448), the best model had an AICc of -59.67 and there were 619 models within 6 AICc. For juvenile whiptails (N = 289), the best model had an AICc of -15.41 and there were >1000 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Adult side-blotched	Year: 2011	0.12	1.15E-03	[0.05, 0.19]
	Year: 2010	0.07	1.07E-03	[0.01, 0.14]
	(Intercept)	0.69	2.29E-02	[0.39, 1.00]
	ENN_MD_MediumAndLarge	-0.08	8.50E-04	[-0.14, -0.02]
Juvenile side-blotched	Year: 2011	0.20	4.71E-03	[-0.34, -0.07]
	Year: 2010	0.18	4.52E-03	[-0.32, -0.05]
	(Intercept)	0.29	3.11E-02	[0.35, 1.06]
Adult whiptail	Year: 2011	0.22	1.94E-03	[0.13, 0.30]
	Year: 2010	0.15	1.82E-03	[0.07, 0.24]
Juvenile whiptail	(Intercept)	0.78	3.76E-02	[0.40, 1.17]

Table 2.3. Model coefficients from the generalized linear models with the proportion of adults with a persistence (number of days between first and last capture) longer than the average for the particular year as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data and model fit (correlation between observed and predicted values, explained deviance of saturated model) is described in the results. For adult side-blotched lizards (N = 242), the model with the lowest AICc had a score of -120.86 and there were 642 models within 6 AICc. For adult whiptails (N = 357), the best model had an AICc of -69.12 and there were 303 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Adult side-blotched	ENN_MD_Large	0.03	9.30E-05	[0.01, 0.05]
Adult whiptail	Year: 2011	-0.15	1.72E-03	[-0.23, -0.06]
	Year: 2010	-0.24	1.61E-03	[-0.32, -0.16]
	(Intercept)	0.58	2.61E-02	[0.26, 0.90]



Table 2.4. Model coefficients from the generalized linear models with the proportion of hatchlings recaptured as juveniles or adults or the proportion of juveniles recaptured as adults as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data and model fit (correlation between observed and predicted values, explained deviance of saturated model) is described in the results. For hatchling side-blotched lizards (N = 453), the model with the lowest AICc had a score of -15.36 and there were 423 models within 6 AICc. For juvenile side-blotched lizards (N = 412), the best model had an AICc of 10.70 and there were 589 models within 6 AICc. For juvenile whiptails (N = 472), the best model had an AICc of -26.58 and there were 471 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Hatchling side-blotched	(Intercept)	0.32	2.56E-02	[0.00, 0.64]
	Road: NM-9	0.13	3.49E-03	[0.01, 0.25]
Juvenile side-blotched	Period: Hatched 2011	-0.22	4.39E-03	[-0.35, -0.09]
	Period: Hatched 2010	-0.17	4.21E-03	[-0.30, -0.04]
	(Intercept)	0.53	3.23E-02	[0.17, 0.88]
Juvenile whiptail	Period: Hatch year 2010	0.15	2.40E-03	[0.06, 0.25]

Table 2.5. Model coefficients from the generalized linear models with the finite annual survival of adults as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data and model fit (correlation between observed and predicted values, explained deviance of saturated model) is described in the results. For adult side-blotched lizards (N = 572 in 2009, 346 in 2010), the model with the lowest AICc had a score of -122.5 and there were 447 models within 6 AICc. For adult whiptails (N = 201 in 2009, 444 in 2010), the best model had an AICc of -30.21 and there were 790 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Adult side-blotched	Period: 2010-2011	0.07	4.46E-04	[0.03, 0.11]
	(Intercept)	0.44	3.68E-02	[0.05, 0.82]
Adult whiptail	Period: 2010-2011	-0.16	1.98E-03	[-0.25, -0.08]
	(Intercept)	0.44	3.68E-02	[0.05, 0.82]

Table 2.6. Model coefficients from the generalized linear models with scaled mass index as the dependent variable. Only the important explanatory variables (as defined in the methods) are reported here. Estimates for all of the variables are available in the supplementary data and model fit (correlation between observed and predicted values) is described in the results. For male side-blotched lizards (N = 1110), the model with the lowest AICc had a score of -83.31 and there were 170 models within 6 AICc. For female side-blotched lizards (N = 992), the best model had an AICc of -393.21 and there were 369 models within 6 AICc. For male whiptails (N = 1567), the AICc of the best model was 5483.18 and there were 231 models within 6 AICc. For female whiptails (N = 999), the AICc of the best model was 3133.15 and there were >1000 models within 6 AICc.

	Explanatory variable	Estimate	Variance	95% CI
Male side-blotched	DistanceToRoad	0.05	2.19E-04	[0.02, 0.08]
	Year: 2011	0.05	3.28E-04	[0.01, 0.09]
	Year: 2010	0.10	3.47E-04	[0.06, 0.14]
	Road: NM-9	-0.15	4.14E-04	[-0.19, -0.11]
	(Intercept)	2.06	7.31E-03	[1.90, 2.23]
	PatchDensity_MediumAndLarge	0.01	1.80E-05	[0.00, 0.02]
	PatchDensity_Large	-0.02	5.98E-05	[-0.04, -0.01]
	PercentageLandscape_Large	0.01	1.91E-05	[0.00, 0.02]
Female side-blotched	ENN_RA_AllSizes	0.02	4.45E-05	[0.01, 0.03]
	Year: 2010	0.06	2.39E-04	[0.03, 0.09]
	(Intercept)	1.65	2.71E-03	[1.55, 1.75]
	ENN_RA_MediumAndLarge	-0.01	1.05E-05	[-0.02, 0.00]
	Road: NM-9	-0.04	2.32E-04	[-0.07, -0.01]
Male whiptails	ENN_RA_Large	0.04	1.71E-04	[0.01, 0.06]
	Year: 2011	-0.53	9.96E-03	[-0.73, -0.33]
	Road: NM-9	-0.50	7.71E-03	[-0.67, -0.32]
	(Intercept)	12.69	1.29E-01	[11.99, 13.40]
Female whiptails	Year: 2011	-0.26	9.71E-03	[-0.46, -0.07]
	Road:NM-9	-0.39	7.69E-03	[-0.57, -0.22]
	(Intercept)	10.08	1.23E-01	[9.40, 10.77]

## 2.9 FIGURES

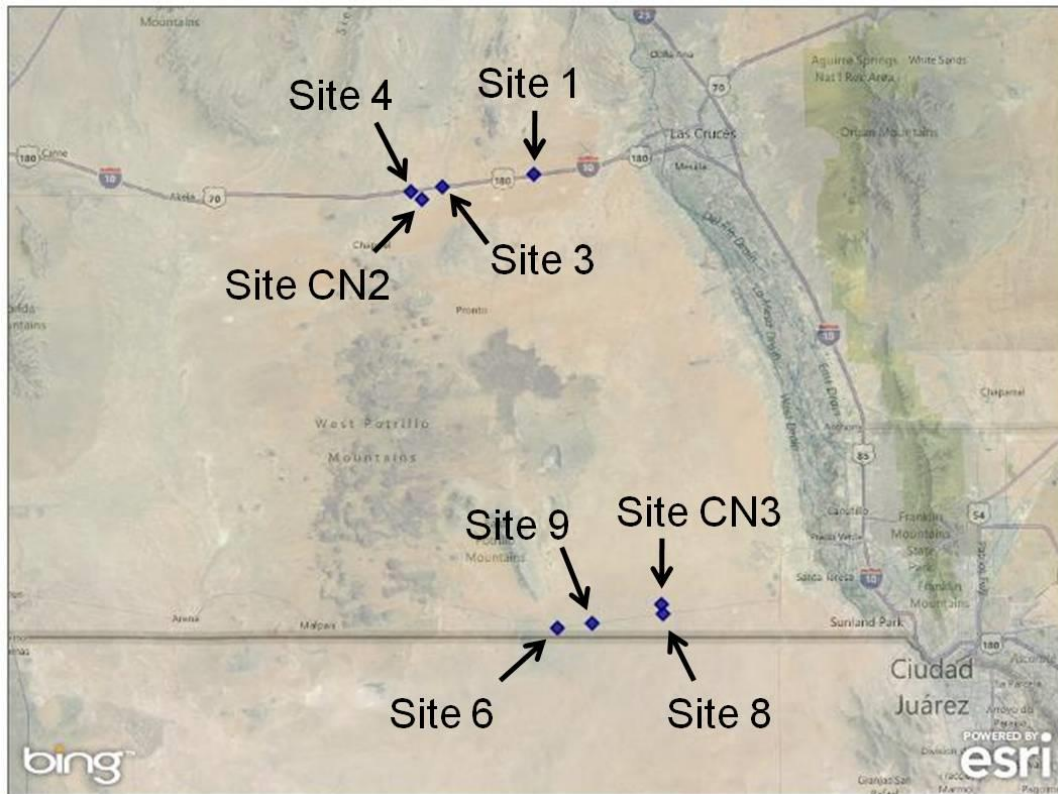


Fig. 2.1. Location of the study sites. Interstate 10 is the northern road and New Mexico Highway 9 is the southern road. The control sites are located approximately 1000 m from each the road. Only sites 3, 4, CN2, 6, 8, and CN3 were used for the genetic study (Chapter 3).

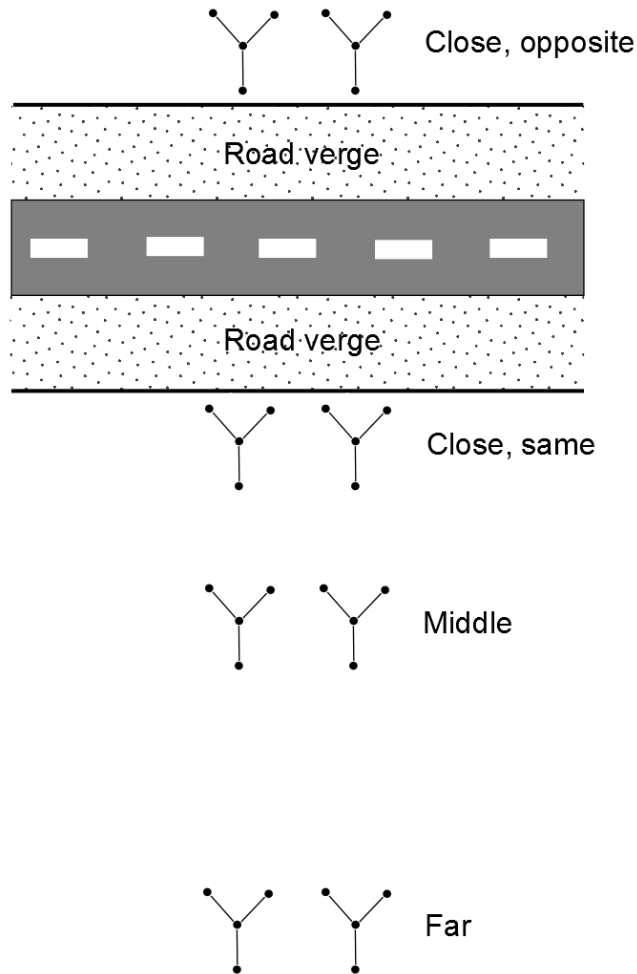


Fig. 2.2. A schematic diagram of the trapping arrays at each site. There are two pitfall trapping arrays at each distance from the road. The distance from the close, same trapping group to the middle group was about 50 m and to the far trapping group was about 125 m. The distance from the close, opposite to the close, same traps was about 50 m for the sites on New Mexico Highway 9 and about 125 m for the Interstate 10 sites. Individuals from a particular trapping distance were considered to be individual populations. For the genetic study (Chapter 3), we genotyped individuals from close, opposite; close, same; and far at the two I-10 sites, and individuals from close, opposite; close, same; and mid for the NM-9 sites. This design allowed for comparison of genetic distance between populations while holding geographic distance constant.

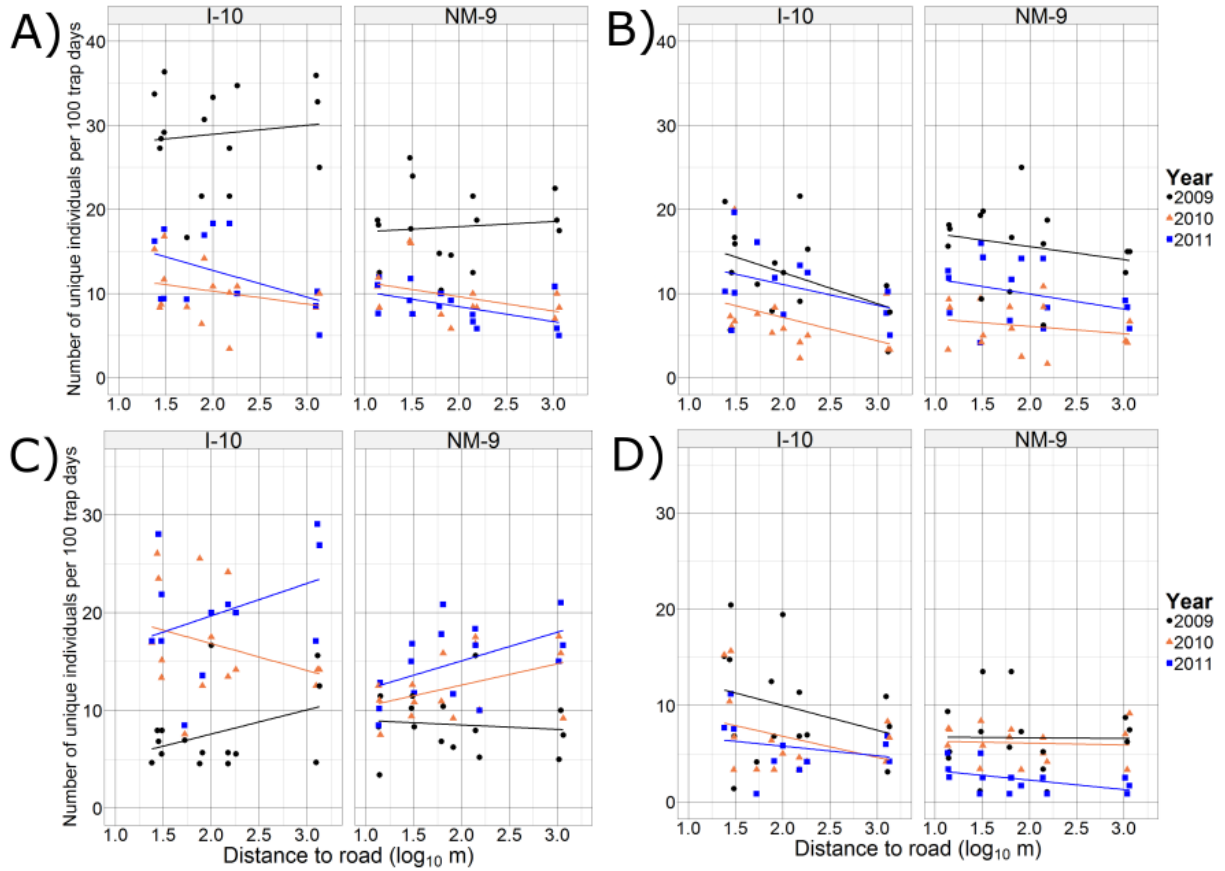


Fig. 2.3. The number of unique individuals by year and distance to the road. A) Adult side-blotched lizards were more abundant near the roads, at the I-10 sites, and in 2009. B) Juvenile side-blotched lizards were also more abundant near the roads and in 2009. C) Adult whiptail abundance was not affected by the distance to the road, but was higher in both 2010 and 2011 than in 2009. D) Juvenile whiptail abundance was highest near the roads and in 2009. Note that the scales on the y-axis for side-blotched and whiptail lizards are different.

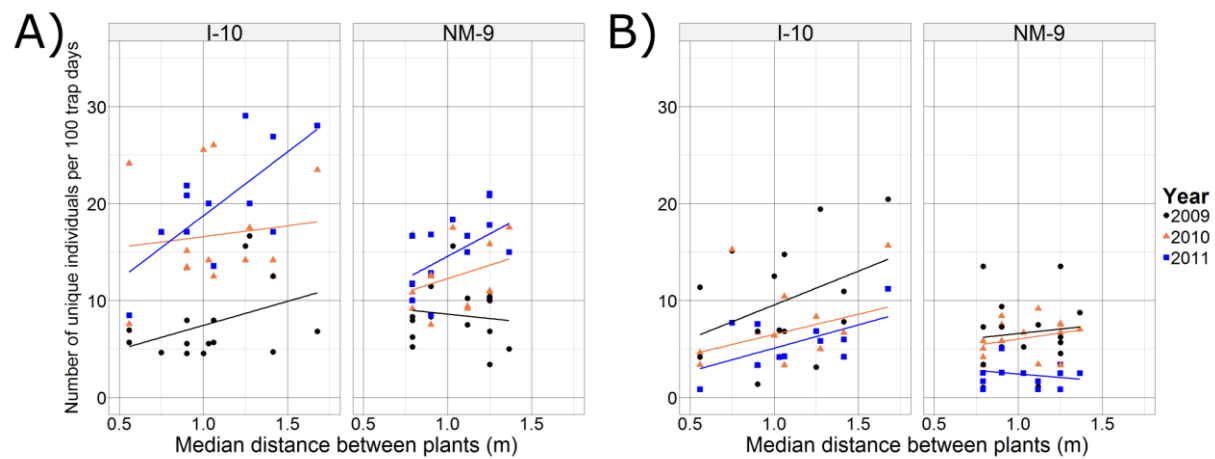


Fig. 2.4. The abundance of whiptail lizards relative to the median distance between plants. Both A) adults and B) juveniles were more abundant in locations with greater distances between plants of all sizes.

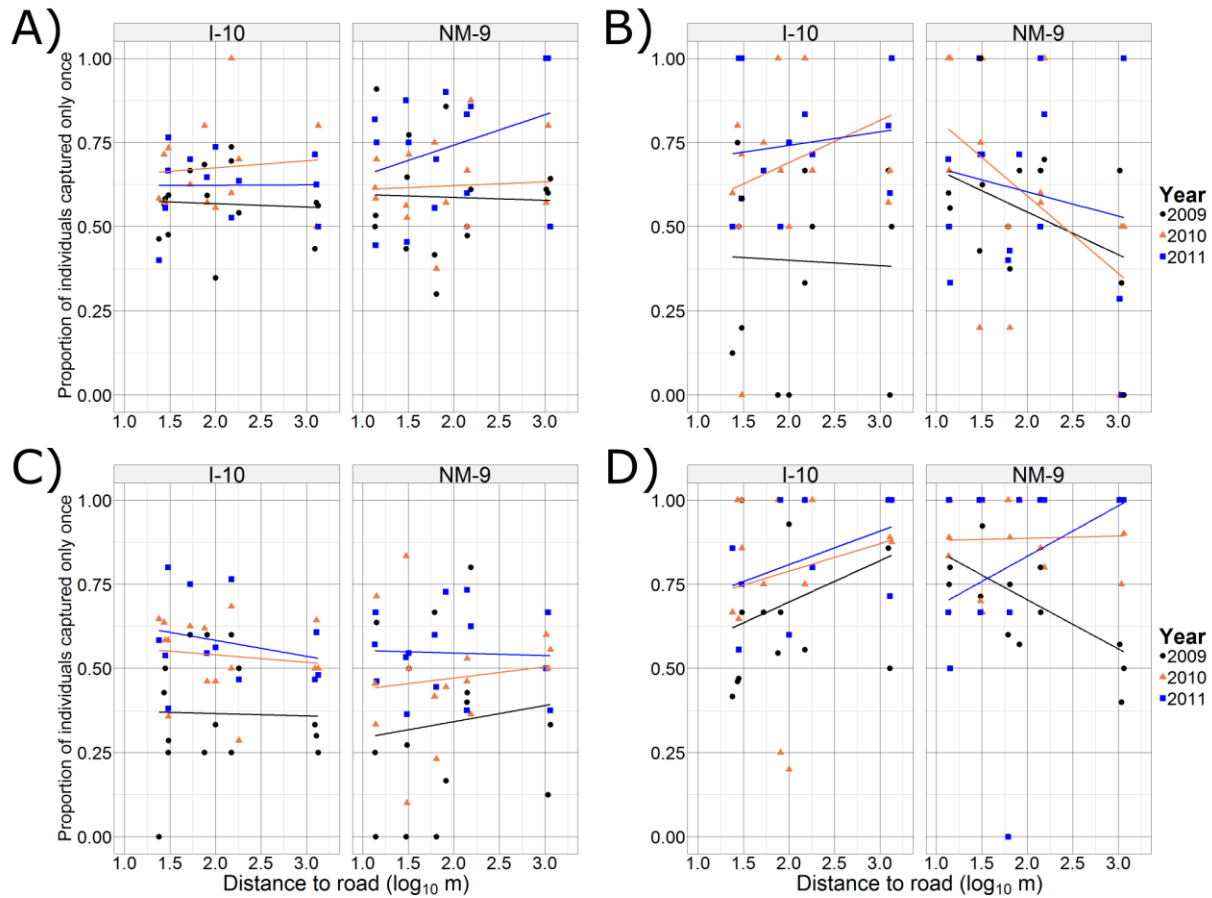


Fig. 2.5. The proportion of individuals only captured once. This metric is the inverse of survival, as individuals only captured once are expected to have lower apparent survival rates than those individuals captured multiple times. The distance to the road was only found to be an important explanatory variable for adult side-blotched lizards (A). Year was also important, with more individuals captured once in 2010 and 2011 than in 2009, as was the distance between plants  $>5 \text{ m}^2$  (not shown in the figure). For B) juvenile side-blotched lizards, there were more individuals captured once in 2009 than in 2010 and 2011. For C) adult whiptails, there were fewer individuals captured once in 2010 and 2011 than in 2009, while for D) juvenile whiptails, the effect of year was not important.



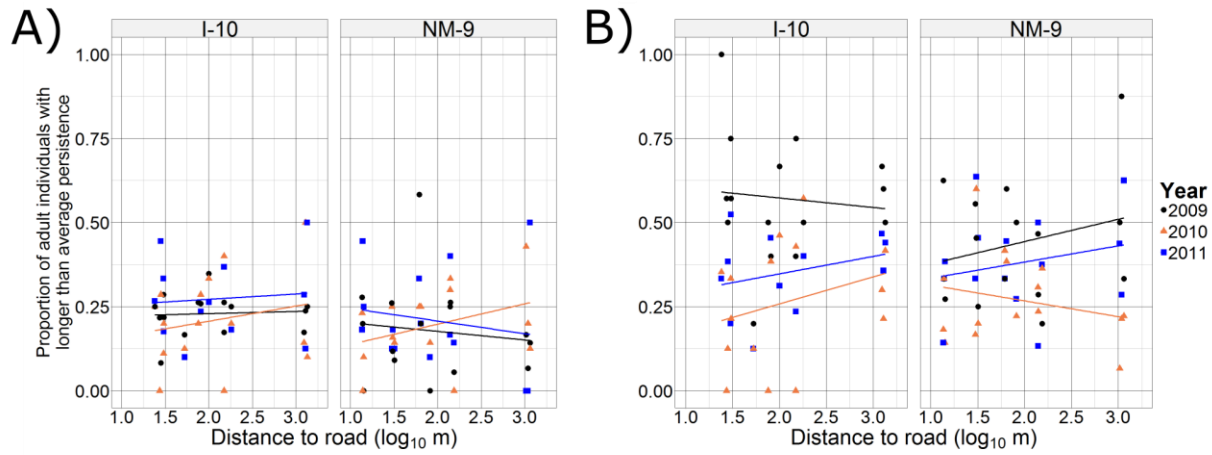


Fig. 2.6. The proportion of adult A) side-blotched lizards and B) whiptails that had persistence lengths (the number of days between the first and last capture) longer than the average for the individuals in the same location and year. Longer than average persistence is expected to reflect increased individual survival. Only the distance between large plants was an important explanatory variable for side-blotched lizards, with neither distance from the road nor year having detectable effects. Year was important for whiptails, and more individuals marked in 2009 had longer than average persistence lengths than those marked in 2010 or 2011. The distance to the road was not an important explanatory variable for either species.

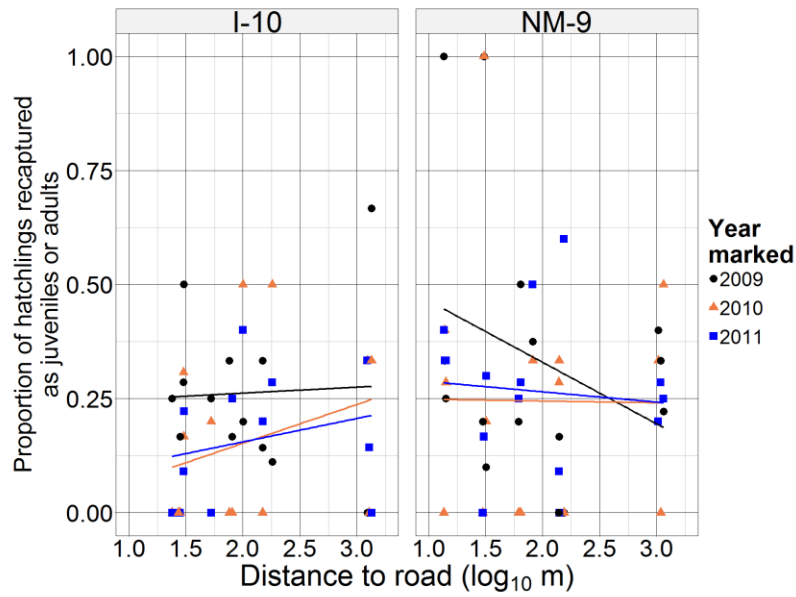


Fig. 2.7. The proportion of side-blotched lizard hatchlings that were recaptured as either juveniles or adults. A greater proportion of hatchlings being recaptured is expected to reflect increased survival rates. Neither the year an individual was marked nor its location relative to the road were important explanatory variables, but fewer hatchlings in the sites along I-10 were later recaptured.

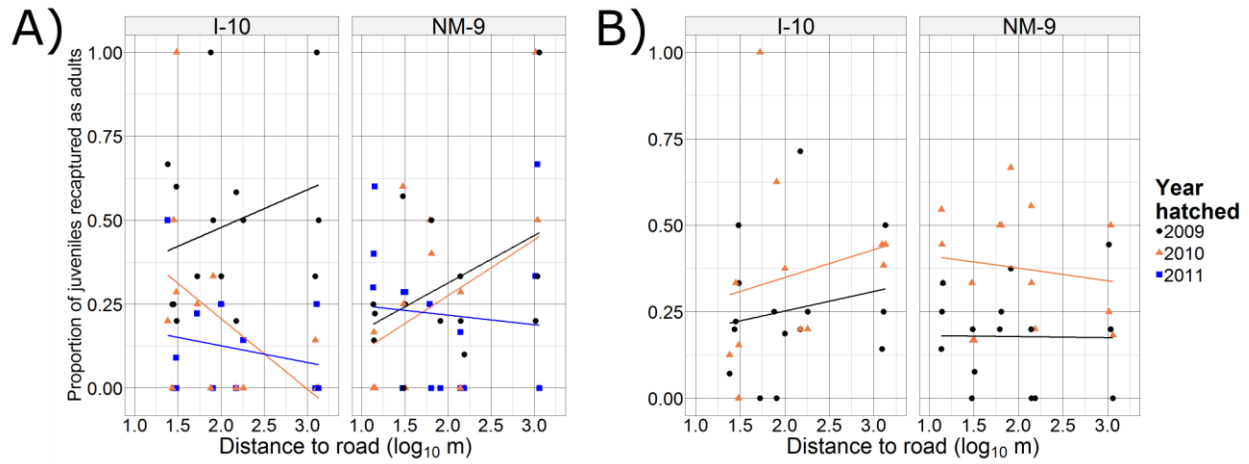


Fig. 2.8. The proportion of A) side-blotched lizard and B) whiptail juveniles that were later recaptured as adults. Side-blotched lizards that hatch early in the summer can reach adult size (>30 mm SVL) that same year, while whiptails typically do not attain adult size (>60 mm SVL) until the year after they hatch. Thus we included the side-blotched lizard hatchlings from 2011, but excluded the whiptails from 2011. A greater number of side-blotched lizards from 2009 were recaptured as adults, while fewer whiptail juveniles from 2009 were recaptured as adults.

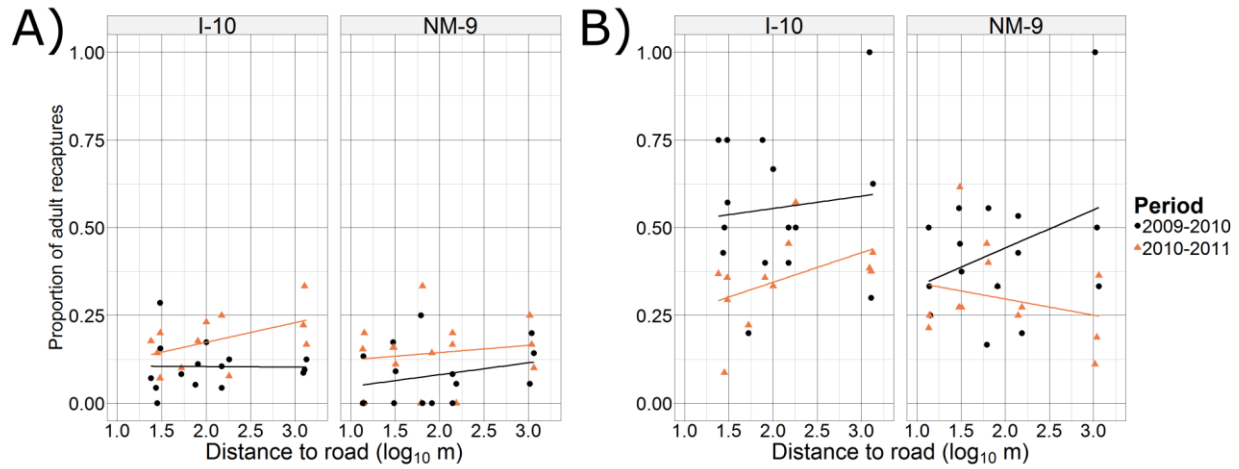


Fig. 2.9. The finite annual survival for A) side-blotched lizard and B) whiptail adults. Finite annual survival is the number of recaptured lizards in year  $t + 1$  relative to total number marked in time  $t$ . Only year was an important explanatory variable for side-blotched lizards, with higher apparent survival from 2010–2011 than 2009–2010. The opposite was true for the whiptails, with higher survival in 2009–2010 than in 2010–2011.

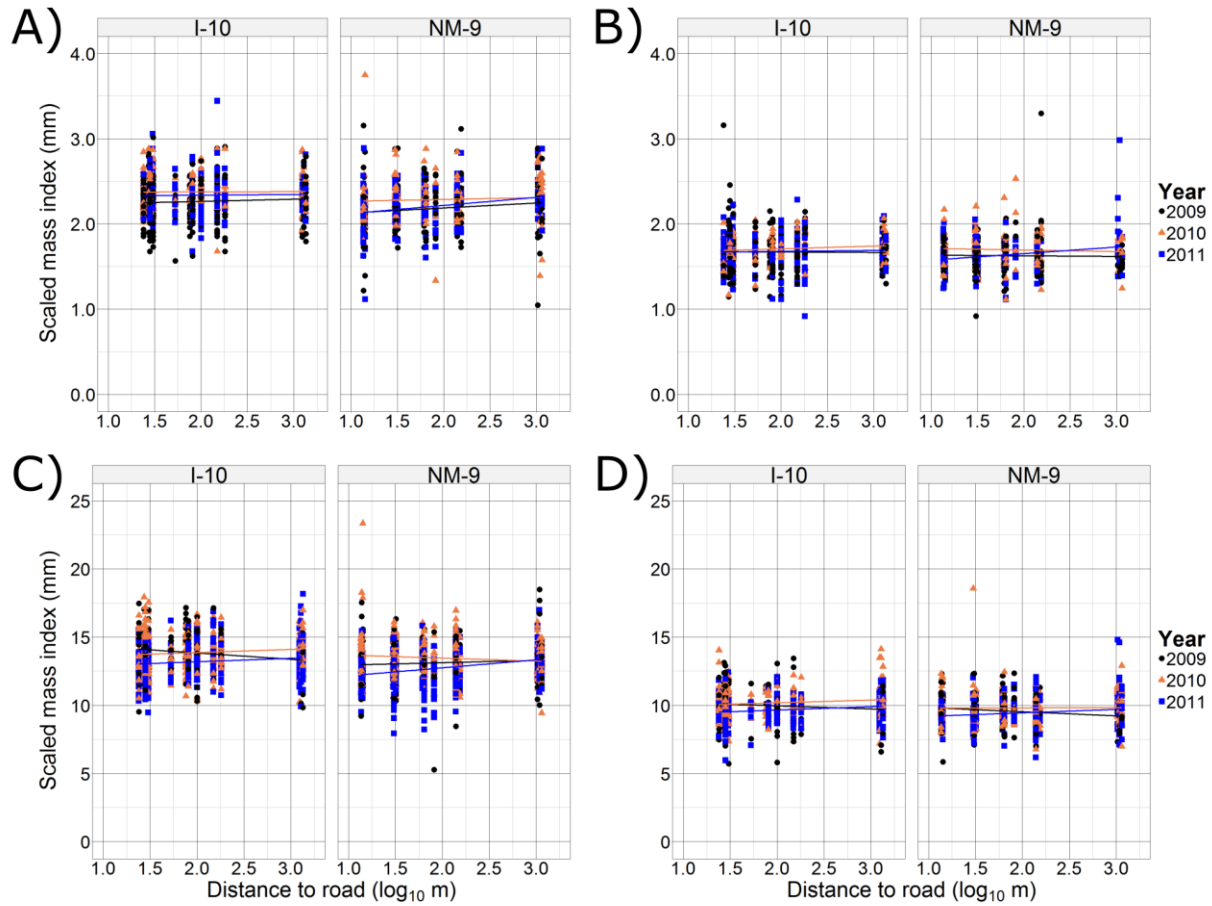


Fig. 2.10. The body condition as measured by the scaled mass index for A) male and B) female side-blotched lizards and for C) male and D) female whiptail lizards. The distance to the pavement was an important explanatory variable for male side-blotched lizards, as was year (higher body condition in 2010 and 2011 than in 2009) and road (higher body condition in the I-10 sites). The year (2010 higher than 2009 and 2011) and road (higher body condition at the I-10 sites) were important variables for female side-blotched lizards. Both male and female whiptails had better body condition in 2009 and 2010 than in 2011 and in the I-10 sites. The outliers shown here were removed prior to analyzing these data. Note the differences in the scales among the panels.

## 2.10 SUPPLEMENTARY DATA

Table S2.1. Trapping effort at each site per year. The trapping effort is reported as trap-days (the number of traps opened times the number of days opened). We stopped trapping at site 4 north in August 2010 because of a near-by road expansion project. The effort reported for 2011 is only for site 4 south of the road, which had eight traps. The control sites (CN2 and CN3) had 24 traps, while the rest of the sites had 32. Some traps were left closed for particular days because of flooding or high levels of nearby ant activity.

Road	Site	2009	2010	2011	Total
I-10	1	288	471	475	1234
	3	352	484	474	1310
	4	352	408	107	867
	CN2	168	360	353	881
NM-9	6	384	480	476	1340
	8	352	473	476	1301
	9	384	479	477	1340
	CN3	240	354	359	953

Table S2.2. Summary statistics for the habitat structure variables. The habitat structure was measured at two different buffer distances around each of the trapping groups (30 groups total): 10 m to reflect the shorter movement distances and smaller home ranges of the side-blotched lizards, and 25 m to reflect the greater movement and larger home ranges of the marbled whiptails. The generalized variance inflation factor (GIVF) measures the multicollinearity for each variable.

	10 m buffer			25 m buffer		
	Mean (SE)	Range	GIVF	Mean (SE)	Range	GIVF
BufferArea	1218.3 (15.3)	1108.5 - 1463.0	NA	3913.6 (31.3)	3694.3 - 4429.9	NA
Perimeter	132.5 (1.1)	124.7 - 150.6	NA	226.8 (1.1)	219.0 - 244.9	NA
PercentageLandscape_ AllSizes	23.8 (1.3)	12.3 - 44.1	18.8	23.6 (1.1)	15.6 - 39.5	22.9
PatchDensity_AllSizes	40.0 (3.4)	16.9 - 84.5	3.2	35.6 (3.3)	14.4 - 87.2	4.8
ENN_MD_AllSizes	1.1 (0.1)	0.6 - 1.8	3.1	1 (0)	0.6 - 1.7	3.5
ENN_RA_AllSizes	3.6 (0.3)	1.0 - 8.0	2.7	4.5 (0.2)	2.4 - 7.3	3.0
PatchDensity_ MediumAndLarge	15.2 (0.8)	7.3 - 25.3	9.2	13.1 (0.6)	7.4 - 21.0	4.8
ENN_MD_ MediumAndLarge	1.8 (0.1)	0.8 - 3.5	3.7	1.5 (0.1)	1.0 - 2.9	2.7
ENN_RA_ MediumAndLarge	5.1 (0.4)	1.5 - 12.4	1.9	6.5 (0.5)	3.3 - 18.7	2.1
PercentageLandscape_ Large	16.9 (1.3)	5.0 - 38.9	24.0	17.1 (1.1)	6.7 - 29.3	26.8
PatchDensity_Large	7.6 (0.5)	4.1 - 13.5	8.7	5.8 (0.3)	2.8 - 9.8	2.8
ENN_MD_Large	2.5 (0.3)	0.5 - 7.2	1.9	2.3 (0.2)	1.0 - 5.3	2.4
ENN_RA_Large	7.8 (0.8)	1.2 - 16.9	1.9	8.8 (0.7)	2.5 - 16.3	1.9

Table S2.3. Model coefficients for all explanatory variables from the generalized linear models with abundance as the dependent variable for side-blotched lizards. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Adult side-blotched</b> N lizards = 1264 Lowest AICc = 483.88 N models = 119	Road: NM-9*	-0.309	4.87E-03	1.000	[-0.45, -0.17]
	Year: 2011*	-0.817	5.08E-03	1.000	[-0.96, -0.68]
	Year: 2010*	-0.860	4.78E-03	1.000	[-1.00, -0.72]
	(Intercept)*	3.562	6.20E-02	1.000	[3.07, 4.06]
	log10(DistanceToRoad)*	-0.146	2.81E-03	1.000	[-0.25, -0.04]
	ENN_MD_Large	0.038	8.22E-04	0.770	[-0.02, 0.09]
	PatchDensity_Large	0.038	1.11E-03	0.675	[-0.03, 0.1]
	PatchDensity_MediumAndLarge	-0.016	2.85E-04	0.614	[-0.05, 0.02]
	ENN_RA_Large	-0.009	9.95E-05	0.568	[-0.03, 0.01]
	PercentageLandscape_AllSizes	-0.014	2.71E-04	0.549	[-0.05, 0.02]
	PercentageLandscape_Large	0.016	3.86E-04	0.517	[-0.02, 0.06]
	PatchDensity_AllSizes	0.000	8.86E-07	0.210	[0.00, 0.00]
	ENN_MD_MediumAndLarge	-0.002	1.37E-04	0.152	[-0.03, 0.02]
	ENN_RA_MediumAndLarge	-0.001	7.91E-06	0.143	[-0.01, 0.0]
	ENN_MD_AllSizes	-0.006	4.93E-04	0.138	[-0.05, 0.04]
	ENN_RA_AllSizes	0.001	2.20E-05	0.135	[-0.01, 0.01]
<b>Juvenile side-blotched</b> N lizards = 927 Lowest AICc = 487.96 N models = 289	Year: 2011*	-0.293	9.45E-03	1.000	[-0.49, -0.10]
	Year: 2010*	-0.772	1.08E-02	1.000	[-0.98, -0.57]
	(Intercept)*	3.163	7.21E-02	1.000	[2.63, 3.70]
	log10(DistanceToRoad)*	-0.202	5.93E-03	0.986	[-0.36, -0.05]
	ENN_RA_AllSizes	-0.019	9.08E-04	0.395	[-0.08, 0.04]
	ENN_RA_Large	0.005	6.87E-05	0.384	[-0.01, 0.02]
	ENN_MD_AllSizes	-0.044	6.97E-03	0.277	[-0.21, 0.12]
	PercentageLandscape_AllSizes	-0.002	1.68E-05	0.202	[-0.01, 0.01]
	PercentageLandscape_Large	0.001	5.77E-06	0.164	[0.00, 0.01]
	PatchDensity_AllSizes	0.000	4.51E-07	0.157	[0.00, 0.00]
	ENN_RA_MediumAndLarge	-0.001	1.74E-05	0.149	[-0.01, 0.01]
	ENN_MD_MediumAndLarge	-0.004	1.50E-04	0.143	[-0.03, 0.02]
	PatchDensity_Large	0.000	9.93E-06	0.136	[-0.01, 0.01]
	Road: NM-9	0.003	2.16E-04	0.136	[-0.03, 0.03]
	PatchDensity_MediumAndLarge	0.000	2.25E-06	0.132	[0.00, 0.00]
	ENN_MD_Large	0.000	1.37E-05	0.127	[-0.01, 0.01]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.



Table S2.4. Model coefficients for all explanatory variables from the generalized linear models with abundance as the dependent variable for marbled whiptails. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
Adult whiptails N lizards = 1252 Lowest AICc = 495.20 N models = 233	ENN_MD_AllSizes*	0.507	2.96E-02	1.000	[0.16, 0.85]
	Year: 2011*	0.745	7.38E-03	1.000	[0.57, 0.92]
	Year: 2010*	0.577	7.40E-03	1.000	[0.41, 0.75]
	(Intercept)*	1.405	1.42E-01	1.000	[0.66, 2.15]
	Road: NM-9*	-0.195	5.87E-03	0.984	[-0.35, -0.04]
	PatchDensity_MediumAndLarge	0.009	1.73E-04	0.437	[-0.02, 0.04]
	PercentageLandscape_AllSizes	0.005	6.42E-05	0.349	[-0.01, 0.02]
	PatchDensity_Large	0.005	1.15E-04	0.244	[-0.02, 0.03]
	PercentageLandscape_Large	-0.002	2.40E-05	0.213	[-0.01, 0.01]
	log10(DistanceToRoad)	0.008	3.87E-04	0.193	[-0.03, 0.05]
	ENN_RA_AllSizes	-0.004	8.09E-05	0.178	[-0.02, 0.01]
	PatchDensity_AllSizes	0.000	4.45E-07	0.156	[0.00, 0.00]
	ENN_RA_MediumAndLarge	0.001	7.18E-06	0.149	[0.00, 0.01]
	ENN_MD_MediumAndLarge	0.006	4.55E-04	0.148	[-0.04, 0.05]
	ENN_MD_Large	-0.001	5.34E-05	0.143	[-0.02, 0.01]
	ENN_RA_Large	0.000	3.32E-06	0.141	[0.00, 0.00]
Juvenile whiptails N lizards = 545 Lowest AICc = 426.32 N models = 121	ENN_MD_AllSizes*	0.870	6.91E-02	1.000	[0.35, 1.39]
	log10(DistanceToRoad)*	-0.360	1.06E-02	1.000	[-0.56, -0.15]
	Road: NM-9*	-0.478	1.50E-02	1.000	[-0.72, -0.23]
	Year: 2011*	-0.789	1.91E-02	1.000	[-1.06, -0.51]
	Year: 2010*	-0.251	1.30E-02	1.000	[-0.48, -0.02]
	(Intercept)*	2.118	3.74E-01	1.000	[0.90, 3.34]
	ENN_RA_Large*	-0.047	3.38E-04	0.984	[-0.08, -0.01]
	ENN_RA_AllSizes	0.061	4.68E-03	0.574	[-0.08, 0.20]
	ENN_RA_MediumAndLarge	-0.007	1.88E-04	0.274	[-0.03, 0.02]
	PercentageLandscape_AllSizes	0.008	2.31E-04	0.264	[-0.02, 0.04]
	ENN_MD_MediumAndLarge	0.045	7.60E-03	0.250	[-0.13, 0.22]
	PatchDensity_AllSizes	0.001	8.24E-06	0.245	[0.00, 0.01]
	PercentageLandscape_Large	-0.007	1.79E-04	0.245	[-0.03, 0.02]
	PatchDensity_Large	-0.009	3.33E-04	0.233	[-0.05, 0.03]
	PatchDensity_MediumAndLarge	0.002	4.73E-05	0.183	[-0.01, 0.02]
	ENN_MD_Large	0.001	8.02E-05	0.123	[-0.02, 0.02]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.5. Model coefficients for all explanatory variables from the generalized linear models with the proportion of side-blotched lizards captured only once as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Adult side-blotched lizards</b> N lizards = 691 Lowest AICc = -102.2 N models = 153	Year: 2011*	0.118	1.15E-03	1.000	[0.05, 0.19]
	Year: 2010*	0.071	1.07E-03	1.000	[0.01, 0.14]
	(Intercept)*	0.694	2.29E-02	1.000	[0.39, 1.00]
	ENN_MD_MediumAndLarge*	-0.079	8.50E-04	0.988	[-0.14, -0.02]
	log10(DistanceToRoad)	0.053	9.01E-04	0.895	[-0.01, 0.11]
	PatchDensity_MediumAndLarge	-0.012	4.72E-05	0.845	[-0.03, 0.00]
	PercentageLandscape_AllSizes	0.013	9.70E-05	0.794	[-0.01, 0.03]
	PercentageLandscape_Large	-0.012	1.09E-04	0.702	[-0.03, 0.01]
	PatchDensity_AllSizes	0.001	1.88E-06	0.578	[0.00, 0.00]
	ENN_MD_Large	-0.009	1.43E-04	0.481	[-0.03, 0.01]
	PatchDensity_Large	-0.004	6.60E-05	0.287	[-0.02, 0.01]
	ENN_RA_Large	-0.001	2.21E-06	0.209	[0.00, 0.00]
	Road: NM-9	-0.006	1.88E-04	0.204	[-0.03, 0.02]
	ENN_MD_AllSizes	0.004	1.73E-04	0.148	[-0.02, 0.03]
	ENN_RA_MediumAndLarge	0.001	2.16E-06	0.133	[0.00, 0.00]
	ENN_RA_AllSizes	0.001	4.30E-06	0.117	[0.00, 0.00]
<b>Juvenile side-blotched lizards</b> N lizards = 250 Lowest AICc = 16.70 N models = 229	Year: 2011*	0.203	4.71E-03	1.000	[-0.34, -0.07]
	Year: 2010*	0.181	4.52E-03	1.000	[-0.32, -0.05]
	(Intercept)*	0.294	3.11E-02	1.000	[0.35, 1.06]
	PatchDensity_AllSizes	0.003	5.97E-06	0.772	[-0.01, 0.00]
	PercentageLandscape_Large	-0.007	1.15E-04	0.363	[-0.01, 0.03]
	PercentageLandscape_AllSizes	0.006	1.03E-04	0.350	[-0.03, 0.01]
	ENN_MD_Large	0.008	2.03E-04	0.348	[-0.04, 0.02]
	ENN_RA_Large	0.002	1.12E-05	0.220	[-0.01, 0.01]
	ENN_MD_AllSizes	-0.016	1.34E-03	0.192	[-0.06, 0.09]
	ENN_RA_AllSizes	0.003	4.36E-05	0.156	[-0.02, 0.01]
	ENN_MD_MediumAndLarge	-0.004	1.17E-04	0.145	[-0.02, 0.03]
	PatchDensity_MediumAndLarge	-0.001	3.39E-06	0.141	[0.00, 0.00]
	PatchDensity_Large	0.000	5.05E-06	0.130	[0.00, 0.00]
	Road: NM-9	-0.002	1.08E-04	0.128	[-0.02, 0.02]
	ENN_RA_MediumAndLarge	0.000	1.97E-06	0.109	[0.00, 0.00]
	log10(DistanceToRoad)	0.000	2.79E-05	0.102	[-0.01, 0.01]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.6. Model coefficients for all explanatory variables from the generalized linear models with the proportion of marbled whiptails captured only once as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
Adult whiptails N lizards = 448 Lowest AICc = -59.67 N models = 619	Year: 2011*	0.215	1.94E-03	1.000	[0.13, 0.30]
	Year: 2010*	0.152	1.82E-03	1.000	[0.07, 0.24]
	(Intercept)	0.238	3.65E-02	1.000	[-0.14, 0.62]
	PercentageLandscape_AllSizes	0.011	8.48E-05	0.778	[-0.01, 0.03]
	Road: NM-9	-0.052	2.56E-03	0.663	[-0.15, 0.05]
	PercentageLandscape_Large	-0.005	6.20E-05	0.457	[-0.02, 0.01]
	ENN_RA_MediumAndLarge	0.004	4.05E-05	0.437	[-0.01, 0.02]
	PatchDensity_Large	-0.006	1.07E-04	0.366	[-0.03, 0.01]
	ENN_MD_MediumAndLarge	-0.012	6.39E-04	0.237	[-0.06, 0.04]
	ENN_MD_AllSizes	-0.014	1.15E-03	0.202	[-0.08, 0.05]
	PatchDensity_AllSizes	0.000	1.45E-07	0.184	[0.00, 0.00]
	PatchDensity_MediumAndLarge	0.000	4.50E-06	0.183	[0.00, 0.00]
	log10(DistanceToRoad)	0.004	1.17E-04	0.181	[-0.02, 0.03]
	ENN_MD_Large	0.002	3.89E-05	0.166	[-0.01, 0.01]
	ENN_RA_Large	-0.001	2.50E-06	0.148	[0.00, 0.00]
	ENN_RA_AllSizes	0.000	9.19E-06	0.140	[-0.01, 0.01]
Juvenile whiptails N lizards = 289 Lowest AICc = -15.41 N models >1000	(Intercept)*	0.784	3.76E-02	1.000	[0.40, 1.17]
	Year: 2011	0.095	4.43E-03	0.847	[-0.04, 0.23]
	Year: 2010	0.111	4.38E-03	0.847	[-0.02, 0.24]
	ENN_MD_Large	-0.038	1.14E-03	0.717	[-0.11, 0.03]
	log10(DistanceToRoad)	0.045	2.27E-03	0.618	[-0.05, 0.14]
	PercentageLandscape_Large	-0.003	2.17E-05	0.362	[-0.01, 0.01]
	PatchDensity_AllSizes	0.001	1.08E-06	0.304	[0.00, 0.00]
	ENN_RA_Large	0.003	2.79E-05	0.292	[-0.01, 0.01]
	PercentageLandscape_AllSizes	-0.001	6.51E-06	0.228	[-0.01, 0.00]
	ENN_RA_MediumAndLarge	-0.002	1.34E-05	0.218	[-0.01, 0.01]
	ENN_MD_AllSizes	-0.022	2.27E-03	0.212	[-0.12, 0.07]
	Road: NM-9	0.007	3.06E-04	0.181	[-0.03, 0.04]
	ENN_MD_MediumAndLarge	-0.008	3.94E-04	0.167	[-0.05, 0.03]
	PatchDensity_MediumAndLarge	0.001	5.43E-06	0.151	[0.00, 0.01]
	ENN_RA_AllSizes	-0.002	2.63E-05	0.149	[-0.01, 0.01]
	PatchDensity_Large	-0.001	1.01E-05	0.135	[-0.01, 0.01]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.7. Model coefficients for all explanatory variables from the generalized linear models with the proportion of adult side-blotched lizards and marbled whiptails with persistence longer than average as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Adult side-blotched lizards</b> N lizards = 242 Lowest AICc = -120.86 N models = 642	(Intercept)	0.178	8.13E-03	1.000	[0.00, 0.36]
	ENN_MD_Large*	0.028	9.30E-05	0.995	[0.01, 0.05]
	PatchDensity_AllSizes	0.000	4.71E-07	0.392	[0.00, 0.00]
	ENN_RA_MediumAndLarge	-0.003	1.95E-05	0.341	[-0.01, 0.01]
	PatchDensity_Large	0.003	2.99E-05	0.326	[-0.01, 0.01]
	PercentageLandscape_AllSizes	-0.002	6.77E-06	0.313	[-0.01, 0.00]
	Road: NM-9	-0.009	2.58E-04	0.284	[-0.04, 0.02]
	ENN_MD_MediumAndLarge	0.007	1.76E-04	0.279	[-0.02, 0.03]
	PercentageLandscape_Large	0.001	5.17E-06	0.257	[0.00, 0.01]
	PatchDensity_MediumAndLarge	-0.001	4.68E-06	0.250	[-0.01, 0.00]
	ENN_RA_Large	0.000	1.05E-06	0.181	[0.00, 0.00]
	ENN_RA_AllSizes	0.001	7.58E-06	0.167	[0.00, 0.01]
	log10(DistanceToRoad)	-0.002	3.64E-05	0.167	[-0.01, 0.01]
	ENN_MD_AllSizes	0.000	4.95E-05	0.148	[-0.01, 0.01]
	Year: 2011	0.001	8.36E-06	0.041	[0.00, 0.01]
	Year: 2010	0.000	1.80E-06	0.041	[0.00, 0.00]
<b>Adult whiptails</b> N lizards = 357 Lowest AICc = -69.12 N models = 303	Year: 2011*	-0.147	1.72E-03	1.000	[-0.23, -0.06]
	Year: 2010*	-0.239	1.61E-03	1.000	[-0.32, -0.16]
	(Intercept)*	0.581	2.61E-02	1.000	[0.26, 0.90]
	PercentageLandscape_AllSizes	-0.024	1.62E-04	0.930	[-0.05, 0.00]
	PercentageLandscape_Large	0.022	1.62E-04	0.897	[0.00, 0.05]
	PatchDensity_Large	0.011	1.68E-04	0.560	[-0.01, 0.04]
	PatchDensity_AllSizes	0.001	2.94E-06	0.514	[0.00, 0.00]
	PatchDensity_MediumAndLarge	0.002	2.88E-05	0.308	[-0.01, 0.01]
	ENN_MD_MediumAndLarge	-0.008	3.58E-04	0.194	[-0.05, 0.03]
	Road: NM-9	-0.006	1.80E-04	0.187	[-0.03, 0.02]
	ENN_MD_AllSizes	0.009	8.12E-04	0.183	[-0.05, 0.07]
	ENN_RA_MediumAndLarge	-0.001	4.51E-06	0.179	[-0.01, 0.00]
	ENN_RA_AllSizes	-0.002	1.91E-05	0.159	[-0.01, 0.01]
	log10(DistanceToRoad)	-0.003	6.39E-05	0.156	[-0.02, 0.01]
	ENN_MD_Large	0.001	9.65E-06	0.133	[-0.01, 0.01]
	ENN_RA_Large	0.000	7.11E-07	0.127	[0.00, 0.00]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.8. Model coefficients for all explanatory variables from the generalized linear models with the proportion of hatchling or juvenile side-blotched lizards recaptured at larger sizes as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
Hatchling side-blotched lizards N lizards = 453 Lowest AICc = -15.36 N models = 423	(Intercept)	0.321	2.56E-02	1.000	[0.00, 0.64]
	Road: NM-9*	0.131	3.49E-03	0.975	[0.01, 0.25]
	PercentageLandscape_Large	-0.010	4.77E-05	0.822	[-0.02, 0.00]
	PatchDensity_Large	0.011	2.35E-04	0.479	[-0.02, 0.04]
	Year Marked: 2011	-0.025	1.80E-03	0.299	[-0.11, 0.06]
	Year Marked: 2010	-0.029	2.20E-03	0.299	[-0.12, 0.06]
	PercentageLandscape_AllSizes	0.000	6.10E-06	0.248	[0.00, 0.00]
	ENN_MD_Large	-0.004	6.59E-05	0.238	[-0.02, 0.01]
	PatchDensity_MediumAndLarge	-0.001	1.10E-05	0.237	[-0.01, 0.01]
	ENN_MD_AllSizes	-0.017	1.21E-03	0.226	[-0.09, 0.05]
	ENN_RA_Large	-0.001	3.47E-06	0.170	[0.00, 0.00]
	ENN_RA_AllSizes	-0.002	2.66E-05	0.157	[-0.01, 0.01]
	PatchDensity_AllSizes	0.000	1.54E-07	0.145	[0.00, 0.00]
	ENN_MD_MediumAndLarge	0.000	3.59E-05	0.131	[-0.01, 0.01]
	log10(DistanceToRoad)	0.000	2.17E-05	0.106	[-0.01, 0.01]
	ENN_RA_MediumAndLarge	0.000	1.29E-06	0.105	[0.00, 0.00]
Juvenile side-blotched lizards N lizards = 412 Lowest AICc = 10.70 N models = 589	Period: Hatched 2011*	-0.222	4.39E-03	1.000	[-0.35, -0.09]
	Period: Hatched 2010*	-0.168	4.21E-03	1.000	[-0.30, -0.04]
	(Intercept)*	0.527	3.23E-02	1.000	[0.17, 0.88]
	ENN_RA_Large	-0.006	5.96E-05	0.561	[-0.02, 0.01]
	PatchDensity_AllSizes	-0.001	3.39E-06	0.513	[-0.01, 0.00]
	PercentageLandscape_Large	0.004	4.80E-05	0.339	[-0.01, 0.02]
	PercentageLandscape_AllSizes	-0.003	3.99E-05	0.281	[-0.02, 0.01]
	PatchDensity_MediumAndLarge	-0.002	1.68E-05	0.276	[-0.01, 0.01]
	ENN_MD_MediumAndLarge	0.010	4.40E-04	0.254	[-0.03, 0.05]
	ENN_MD_Large	-0.004	7.42E-05	0.227	[-0.02, 0.01]
	PatchDensity_Large	-0.001	1.30E-05	0.169	[-0.01, 0.01]
	ENN_RA_AllSizes	0.002	3.19E-05	0.159	[-0.01, 0.01]
	ENN_MD_AllSizes	0.006	4.36E-04	0.158	[-0.04, 0.05]
	ENN_RA_MediumAndLarge	0.001	5.35E-06	0.131	[0.00, 0.01]
	log10(DistanceToRoad)	-0.001	4.22E-05	0.122	[-0.01, 0.01]
	Road: NM-9	0.000	5.56E-05	0.120	[-0.02, 0.01]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.9. Model coefficients for all explanatory variables from the generalized linear models with the proportion of hatchling marbled whiptails recaptured as adults as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Juvenile whiptails</b> N lizards = 472 Lowest AICc = -26.58 N models = 471	Period: Hatched 2010*	0.153	2.40E-03	1.000	[0.06, 0.25]
	(Intercept)	0.266	4.31E-02	1.000	[-0.15, 0.68]
	PercentageLandscape_Large	-0.006	4.47E-05	0.593	[-0.02, 0.01]
	ENN_MD_Large	0.014	4.89E-04	0.364	[-0.03, 0.06]
	PercentageLandscape_AllSizes	-0.002	2.21E-05	0.329	[-0.01, 0.01]
	ENN_MD_MediumAndLarge	0.026	2.07E-03	0.296	[-0.07, 0.12]
	log10(DistanceToRoad)	0.014	6.57E-04	0.275	[-0.04, 0.07]
	Road: NM-9	0.009	4.17E-04	0.191	[-0.03, 0.05]
	ENN_MD_AllSizes	0.016	1.43E-03	0.178	[-0.06, 0.09]
	ENN_RA_AllSizes	-0.002	2.71E-05	0.150	[-0.01, 0.01]
	PatchDensity_MediumAndLarge	-0.001	5.02E-06	0.148	[-0.01, 0.00]
	PatchDensity_AllSizes	0.000	1.54E-07	0.147	[0.00, 0.00]
	ENN_RA_MediumAndLarge	0.000	2.99E-06	0.135	[0.00, 0.00]
	PatchDensity_Large	-0.001	7.21E-06	0.124	[-0.01, 0.00]
	ENN_RA_Large	0.000	1.20E-06	0.119	[0.00, 0.00]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.10. Model coefficients for all explanatory variables from the generalized linear models with the finite annual survival of adult side-blotched lizards and marbled whiptails as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Adult side-blotched lizards</b> N lizards 2009 = 572 N lizards 2010 = 346 Lowest AICc = -122.5 N models = 477	Period: 2010-2011*	0.068	4.46E-04	1.000	[0.03, 0.11]
	(Intercept)	0.093	4.44E-03	1.000	[-0.04, 0.23]
	ENN_RA_Large	-0.003	9.27E-06	0.580	[-0.01, 0.00]
	log10(DistanceToRoad)	0.010	2.22E-04	0.389	[-0.02, 0.04]
	Road: NM-9	-0.011	3.16E-04	0.365	[-0.05, 0.02]
	PatchDensity_AllSizes	0.000	2.25E-07	0.327	[0.00, 0.00]
	ENN_MD_AllSizes	0.006	1.68E-04	0.201	[-0.02, 0.03]
	PercentageLandscape_Large	0.000	5.57E-07	0.191	[0.00, 0.00]
	PatchDensity_Large	0.001	2.62E-06	0.178	[0.00, 0.00]
	PercentageLandscape_AllSizes	0.000	2.20E-07	0.151	[0.00, 0.00]
	ENN_MD_MediumAndLarge	0.001	1.20E-05	0.139	[-0.01, 0.01]
	ENN_MD_Large	0.000	1.92E-06	0.127	[0.00, 0.00]
	PatchDensity_MediumAndLarge	0.000	2.21E-07	0.124	[0.00, 0.00]
	ENN_RA_AllSizes	0.000	1.27E-06	0.121	[0.00, 0.00]
	ENN_RA_MediumAndLarge	0.000	3.25E-07	0.106	[0.00, 0.00]
<b>Adult whiptails</b> N lizards 2009 = 201 N lizards 2010 = 444 Lowest AICc = -37.70 N models = 283	Period: 2010-2011*	-0.164	1.98E-03	1.000	[-0.25, -0.08]
	(Intercept)*	0.394	3.32E-02	1.000	[0.03, 0.76]
	Road: NM-9	-0.078	4.06E-03	0.758	[-0.21, 0.05]
	ENN_MD_MediumAndLarge	0.090	5.55E-03	0.749	[-0.06, 0.24]
	ENN_MD_Large	-0.020	7.41E-04	0.460	[-0.07, 0.03]
	ENN_RA_MediumAndLarge	-0.002	1.90E-05	0.248	[-0.01, 0.01]
	ENN_RA_AllSizes	0.004	7.32E-05	0.215	[-0.01, 0.02]
	log10(DistanceToRoad)	0.005	1.27E-04	0.161	[-0.02, 0.03]
	PatchDensityPer1000m_Large	0.002	1.86E-05	0.155	[-0.01, 0.01]
	ENN_MD_AllSizes	0.009	6.93E-04	0.149	[-0.04, 0.06]
	PatchDensityPer1000m_MediumAndLarge	0.001	6.61E-06	0.149	[0.00, 0.01]
	PercentageLandscape_AllSizes	0.000	1.01E-06	0.124	[0.00, 0.00]
	PercentageLandscape_Large	0.000	1.05E-06	0.119	[0.00, 0.00]
	PatchDensityPer1000m_AllSizes	0.000	5.33E-08	0.111	[0.00, 0.00]
	ENN_RA_Large	0.000	6.97E-07	0.099	[0.00, 0.00]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.

Table S2.11. Model coefficients for all explanatory variables from the generalized linear models with the scaled mass index of male and female side-blotched lizards as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
<b>Male side-blotched lizards</b>	log10(DistanceToRoad)*	0.048	2.19E-04	1.000	[0.02, 0.08]
N lizards = 1110	Year: 2011*	0.049	3.28E-04	1.000	[0.01, 0.09]
Lowest AICc = -83.31	Year: 2010*	0.101	3.47E-04	1.000	[0.06, 0.14]
N models = 170	Road: NM-9*	-0.146	4.14E-04	1.000	[-0.19, -0.11]
	(Intercept)*	2.063	7.31E-03	1.000	[1.9, 2.23]
	PatchDensity_MediumAndLarge*	0.013	1.80E-05	0.998	[0.00, 0.02]
	PatchDensity_Large*	-0.025	5.98E-05	0.998	[-0.04, -0.01]
	PercentageLandscape_Large	0.011	1.91E-05	0.998	[0.00, 0.02]
	ENN_RA_MediumAndLarge	-0.005	1.90E-05	0.677	[-0.01, 0.00]
	ENN_RA_AllSizes	0.006	6.67E-05	0.514	[-0.01, 0.02]
	PercentageLandscape_AllSizes	-0.003	1.45E-05	0.501	[-0.01, 0.00]
	ENN_MD_AllSizes	0.006	2.18E-04	0.288	[-0.02, 0.03]
	PatchDensity_AllSizes	0.000	7.53E-08	0.288	[0.00, 0.00]
	ENN_MD_MediumAndLarge	0.003	4.71E-05	0.281	[-0.01, 0.02]
	ENN_RA_Large	0.000	9.46E-07	0.279	[0.00, 0.00]
	ENN_MD_Large	0.001	4.31E-06	0.251	[0.00, 0.00]
<b>Female side-blotched lizards</b>	ENN_RA_AllSizes*	0.021	4.45E-05	1.000	[0.01, 0.03]
N lizards = 992	Year: 2011	0.011	2.27E-04	1.000	[-0.02, 0.04]
Lowest AICc = -393.21	Year: 2010*	0.058	2.39E-04	1.000	[0.03, 0.09]
N models = 369	(Intercept)*	1.649	2.71E-03	1.000	[1.55, 1.75]
	ENN_RA_MediumAndLarge*	-0.009	1.05E-05	0.999	[-0.02, 0]
	Road: NM-9*	-0.040	2.32E-04	0.977	[-0.07, -0.01]
	ENN_MD_AllSizes	-0.023	7.39E-04	0.581	[-0.08, 0.03]
	ENN_RA_Large	0.001	2.83E-06	0.481	[0.00, 0.00]
	ENN_MD_Large	0.001	8.25E-06	0.320	[0.00, 0.01]
	ENN_MD_MediumAndLarge	0.002	2.84E-05	0.284	[-0.01, 0.01]
	PercentageLandscape_AllSizes	0.000	1.05E-06	0.281	[0.00, 0.00]
	PercentageLandscape_Large	0.000	3.64E-07	0.236	[0.00, 0.00]
	PatchDensity_AllSizes	0.000	2.35E-08	0.230	[0.00, 0.00]
	PatchDensity_MediumAndLarge	0.000	2.10E-07	0.209	[0.00, 0.00]
	PatchDensity_Large	0.000	5.23E-07	0.206	[0.00, 0.00]
	log10(DistanceToRoad)	0.000	7.29E-06	0.202	[-0.01, 0.01]

\* Variables with importance >0.8 and 95% CI that do not overlap 0.



Table S2.12. Model coefficients for all explanatory variables from the generalized linear models with the scaled mass index of male and female marbled whiptails as the dependent variable. The number of individuals, lowest AICc score, and the number of models within 6 AICc of the lowest score are reported in the table. The importance is the sum of the Akaike weights per model summed over all models in which that variable occurs.

	Explanatory variable	Estimate	Variance	Importance	95% CI
Male marbled whiptails N lizards = 1567 Lowest AICc = 5483.18 N models = 231	ENN_RA_Large*	0.038	1.71E-04	1.000	[0.01, 0.06]
	Year: 2011*	-0.531	9.96E-03	1.000	[-0.73, -0.33]
	Year: 2010	0.148	1.00E-02	1.000	[-0.05, 0.34]
	Road: NM-9*	-0.497	7.71E-03	1.000	[-0.67, -0.32]
	(Intercept)*	12.694	1.29E-01	1.000	[11.99, 13.40]
	log10(DistanceToRoad)	0.134	5.34E-03	0.907	[-0.01, 0.28]
	PercentageLandscape_Large	0.025	1.99E-04	0.850	[0.00, 0.05]
	PatchDensity_MediumAndLarge	-0.009	1.82E-04	0.422	[-0.04, 0.02]
	ENN_MD_MediumAndLarge	0.045	6.08E-03	0.372	[-0.11, 0.20]
	PercentageLandscape_AllSizes	0.003	1.04E-04	0.331	[-0.02, 0.02]
	ENN_MD_Large	-0.009	4.30E-04	0.253	[-0.05, 0.03]
	PatchDensity_AllSizes	0.000	5.13E-07	0.204	[0.00, 0.00]
	PatchDensity_Large	-0.001	4.88E-05	0.204	[-0.01, 0.01]
	ENN_RA_AllSizes	-0.002	7.28E-05	0.202	[-0.02, 0.02]
	ENN_MD_AllSizes	0.008	1.95E-03	0.199	[-0.08, 0.09]
	ENN_RA_MediumAndLarge	-0.001	1.03E-05	0.191	[-0.01, 0.01]
Female marbled whiptails N lizards = 999 Lowest AICCc = 3133.15 N models >1000	Year: 2011*	-0.262	9.71E-03	1.000	[-0.46, -0.07]
	Year: 2010	0.190	9.63E-03	1.000	[0.00, 0.38]
	Road: NM-9*	-0.394	7.69E-03	1.000	[-0.57, -0.22]
	(Intercept)*	10.083	1.23E-01	1.000	[9.40, 10.77]
	PatchDensity_AllSizes	-0.003	1.11E-05	0.541	[-0.01, 0.00]
	log10(DistanceToRoad)	0.047	4.12E-03	0.484	[-0.08, 0.17]
	PatchDensity_Large	-0.012	4.36E-04	0.372	[-0.05, 0.03]
	ENN_RA_MediumAndLarge	0.006	1.28E-04	0.336	[-0.02, 0.03]
	ENN_MD_Large	-0.017	1.02E-03	0.329	[-0.08, 0.05]
	PatchDensity_MediumAndLarge	-0.006	1.05E-04	0.324	[-0.03, 0.01]
	PercentageLandscape_Large	0.003	4.13E-05	0.312	[-0.01, 0.02]
	ENN_RA_AllSizes	-0.014	7.36E-04	0.295	[-0.07, 0.04]
	ENN_RA_Large	0.003	3.80E-05	0.252	[-0.01, 0.01]
	PercentageLandscape_AllSizes	0.000	1.06E-05	0.231	[-0.01, 0.01]
	ENN_MD_AllSizes	-0.001	1.92E-03	0.200	[-0.09, 0.09]
	ENN_MD_MediumAndLarge	0.010	1.06E-03	0.195	[-0.05, 0.07]

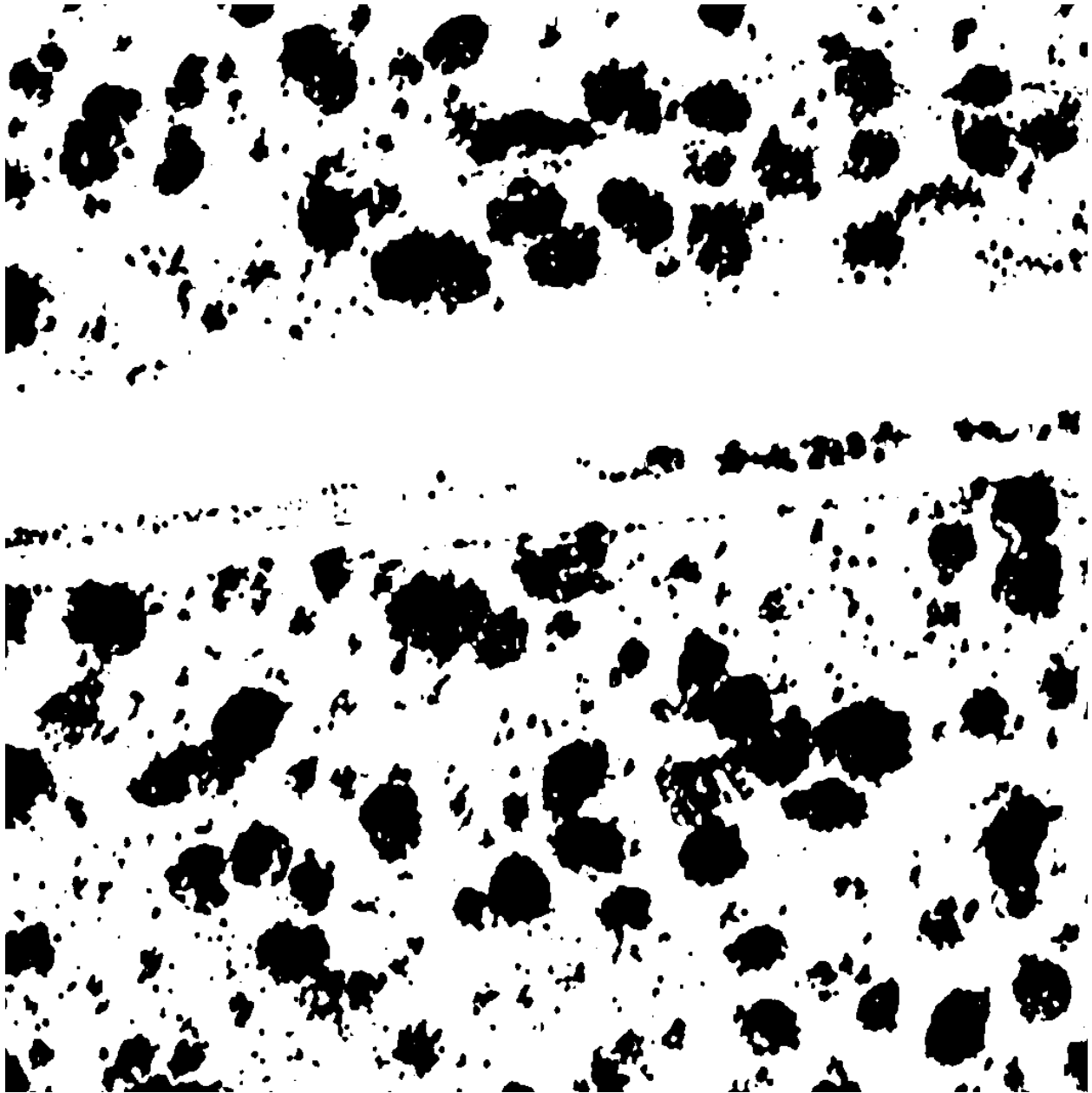
\* Variables with importance >0.8 and 95% CI that do not overlap 0.

## **Supplementary 2.1 – Generation of habitat structure shapefiles**

To examine the effects of habitat structure on lizard abundance and survival, we quantified the number and area of the plants within a 90 m buffer around the trapping arrays for each site. We used the Arc2Earth plugin (Arc2Earth, Somerset, NJ, USA) in ArcMap 10.2 (ESRI, Redlands, CA, USA) to save high-resolution Google Earth imagery (map data from April 2013 from Google and the Mexican National Institute of Statistics and Geography [INEGI]) in ArcMap 10.2 (ESRI, Redlands, CA, USA) as tiff files (A). We converted the color files into black and white using the automatic thresholding function in ImageJ version 1.46 (Rasband 1997-2014)(B). We used the feature extraction function in ENVI Zoom version 4.4 (Exelis Visual Information Solutions, Boulder, CO, USA) to create shapefiles of vegetation objects. Shapefiles were manually edited in ArcMap to remove known human artifacts (i.e., shadows from powerlines) and to match objects in the shapefile to the actual plants visible in Google Earth imagery. For example, shadows often connected two obviously separate plants in the Google Earth imagery, and those two plants were classified as a single object in ENVI Zoom. Manual editing split the object into the two separate plants (C). The objects  $<0.5 \text{ m}^2$  were removed, and the shapefile was clipped by the 10 m buffer around each trapping group for use in the side-blotched lizard analyses (D) and the 25 m buffer for the whiptail analyses (E). The resulting shapefiles were converted to rasters for habitat structure analyses in FRAGSTATS (McGarigal et al. 2012).

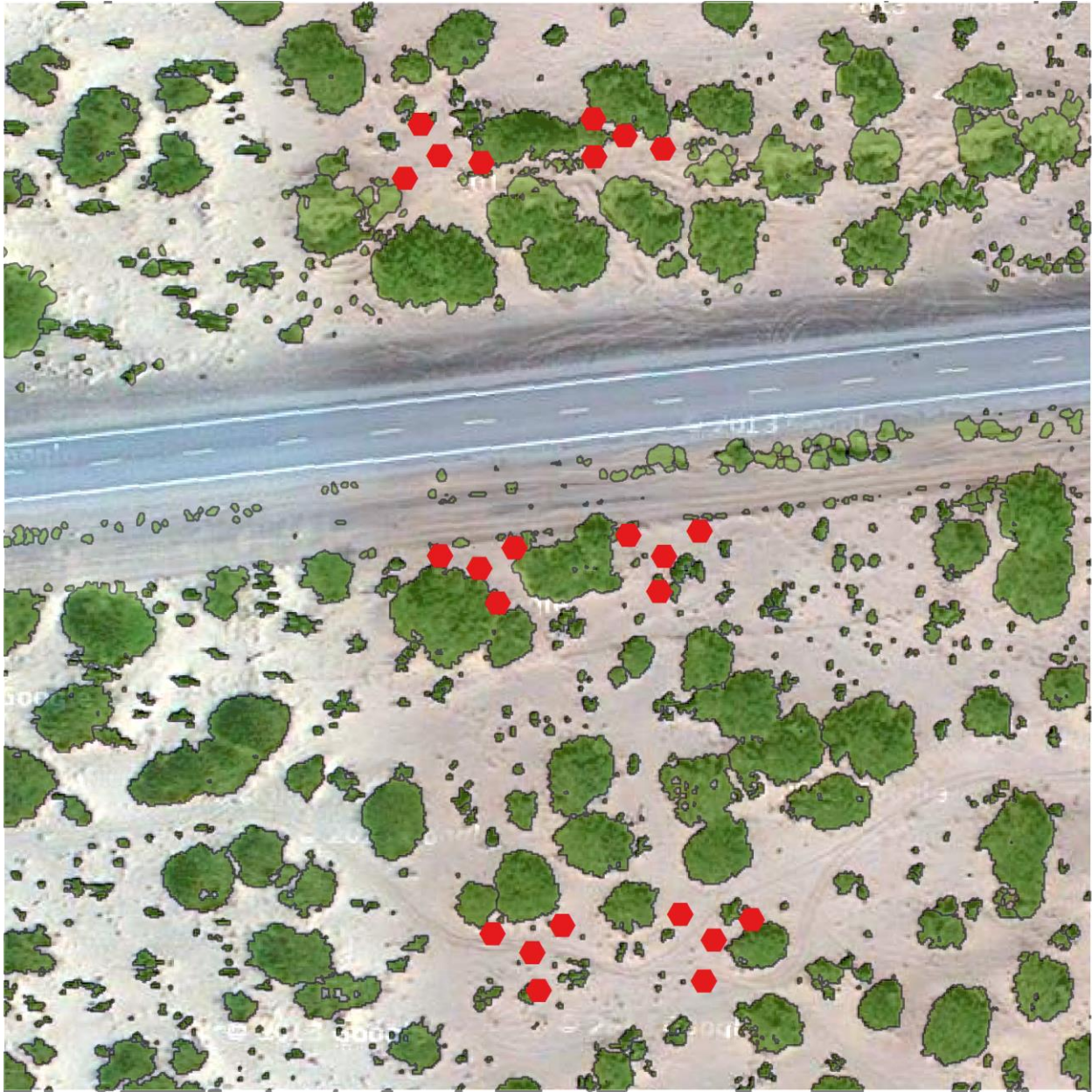


A) Google Earth image of part of site 8 on NM-9. The area in the figure in  $135\text{ m} \times 135\text{ m}$ .



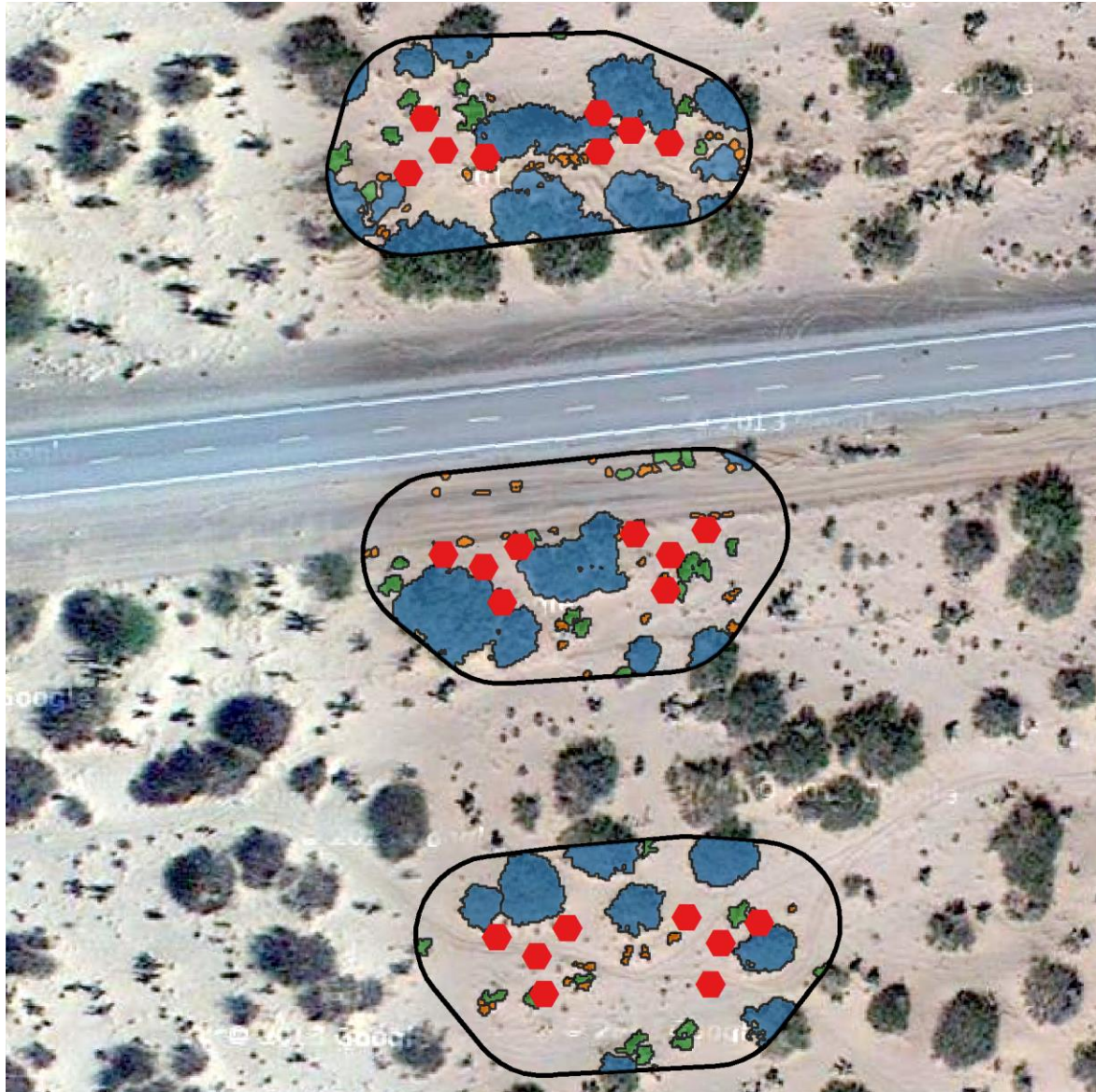
B) Image converted to black and white in ImageJ. These images were used in ENVI to generate shapefiles of the plants.





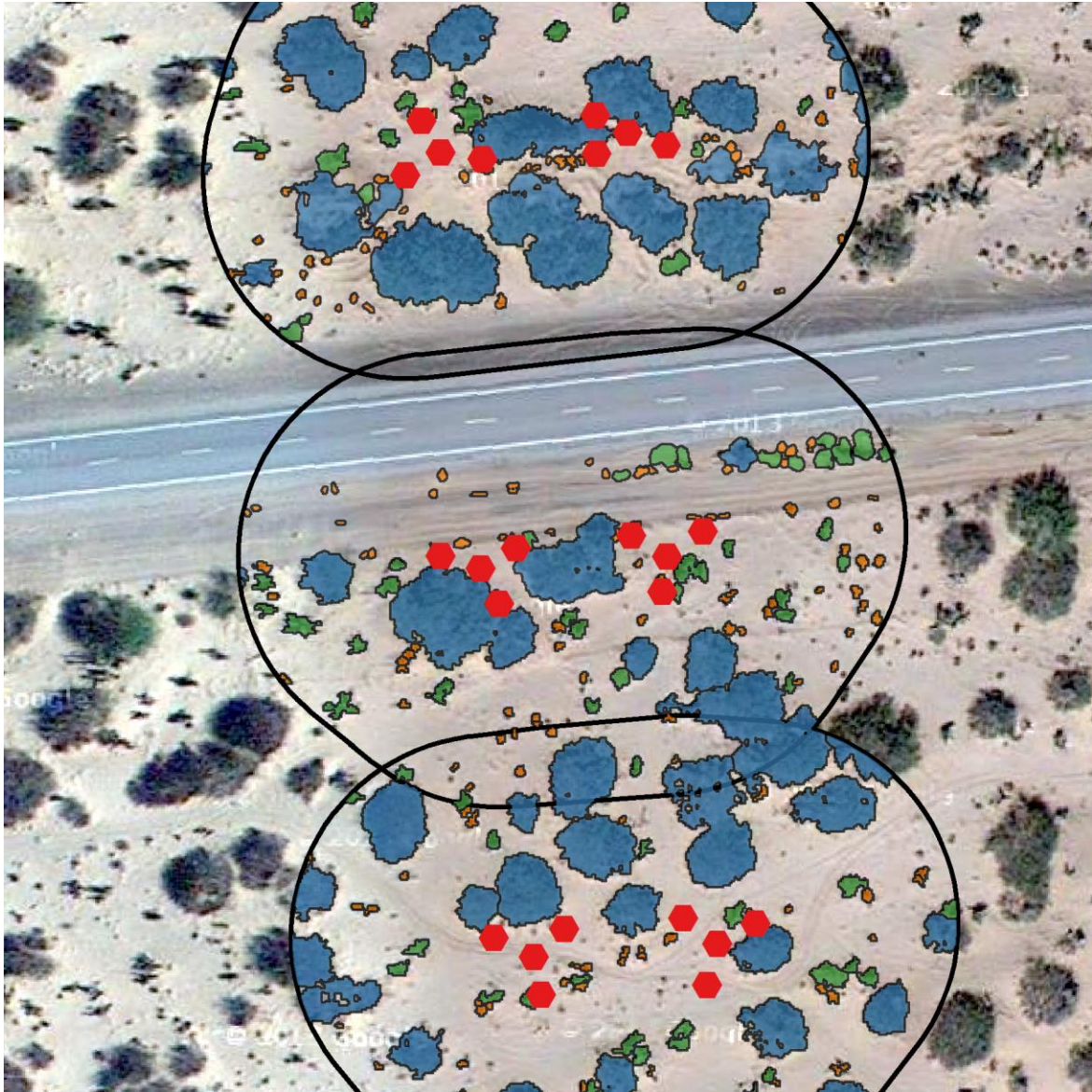
C) The shapefile from ENVI. Objects were manually edited to remove artifacts and split connected plants into individual objects. The location of the traps is marked with red hexagons.





D) The plants within the 10 m buffer of the different trapping groups. The blue are plants with areas  $\geq 10 \text{ m}^2$ , the green for areas  $2\text{--}10 \text{ m}^2$ , and the orange for plants  $0.5\text{--}2 \text{ m}^2$ .

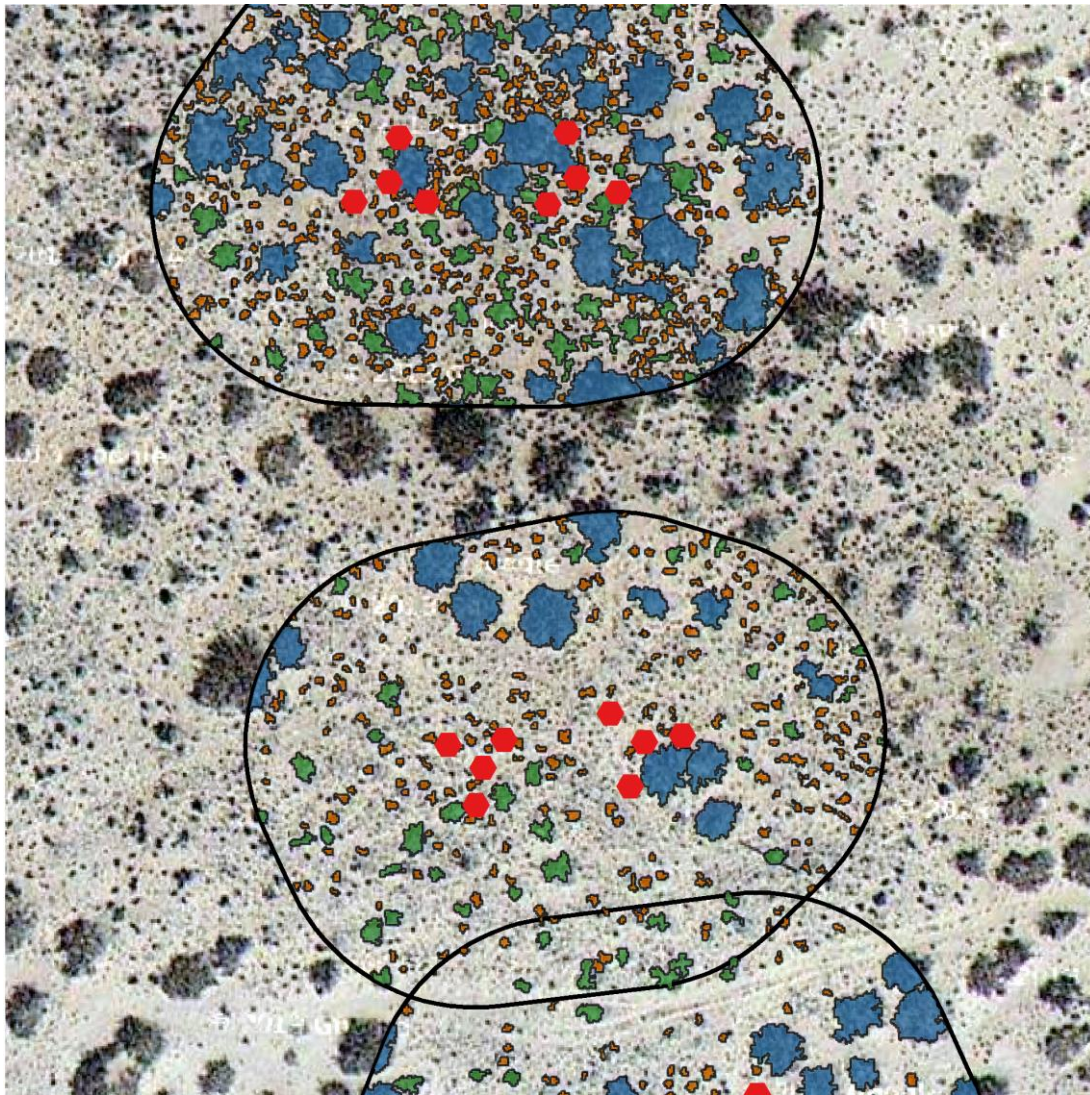




E) Plants within the 25 m buffer around each trapping group. The colors are the same as in D.



## Supplementary 2.2 – Examples of sites with varying levels of plant cover



Site 4 north, far, had the highest percentage of area within the 25 m covered by plants of all sizes (39.5%), and the second highest density of plants of all sizes (70.4 per 1000 m<sup>2</sup>). However, it was only 8<sup>th</sup> for percent cover of only large plants (21.0%) and 3<sup>rd</sup> for density of large plants (9.5 per 1000 m<sup>2</sup>). The high density of smaller plants (orange) was unusual across all sites. In contrast, site 8 north, close (the trapping group at the top of the figures in supplementary data 2.1), had the 5<sup>th</sup> highest percent cover of plants of all sizes (30.8%) and had the 26<sup>th</sup> highest density of plants of all sizes (17.9 per 1000m<sup>2</sup>). It had the 4<sup>th</sup> highest percent cover for only large plants (26.9%) and the 9<sup>th</sup> highest density of large plants (7.0 per 1000 m<sup>2</sup>). These two locations highlight some of the differences between locations with few, mostly large plants and those with lots of smaller plants.



## **Chapter 3: The Impact of Roads on the Population Genetic Structure of Two Species of Lizards in Southern New Mexico**

### **3.1 ABSTRACT**

Roads fragment habitats and increase mortality rates of wildlife. Both effects can create small isolated populations that are at risk for decreased genetic diversity due to genetic drift and inbreeding. More mobile species are expected to encounter roads more often than sedentary species, and should experience increased vehicle-caused mortality. The effects of roads on the population genetic structure of reptiles has not been well studied, particularly for lizards. We used microsatellite markers to assess changes in the genetic diversity and population structure of two species of lizards in southern New Mexico. We compared the road impacts on an active forager (marbled whiptails, *Aspidoscelis marmorata*) and a sit-and-wait predator (side-blotched lizards, *Uta stansburiana*) to test the hypothesis that more mobile animals will experience more negative impacts. We also compared the effects of two roads of different size and traffic volumes to test the hypothesis that larger roads with higher traffic volumes have greater impacts on wildlife populations. Neither road had measurable impacts on the genetic diversity nor the population genetic structure for either lizard. Although the smaller road was a partial barrier to both species, we did detect several successful crossing events during the study. This connectivity can maintain genetic diversity and prevent increased population differentiation. Although we did not detect any individual lizards successfully crossing the larger road, we detected only a few movements across unfragmented habitat of a long enough distance to successfully cross the larger road. Despite the possibility that enough lizards cross the larger road to maintain genetic connectivity, a more likely explanation for the lack of detectable effects of the larger road is that both species of lizards have large and mostly continuous populations in the study region. Other than the two study roads and a railroad, there is little disturbance of the desert shrubland in this region. The effective population sizes of whiptail lizards were >1000 per population, and >350 per population for the side-blotched lizards. Detectable genetic changes are slower to arise when effective population sizes are large. The larger road has existed for >50 generations of side-blotched lizards and >25–50 generations

of whiptails, but this timeframe apparently is insufficient for detectable changes to accumulate. Additionally, there were no barriers between the populations near the road and those away from the road, likely allowing sufficient gene flow from the unaffected populations in the hinterlands to counteract any potential changes in the near-road populations. Our results indicate that even for animals with short generation times, large populations can make the detection of barrier effects from roads difficult. This is promising for conservation efforts, as it suggests large population sizes by themselves reduce negative road effects in some instances. Limited funds for mitigating negative road impacts could thus be directed to where greater levels of road density and habitat fragmentation have created the small isolated populations that are expected to suffer from decreased genetic diversity and lower probabilities of population persistence.

## **3.2 INTRODUCTION**

### **3.2.1 Potential impacts of roads on wildlife**

Transportation infrastructure has many negative impacts to wildlife populations (Trombulak and Frissell 2000, Forman et al. 2003). Increased mortality rates can decrease population sizes (Fahrig and Rytwinski 2009). The barrier effect of roads, either through increased mortality rates or behavioral avoidance (Jaeger et al. 2005), reduces connectivity and can lead to genetic isolation (Balkenhol and Waits 2009, Holderegger and Di Giulio 2010, Simmons et al. 2010). Isolation, in combination with reduced population sizes, can lead to loss of genetic diversity, primarily through genetic drift or inbreeding (Frankham 1996, Holderegger and Di Giulio 2010). It well known that isolated populations are at greater risk for local extinction through both demographic and genetic processes (Frankham 2005).

Roads are a relatively recent feature on the landscape and consequently changes in population sizes and gene flow may be difficult to detect with current molecular markers (Holderegger and Di Giulio 2010). Despite this concern many studies have found evidence for reduced gene flow across roads (e.g., Epps et al. 2005, Clark et al. 2010, Delaney et al. 2010, Breyne et al. 2014) and several have found reduced genetic diversity in populations isolated by roads (e.g., Keller et al. 2005, Lesbarreres et al. 2006, Hepenstrick et al. 2012, Reid and Peery 2014). In addition, a recent simulation study found that changes in genetic structure of populations could be detected within one generation of the formation of a barrier, although the number of generations needed for detectable differentiation increased as the average individual dispersal distance decreased (Landguth et al. 2010).

### **3.2.2 Comparisons among species**

Species are expected to react differently to roads. Animals that avoid crossing roads should not experience greater mortality, but will be isolated from other populations (Jaeger et al. 2005).

Animals that readily attempt to cross roads will likely suffer greater mortality, but may still maintain connectivity to other populations. The size and traffic volume of the road also matters, as wider roads with heavier traffic volumes are likely to be complete barriers to most species, while narrower roads with lighter traffic volumes should remain permeable for at least some species (Oxley et al. 1974, Trombulak and Frissell 2000, Clevenger et al. 2003, Farmer and Brooks 2012, Brehme et al. 2013, Rhodes et al. 2014). Other landscape features, such as canopy cover or rock outcrops, can also differentially influence movement of species that have varied habitat requirements (e.g., Levy et al. 2013, Peterman et al. 2015), influencing population genetic structure. Among the studies that have compared multiple species in the same region, some have found that species have similar responses to roads and landscape structure (e.g., Delaney et al. 2010, Coster et al. 2015), while others have found different responses (e.g., Goldberg and Waits 2010, Frantz et al. 2012, Whiteley et al. 2014). Additional studies comparing multiple species in the same region will improve our understanding of how different dispersal behavior and use of landscape features affect responses to roads.

### **3.2.3 Impacts of roads on the genetics of reptiles**

Reptiles, snakes and turtles in particular, experience predominately negative road impacts on abundance and survival (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). Although the number of studies investigating the impacts of roads on population genetics have increased in recent years (Holderegger and Di Giulio 2010), very few have included reptiles. None of the studies reviewed by Holderegger and Di Giulio (2010) investigated impacts to reptiles, and to our knowledge, only 10 studies specifically focused on molecular road ecology with reptiles have been published since (Clark et al. 2010, Delaney et al. 2010, Latch et al. 2011, Dileo et al. 2013, Laporte et al. 2013, Munguia-Vega et al. 2013, Cureton II et al. 2014, Reid and Peery 2014, Tucker et al.

2014, Weyer et al. 2014). There were mixed results in the three studies on snakes. Timber rattlesnakes (*Crotalus horridus*) in hibernacula isolated by roads had lower genetic diversity and increased differentiation relative to those in continuous habitat (Clark et al. 2010). Eastern massasauga rattlesnakes (*Sistrurus c. catenatus*) had reduced gene flow across roads, but some roads paralleled water bodies that also restrict gene flow, so it was unclear which landscape feature caused the reduced gene flow, and to what degree (Dileo et al. 2013). There were no detectable road impacts on genetic diversity or differentiation for prairie rattlesnakes (*Crotalus viridis*) despite the low probability of individuals successfully crossing the roads in the study region (Weyer et al. 2014). The authors attributed the conflicting results to the historically large populations maintaining large effective population sizes, which decreases the rate at which genetic structuring will be detectable (Weyer et al. 2014).

Detecting the effects of recent landscape changes, including roads, on the genetics of turtles is particularly challenging due to their long generation times. Latch et al. (2011) found that both roads and slopes caused weak but significant population differentiation in desert tortoises (*Gopherus agassizii*). Painted turtles (*Chrysemys picta*) living near roads had lower mitochondrial diversity than those living in natural areas, but there were no significant differences in the diversity measured by nuclear microsatellite markers (Laporte et al. 2013). In a study comparing the genetic diversity of painted turtles, snapping turtles (*Chrysemys serpentina*), and Blanding's turtles (*Emydoidea blandingii*) found that only Blanding's turtles had lower genetic diversity in areas with greater road density (Reid and Peery 2014). Blanding's turtles had more terrestrial movements than the other species, increasing road encounter rates and associated mortality. Reid and Peery (2014) attributed the difference in results for painted turtles to their choice of sites that had lower traffic densities than those used by Laporte et al. (2013). Cureton II et al. (2014) found

no evidence for decreased genetic diversity in an urban population of ornate box turtles (*Terrapene ornate*), and only weak evidence for increased differentiation across a large highway. They attributed the lack of detectable effects to the long generation times of the turtles, and in a simulation study they found no significant changes in genetic diversity after 200 years of complete isolation among populations.

Delaney et al. (2010) found increased genetic differentiation in populations of three species of lizards (side-blotched lizards (*Uta stansburiana*), western skinks (*Plestiodon skiltonianus*), and western fence lizards (*Sceloporus occidentalis*)) separated by large highways in southern California, but found no impact of the separation on genetic diversity. Areas cleared of native vegetation, including highways, were found to be partial barriers to gene flow in black-tailed brush lizards (*Urosaurus nigricaudus*) in Baja California (Munguia-Vega et al. 2013). They did not find significant changes in genetic diversity, but did find localized extinctions in habitat patches that were both small and far from the nearest continuous habitat area. Tucker et al. (2014) found only weak evidence that a highway was acting as a barrier to Florida scrub lizards (*Sceloporus woodi*), even though these lizards are habitat specialists with low dispersal ability. The authors suggested that the lack of gene flow across the entire landscape limited their ability to distinguish between potential barrier effects of the highway with the barrier effects of overgrown forest. In sum, the molecular evidence for roads acting as barriers to the movements of different reptiles is mixed, even though reptiles are expected to be heavily impacted by roads due to their generally slow movement speeds and possible attraction to roads for thermoregulation or nesting (Andrews et al. 2008, Rytwinski and Fahrig 2012).

### 3.2.4 Study system

Lizards are important consumers (Turner et al. 1976) and have high species diversity in desert systems (Pianka 1967, 1969), but have not been well-studied in the road ecology literature (Andrews et al. 2008, Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). We compared the effects of two roads on the genetic diversity and differentiation on two species of lizards potentially impacted by roads. We focused on the side-blotched lizard (*Uta stansburiana*), a sedentary sit-and-wait predator that does not move large distances (Tinkle 1967, Peterson and Whitford 1987), and the marbled whiptail lizard (*Aspidoscelis marmorata*), a more wide-ranging, active forager (Parker 1972, Peterson and Whitford 1987, Anderson 1993). Side-blotched lizards are essentially annual, with most breeding adults consisting of the previous year's hatchlings (Tinkle 1967). Marbled whiptails live 3–4 years on average (Turner et al. 1969), and become sexually mature 1–2 years after they hatch (Pianka 1970, Parker 1972). Because traffic volume and road size are expected to differentially affect mortality risks and crossing behavior, we estimated the impacts that a wider interstate highway (Interstate 10, hereafter I-10) and a smaller rural highway (New Mexico State Highway 9, hereafter NM-9) had on populations of these lizards in southern New Mexico.

### 3.2.5 Question and hypothesis

We used microsatellite markers to assess if these species have been impacted by either road. We predicted that whiptails would have greater mortality rates near the roads due their greater likelihood of undertaking foraging forays across the roads. Reduced population sizes would then cause decreased genetic diversity (Jackson and Fahrig 2011). However, even if whiptails experienced greater road mortality rates, even a few successful crossings per generation would maintain genetic connectivity (Mills and Allendorf 1996), and we would find little evidence of differentiation. Because side-blotched lizards do not typically forage over large distances, we

expected that they would not experience increased mortality on roads, and would not show decreases in genetic diversity due to this factor. However, their reduced movement propensity would likely cause the roads to effectively act as complete barriers, and we predicted they would show increased differentiation across the road. We further predicted that barrier effects of I-10 should be greater than those of NM-9, and this difference would be evident in stronger differentiation among individuals inhabiting desert intersected by interstate highway than those living in proximity to the smaller state road.

### **3.3 METHODS**

#### **3.3.1 Sample collection**

The study region is typical Chihuahuan Desert shrubland. Site descriptions and trapping methods are detailed in Chapter 2 (sections 2.3.1 and 2.3.2). Briefly, we trapped lizards from 2009-2011 at a total of eight sites, three adjacent to I-10 and three adjacent to NM-9, along with control sites located 1000 m from each road (a distance much greater than the lizards are expected to move; Fig. 2.1, Supplementary Table S3.1). I-10 had an average annual daily traffic volume (AADT) of 15,187 vehicles in 2010 and 19,731 in 2011 (New Mexico Department of Transportation [NMDOT], <http://www.dot.state.nm.us/en/Planning.html>, accessed 16 March 2014) while NM-9 had an AADT of about 500 vehicles in 2010 (our measurements using a TRAX Apollyon, JAMAR Technologies, Inc., Hatfield, PA, USA). In the study region, neither road parallels any geographic features that might act as barriers (e.g., rivers, ridgelines) and lead to difficulties in teasing apart which landscape feature is actually reducing gene flow (e.g., Dileo et al. 2013). The roads bisect otherwise continuous habitat, which allows inference to be drawn about the effects of roads separate from differences in population sizes caused by different patch areas. Pitfall trapping arrays were located at three distances from the road, all outside of the road right-



of-way. There were trapping arrays close to the road on both sides (“close”), with additional traps approximately 50 m (“mid”) and 125 m (“far”) from the close group on just one side of the road (the particular side was chosen haphazardly and to avoid contact with any potential anthropogenic disturbances such as lightly travelled dirt roads, Fig. 2.2). At the control sites the array closest to the road was located approximately 1000 m away, with the additional traps at 50 and 125 m away from that array as for the other sites. Captured lizards were given unique marks by removing unique combinations of toes (Tinkle 1967), and toes were stored in 95% ethanol for DNA extraction.

### **3.3.2 Molecular methods**

We genotyped individuals from trapping distances that allowed us to compare the effects of the roads independently from that of geographical distance. As lizards were expected to move between the two groups of traps at each array, we calculated the center point of the minimum convex polygon connecting the traps to use as the geographic capture location in ArcGIS 10.2 (ESRI, Redlands, CA, USA). Although there were some movements between adjacent trapping arrays for both species (side-blotched lizards: 1.8% of 559 individuals, whiptails: 9.8% of 644 individuals), individuals were assigned to the location where they were first captured. The distance between the traps close to the road for I-10 was 106–140 m, and for NM-9 was 52–56 m. Thus, for the two sites adjacent to I-10 we genotyped individuals from the two close groups on opposite sides of the road, plus the group 125 m away, while for NM-9 we genotyped the individuals from the close groups and 50 m away (Fig. 2.2). This sampling strategy allowed for comparison of the genetic structure of populations approximately the same distance apart, but one comparison where individuals would cross open desert habitat and one where they would need to cross a road. Each trapping distance sampled was treated as a population in our analyses. Our goal was to genotype

>25 individuals for each group, but we were limited by the number of lizards captured at a few locations (Tables 3.1, 3.2). To minimize the probability of sampling both parents and offspring, we used samples from 2009 for the side-blotched lizards. Because of smaller population sizes, we used samples from 2009 and 2010 for whiptails in order to genotype at least 20 individuals per population. If there were >28 tissue samples for a location, we randomly selected the 28 samples for amplification using a random number generator. We also obtained tissue samples for both species from outside of the study area for comparison (Table S3.1). These outgroup samples were generally collected over a larger area than our sampling arrays, but were usually all within several 100 m of each other (C-Y. Kuo, pers. comm., W. Lukefahr, pers. comm.)

We extracted DNA from single toes using MasterPure DNA extraction kits (Epicentre, Madison, WI), with an overnight incubation of the toe in 300  $\mu$ L lysis buffer with 100 mg/ml proteinase K at 55°C, with occasional vortexing and maceration with sterile plastic or glass pestles, which assisted with degrading the scales and bone in the toe. We quantified the concentration of purified DNA with a Nanodrop 1000 (ThermoScientific, Wilmington, DE) and adjusted the concentration to approximately 30 ng / $\mu$ L. We amplified nine microsatellite loci for side-blotched lizards (Zamudio and Sinervo 2000), and all produced repeatable results. We screened 13 loci for whiptails (Crawford et al. 2008), using the eight loci that produced consistent results. The PCR amplifications were conducted in two multiplex reactions for each species using Multiplex PCR kits (QIAGEN, Valenica, CA), with fluorescently-tagged primers. For the side-blotched lizards, each primer was uniquely tagged, while for whiptails we used the “tailed” method of Blacket et al. (2012). Amplification conditions are reported in supplementary data (Table S3.2). The amplified products were diluted 1:20 with double-distilled water and then visualized with an ABI 3130xl automated sequencer (Applied Biosystems, Foster City, CA) using the 600LIZ internal size

standard. We ran negative and positive controls with each set of PCRs, and manually scored genotypes with Peak Scanner v 1.0 (Life Technologies, Grand Island, NY). Samples with ambiguous or missing alleles were re-amplified, and if there were still inconsistencies that individual was removed from the analyses. Individuals with  $\leq 2$  loci with consistently missing alleles were retained for the detection of null alleles, but individuals missing data for  $>3$  loci were removed.

### 3.3.3 Data analysis

We re-amplified a randomly selected subset of 45 individuals of each species (about 10% of the total sample size) to check for consistency and repeatability. We binned the raw allele sizes using TANDEM (Matschiner and Salzburger 2009). We checked for potential scoring errors and null alleles with MICROCHECKER using Bonferroni corrections (van Oosterhout et al. 2004). We tested for departures from Hardy-Weinberg equilibrium and pairwise genotypic linkage disequilibrium within each population with GENEPOP Web Service (<http://genepop.curtin.edu.au/>; Raymond and Rousset 1995, Rousset 2008). *P*-values were adjusted using a sequential Bonferroni correction for multiple tests (Rice 1989).

### *Genetic diversity*

We assessed genetic diversity using several metrics: number of alleles per locus, allelic richness rarefied to the smallest number of samples in a population, and observed and expected heterozygosities, all estimated with the R package *diveRsity* (Keenan et al. 2013) in R version 3.2.0 (R Core Team 2014). We compared each metric among the populations using Kruskal-Wallis rank sum tests because the data for all metrics were non-normal and had heterogeneous variances. We used the post-hoc multiple comparison test in *pgirmess* version 1.6.2 (Giraudoux 2015) if the Kruskal-Wallis test was significant to determine which populations were different. The outgroup

populations were included in these analyses. There are several relatedness estimators available, and their performance varies based on the data analyzed (Van de Castele et al. 2001, Csilléry et al. 2006). We compared three common estimators (Queller and Goodnight 1989, Lynch and Ritland 1999, Wang 2002), calculated using the R package *related* (Pew et al. 2015), which is based on the program COANCESTRY (Wang 2011). Outgroups were excluded from these calculations. We assessed whether individuals in each population had higher than expected relatedness using the permutation test in *related* with 1000 iterations. We used linear regression implemented in R to test whether individuals near the roads had a higher degree of relatedness than those away from the road, as might occur if individuals were unable to disperse across the road. We estimated the effective population size ( $N_e$ ) using the step-wise mutation model of Ohta and Kimura (1973) with the microsatellite mutation rate estimate of  $5 \times 10^{-4}$  (Garza and Williamson 2001).

### ***Population genetic structure***

We assessed overall population genetic structure using three methods: pairwise population differentiation, isolation by distance, and Bayesian clustering. There are several estimators for measuring population differentiation (Meirmans and Hedrick 2011). We calculated  $F_{ST}$  (Weir and Cockerham 1984),  $G''_{ST}$ , the analogue standardized by the maximum value of  $G_{ST}$  given the observed within population diversity (Hedrick 2005, Meirmans and Hedrick 2011), and Jost's  $D$ , a measure of differentiation based on the effective number of alleles (Jost 2008) using the R package *diveRsity* (Keenan et al. 2013). Statistical significance of each pairwise comparison was assessed with biased-corrected 95% confidence intervals determined with 1000 bootstrap iterations in *diveRsity*. Comparisons with 95% CIs that did not overlap 0 were considered to be significant. The three metrics were highly correlated for side-blotched lizards (pairwise

comparisons of the metrics, all Pearson's  $r > 0.854$ ), with  $F_{ST}$  and  $G'_{ST}$  especially so ( $r = 0.996$ ). We report the values for  $F_{ST}$  instead of  $G'_{ST}$  to facilitate comparisons with past studies, along with the values for  $D$ , as recommended by Meirmans and Hedrick (2011). Isolation by distance (Wright 1943) was assessed for each metric using Mantel tests implemented with the *vegan* package (Oksanen et al. 2015) using 10,000 permutations to determine significance (5040 permutations when testing just the sites near I-10 and those near NM-9, which is the maximum given seven populations). Both genetic and Euclidean distances were transformed ( $F_{ST}/(1 - F_{ST})$  and  $\ln$ , respectively) prior to performing the Mantel tests (Rousset 1997). We analyzed the data with all populations, just those in the study region, just those along I-10, and just those along NM-9. Bayesian clustering analysis was conducted using STRUCTURE v2.3.4 (Pritchard et al. 2000, Falush et al. 2003, Hubisz et al. 2009), which clusters individuals into user-specified numbers of groups ( $K$ ) that best fit Hardy-Weinberg and linkage equilibrium. We ran a modified version of hierarchical structure analysis (Evanno et al. 2005). Typically clusters that are identified at broad spatial scales are then analyzed separately, repeating the process until no finer spatial scales of structure are found (Evanno et al. 2005). We used *a priori* spatial scales that corresponded to expected genetic groups: all sites, study sites only, sites near I-10, and sites near NM-9. Removing the more distant individuals was expected to increase our ability to detect structure when low levels of differentiation were present (Hubisz et al. 2009). We used the correlated allele frequency model with admixture of individuals allowed, with the LOCPRIOR option to increase the detection of weak population structure (Hubisz et al. 2009). We performed 10 independent runs for each  $K$  value, with 500,000 burn-in and 500,000 Markov chain Monte Carlo (MCMC) repetitions. The number of potential clusters was 1–7 for all populations of side-blotched lizards and 1–4 for all populations of whiptails because of the smaller number of outgroup populations. For the study

site populations, we tested  $K = 1-4$  for both species, and tested  $K = 1-3$  for the road-specific populations. The widely used  $\Delta K$  method for estimating the number of clusters cannot find the best  $K$  if the true  $K = 1$  (Evanno et al. 2005), so we used *ad hoc* methods to determine the best value of  $K$ , including  $\Delta K$ , the  $\ln P(K)$ , and examination of the bar plots. We assumed the true  $K$  to be 1 if  $\Delta K$  identified  $K = 2$ , the values for the  $\ln P(K)$  were similar or lower for  $K = 1$  vs.  $K = 2$ , and the bar plot of  $K = 2$  showed all individuals to be admixed. We used STRUCTURE HARVESTER (Earl and vonHoldt 2012) to determine  $\ln P(K)$  and  $\Delta K$ , followed by CLUMPP (Jakobsson and Rosenberg 2007) to align the 10 iterations and DISTRICT (Rosenberg 2004) to visualize the bar plots.

### ***Detection of roads as barriers***

While the assessment of the population genetic structure could reveal patterns caused by roads acting as barriers, other processes like isolation by distance could mask weak road effects. We used three methods specifically to investigate increased population differentiation across roads (excluding outgroups from all analyses). First, we used partial Mantel tests for correlations between the pairwise population genetic differentiation and the side of the road (“roadside”, a matrix of dummy pairwise variables with 1 = same side of the road, 2 = opposite sides of the road, and 3 = opposite sides of both I-10 and NM-9 [north of I-10 and south of NM-9]), controlling for Euclidean distance. We used the *vegan* package (Oksanen et al. 2015) with 10,000 permutations to assess significance for all populations within the study site, and used 5040 permutations for populations near I-10 and NM-9 separately. We used transformed genetic distances (Rousset 1997) as above. Second, within each site we compared the statistical significance of the pairwise genetic distance for populations on the same side of the road with those on opposite sides of the roads. Our study design controlled for Euclidean distance, so significant differentiation between

populations on opposite sides of the road without significant differentiation between those on the same side of the road was taken as evidence that the road reduced gene flow. Finally, we ran STRUCTURE analyses for each site individually, using only  $K = 1$  and  $K = 2$ , with the same program options and run lengths as above. This approach has been shown to be effective in detecting barriers in both simulation (Blair et al. 2012) and empirical studies (Munguia-Vega et al. 2013). We again used the *ad hoc* method of assessing  $\ln P(K)$  and bar plots to determine the most likely number of clusters.

### **3.4 RESULTS**

#### **3.4.1 Sample size and quality of genotyping**

For side-blotched lizards, we genotyped 452 individuals from 19 populations (371 from the 14 populations in the study area and 81 from the 5 outgroups, Table 3.1). The error rate on repeat-genotyped individuals was 2.2% across all nine loci for 45 randomly selected individuals (total of 810 individual alleles). Data entry errors accounted for most of the errors (1.7%), and only 0.24% of individual alleles were different relative to the original genotyping. Data entry errors were usually found during the re-check of data before performing analyses. This overall error rate is similar to that found by Hoffman and Amos (2005) in a study focused on measuring genotyping errors. The 10,000M's locus failed to amplify for almost all individuals from the outgroup populations from Oregon and Washington, but all other loci amplified consistently. We tested for null alleles in the rest of the loci for those two populations separately, after removing the 10,000M's locus from the data. There were five loci identified as having an excess of homozygotes, possibly due to the presence of null alleles. However, four of the loci showed evidence of potential null alleles in only one population each, and the fifth (IGs) had possible null alleles in two populations. Thus we retained all loci for subsequent analyses. Only the PLkn locus

in population 4S\_close (see table S3.1 for information regarding population names) was not in Hardy-Weinberg equilibrium following the sequential Bonferroni adjustment ( $P = 0.0076$ , adjusted  $\alpha = 0.0083$ ). None of the loci were in linkage disequilibrium following the Bonferroni correction.

We genotyped 400 marbled whiptails from 16 populations (362 from within the study area and 38 from two populations in Texas, Table 3.2). The error rate on the 45 repeat-genotyped individuals was 1.5% across all eight loci. Data entry (0.42%) and differences in peak size determination (1.11%) were the only types of errors. All of the errors were in two loci, Ai5013 and Ai5072, and both were omitted from later analyses because of other problems. Two loci (Acos2 and Ai5072) were monoallelic in these samples and were not included in subsequent analyses. One locus (Ai5013) was reported as a tetranucleotide repeat (Crawford et al. 2008), but we consistently amplified alleles that differed by 1, 2, and 3 base pairs. This pattern could be caused by point mutations in the microsatellite itself or the flanking region. Because we could not assign the odd alleles to the “correct” allele size, we did not use that locus. One locus (Acos3) was identified as possibly containing null alleles in 44% of the populations. Although Acos3 contained only four alleles across all populations, we assessed the loss of information by excluding that locus by calculating the probability of identity ( $P_{(ID)}$ ) both with and without Acos3 using GENALEX 6.5 (Peakall and Smouse 2006, Peakall and Smouse 2012). Including Acos3 changed  $P_{(ID)}$  from  $4.2 \times 10^{-4}$  to  $1.6 \times 10^{-4}$ . We ran subsequent analyses both with and without that locus. When Acos3 was included, there were only two populations with loci not in Hardy-Weinberg equilibrium following the sequential Bonferroni correction: OG\_TX for Acos3 ( $P = 0.002$ , adjusted  $\alpha = 0.0125$ ) and CN2 for Acos5 ( $P = 0.002$ , adjusted  $\alpha = 0.01$ ). Acos5 still significantly deviated from Hardy-Weinberg equilibrium for CN2 when the analysis was run without Acos3.



None of the loci were in linkage disequilibrium following the Bonferroni adjustment when Acos3 was included, and only one population had a pair of loci significantly linked when Acos3 was excluded (Ai5062 and Ai5043 in OG\_TX,  $P = 0.0102$ , adjusted  $\alpha = 0.0167$ ). Removing Acos3 had little impact on the overall results (data not shown). Excluding Acos3 increased the average values for the genetic diversity metrics, but there were no changes in the relative genetic diversity among populations when compared to including Acos3. The different measurements of genetic structure also had small changes to the absolute values, but the relative values were similar with and without Acos3. We report only the results of the analyses including Acos3.

### **3.4.2 Genetic diversity**

#### ***Side-blotched lizards***

The overall genetic diversity was greatest in the AZ and NV outgroup populations, and lowest in the OR and WA populations (Table 3.1). There were significant differences in both the number of alleles and allelic richness (number of alleles: Kruskal-Wallis  $\chi^2 = 31.86$ , d.f. = 18,  $P = 0.023$ ; allelic richness:  $\chi^2 = 33.63$ , d.f. = 18,  $P = 0.014$ ). The only significant differences between populations were between AZ and OR (number of alleles, allelic richness), AZ and WA (number of alleles, allelic richness), NV and OR (allelic richness), and NV and WA (number of alleles, allelic richness, Table 3.1). The observed heterozygosity was marginally significant ( $\chi^2 = 28.35$ , d.f. = 18,  $P = 0.057$ ), with only AZ vs OR and NV vs WA different in the multiple comparisons test. There were no significant differences among the populations for expected heterozygosity ( $\chi^2 = 25.49$ , d.f. = 18,  $P = 0.112$ ). We confirmed that there were no significant differences among the populations in the study region by running the Kruskal-Wallis tests excluding the outgroups ( $\chi^2$  values  $< 2$ , d.f. = 13,  $P > 0.99$  for all four metrics).

Four populations had higher than expected relatedness using the Lynch and Ritland estimator (Table 3.1). Only one population had higher than expected relatedness using the Queller and Goodnight estimator, and there was no increase in relatedness found using the Wang estimator (Table 3.1). The regression of individual pairwise relatedness against the distance to the road ( $\log_{10}$  transformed) was only significant for the Wang estimator ( $\beta = -0.025$ ,  $t = -3.11$ ,  $P = 0.002$ ). However, the explained variation was very low ( $R^2 = 0.002$ ) and likely not biologically significant. Overall there was limited evidence for increased relatedness within populations near either road.

The effective population size was largest for the AZ and NV populations ( $N_e = 1679$  and  $1481$ , respectively) and lowest for the OR and WA populations ( $N_e = 33$  and  $34$ , respectively; Table 3.1). The average  $N_e$  in the study region was  $373 \pm 16$  (SE; Table 3.1).

### ***Marbled whiptails***

There were no significant differences in any of the four metrics among all the populations of marbled whiptails (number of alleles: Kruskal-Wallis  $X^2 = 1.00$ , d.f. = 15,  $P = 1.00$ ; allelic richness: Kruskal-Wallis  $X^2 = 0.65$ , d.f. = 15,  $P = 1.00$ ; observed heterozygosity: Kruskal-Wallis  $X^2 = 1.46$ , d.f. = 15,  $P = 1.00$ ; expected heterozygosity: Kruskal-Wallis  $X^2 = 1.55$ , d.f. = 15,  $P = 1.00$ ; Table 3.2). This lack of significant difference was also true when only comparing the populations in the study region (data not shown, Kruskal-Wallis  $X^2$  lower than for comparisons among all populations for each metric).

Four populations had higher than expected relatedness using the Lynch and Ritland estimator (Table 3.2), and all were close to a road. Only two populations had higher than expected relatedness using the Queller and Goodnight estimator, one of which was close to the road and one far from the road (both at site 4 on I-10). None of the populations had greater than expected

relatedness using the Wang estimator. The pairwise relatedness values were negatively associated with the distance from the road ( $\log_{10}$  transformed) for all three estimators (Lynch and Ritland:  $\beta = -0.013$ ,  $t = -2.59$ ,  $P = 0.010$ ; Queller and Goodnight:  $\beta = -0.014$ ,  $t = -1.98$ ,  $P = 0.048$ ; Wang:  $\beta = -0.017$ ,  $t = -2.62$ ,  $P = 0.009$ ). However, the estimates of the slopes were small in all cases, and the explained variation was very low (adjusted  $R^2 = 0.001$  for all estimators). As for the side-blotched lizards, there was limited evidence for increased relatedness near the roads.

The average  $N_e$  in the study region was  $1024 \pm 61$  (SE, Table 3.2). The two TX outgroup populations had lower effective populations sizes ( $N_e = 607$  and  $640$ , respectively; Table 3.2).

### 3.4.3 Population structure

#### *Side-blotched lizards*

There was very low genetic differentiation among the populations of side-blotched lizards within the study region for either metric (Table 3.3). The average pairwise  $F_{ST}$  was  $0.009 \pm 0.0009$  (SE), and the average Jost's  $D$  was  $0.002 \pm 0.0003$  (SE). Only two population comparisons were significant (i.e., had 95% CI that did not overlap 0) for  $F_{ST}$  (3S\_close vs. 8N\_close, 3S\_close vs. 8S\_close), and only one comparison was significant for  $D$  (4S\_close vs. CN2). All of the study region populations were significantly differentiated from the outgroups according to  $F_{ST}$  values, and for all but three study region populations using  $D$  (Table 3.3). All of the outgroups were significantly differentiated from each other for both metrics. The Texas outgroup was the least differentiated from the study region populations. Pairwise comparisons between the AZ and NV populations revealed relatively low differentiation for both metrics, while the OR and WA comparison showed high differentiation as measured by  $F_{ST}$ , but relatively low differentiation as measured by  $D$ . This pattern of differentiation was also apparent in the Mantel tests of isolation by distance. There was a strong pattern of IBD for all populations ( $F_{ST}$ : Mantel's  $r = 0.671$ ,  $P$

<0.001;  $D$ : Mantel's  $r = 0.699$ ,  $P < 0.001$ ; Table 3.5a, Fig. 3.2a, b). The pattern was less pronounced, but significant for the populations in the study region ( $F_{ST}$ : Mantel's  $r = 0.415$ ,  $P < 0.001$ ;  $D$ : Mantel's  $r = 0.412$ ,  $P = 0.001$ ; Table 3.5a, Fig 3.2c, d). For the populations adjacent to I-10, there was significant IBD only with  $F_{ST}$  (Mantel's  $r = 0.398$ ,  $P = 0.031$ ;  $D$ : Mantel's  $r = 0.274$ ,  $P = 0.115$ ; Table 3.5a). There was no significant IBD for the NM-9 populations, although there was marginal significance with  $D$  (Mantel's  $r = 0.309$ ,  $P = 0.051$ ;  $F_{ST}$ : Mantel's  $r = 0.172$ ,  $P = 0.153$ ; Table 3.5a).

Bayesian clustering analysis for all populations found four clusters, with the OR and WA outgroups and the AZ and NV outgroups well defined, and the TX outgroup and the study region populations differentiated but with some weakly admixed individuals in several populations (Fig. 3.3a). There was likely only one genetic cluster at the scale of the study region. Although both  $\ln P(K)$  and  $\Delta K$  support  $K = 2$ , the bar plot showed most individuals assigned completely to one cluster with some individuals weakly admixed with the second cluster (Fig. 3.3b). None of the individuals were fully assigned to the second cluster. The same pattern was found for the I-10 and NM-9 populations.

### ***Marbled whiptails***

There was also very low genetic differentiation among the study region populations of marbled whiptails based on either metric (Table 3.4). Both the average pairwise  $F_{ST}$  and Jost's  $D$  were  $0.008 \pm 0.001$  (SE). Within the study region there were four significant pairwise comparisons (out of 91 total) with both  $F_{ST}$  and with  $D$  (Table 3.4). Unlike the side-blotched lizards, there was also low differentiation between the study populations and the two Texas outgroups >200 km away. Of the 29 pairwise comparisons, only 7 had 95% CI that did not overlap 0 for  $F_{ST}$  and 6 for  $D$ . There was significant isolation by distance for all populations ( $F_{ST}$ : Mantel's  $r = 0.468$ ,  $P$

$<0.001$ ;  $D$ : Mantel's  $r = 0.427$ ,  $P < 0.001$ ; Table 3.5a, Fig. 3.3a, b) and just the populations within the study region ( $F_{ST}$ : Mantel's  $r = 0.299$ ,  $P < 0.001$ ;  $D$ : Mantel's  $r = 0.241$ ,  $P = 0.003$ ; Table 3.5a, Fig. 3.3c, d). There was no evidence for IBD at the finer spatial scale of just populations near each road (Table 3.5a).

Bayesian clustering analysis for all populations found three clusters (Fig. 3.3c). However, all individuals had some degree of admixture. The general pattern was for the two outgroups from TX forming one cluster, and the individuals from the study region forming two clusters (Fig. 3.3c). The individuals from the populations near I-10 and those from near NM-9 tended to have different proportions in each cluster, which indicates potentially weak differentiation (Fig. 3.3d). However, when analyzing the populations within the study region, the  $\ln P(K)$  supported  $K = 1$ . Although the  $\Delta K$  method indicated  $K = 2$ , the bar plot for  $K = 2$  showed high levels of admixture for most individuals (Fig. 3.3d). The results were similar for analyses of the populations near I-10 and those near NM-9 separately (results not shown). Overall there is support for genetic differentiation between the study site populations and the TX outgroups, there is only little support for genetic differentiation among populations within the study region.

#### **3.4.4 Barrier effects**

The results on the tests for roads as barriers were qualitatively similar for both species, and there was no evidence that either road was a barrier to gene flow. The partial Mantel tests were not significant for any spatial grouping or for either genetic distance metric (Table 3.5b). None of the comparisons of genetic differentiation within a site were significant (Tables 3.3, 3.4), indicating that individuals on opposite sides of the road were not more genetically isolated than those a comparable distance away on the same side of the road. Bayesian clustering analysis of

each site independently did not find any evidence for more than one genetic cluster (Fig. 3.4), again indicating no genetic structure caused by either road.

### **3.5 DISCUSSION**

Roads have generally been thought to negatively impact many taxa, with reptiles in particular suffering increased mortality on roads (Andrews et al. 2008, Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). Particularly high mortality rates can cause roads to act as both population sinks and barriers (Jackson and Fahrig 2011). Some animals can show behavioral avoidance of roads, which reduces mortality rates but increases the barrier effects (Jaeger et al. 2005). Reduced population sizes and/or reduced connectivity can increase the rate at which genetic drift can reduce genetic diversity and increase population differentiation (Wright 1931). Despite these predicted effects, we did not find evidence that either road reduced diversity or increased differentiation for either lizard species.

#### **3.5.1 No impact on genetic diversity**

All three measures of genetic diversity were similar in all populations in the study region. Decreases in genetic diversity are closely tied to population size and degree of isolation. Population size appears to contribute more to decreased diversity. A simulation study by Jackson and Fahrig (2011) found that road mortality had a much greater impact on reducing genetic diversity than did barrier effects. Mortality can continually decrease population sizes, while a barrier effect caused by behavioral avoidance maintains a relatively constant abundance. We estimated abundance and survival for both lizards as a part of the broader study (Chapter 2, sections 2.4.2 and 2.4.3). The minimum number of unique individuals (an abundance index) averaged  $23.2 \pm 1.9$  (SE) per population per year for side-blotched lizards and  $18.6 \pm 0.8$  for whiptails, and abundance was slightly increased near the roads (Chapter 2, section 2.4.2). There was no evidence

for increased mortality near either road for either species. With abundant lizards and low mortality, the lack of impact on diversity is consistent with the results of Jackson and Fahrig (2011). The estimates of the effective population sizes were also relatively large for both species (Tables 3.1, 3.2). The  $N_e$  was lower for the side-blotched lizards than the whiptails in the study region, as expected given their smaller home ranges and dispersal distances.

The study region is largely continuous desert shrubland, with only a railroad and dirt roads bisecting the approximately 50 km between I-10 and NM-9. Thus there are few barriers to gene flow between populations near the roads and those away from the roads, and movement between them can maintain genetic diversity near the roads (Kuehn et al. 2007). Jackson and Fahrig (2014) used simulations to show that genetic diversity responds to the amount of habitat at large spatial scales, regardless of overall habitat fragmentation and individual dispersal abilities. Because the study sites are located in a large area of continuous habitat, even small movements by individual lizards can lead to connectivity across the landscape at the temporal scale (i.e., many generations) where decreases in diversity might otherwise be expected (Jackson and Fahrig 2014).

In a review by Holderegger and Di Giulio (2010), 14 of the 19 studies that measured the effects of roads on genetic diversity found measurable decreases. We found an additional 28 studies since then that assessed diversity in 35 taxa, and only nine found decreased diversity. Several of the studies that found decreased diversity found greater decreases in smaller and/or more isolated populations (e.g., Epps et al. 2005, Hopenstrick et al. 2012, Munshi-South et al. 2013). The reasons for no change in diversity included large population sizes (e.g., Keller et al. 2004, Gauffre et al. 2008, Weyer et al. 2014) or some amount of dispersal across roads (e.g., Sackett et al. 2012, Sotiropoulos et al. 2013). Finally, changes in genetic diversity generally take longer to detect than barrier effects (Jackson and Fahrig 2011).

### 3.5.2 Limited barrier effect

Genetic divergence generally responds to road effects more quickly than does genetic diversity (Keyghobadi et al. 2005, Keyghobadi 2007, Jackson and Fahrig 2011). However, we did not find evidence of increased divergence caused by the roads in our study. One possible explanation is that the roads are not actually barriers, and lizards successfully cross often enough to maintain genetic connectivity (Mills and Allendorf 1996). This explanation is certainly the case for the smaller road. Both species were detected crossing NM-9 less frequently than expected based on movement rates across similar distances of natural habitat, but two side-blotched lizards and five whiptails were trapped on both sides of the road during the three years of the study (Chapter 2, section 2.4.5). This rate likely underestimates the true crossing frequency because it is based on pitfall trapping, not direct observations or radio-tracking. We did not detect any individuals of either species crossing the larger road. We also did not detect many movements of the length required for an individual to be trapped on both sides of I-10, so it is difficult to say if some lizards are actually able to successfully cross. Although limited, there is evidence that lizards behaviorally avoid crossing roads (Klingensböck et al. 2000, Koenig et al. 2001, Tanner and Perry 2007, Brehme et al. 2013). It thus seems likely that few, if any, of these lizards are attempting to cross I-10 in the study region. There are drainage culverts and some vegetated underpasses in the area (although not within the study sites) that could be used by lizards to maintain connectivity. There are few studies on lizard use of such structures. Some lizards were detected using underpasses in Portugal (< 4% of all detected vertebrate crossings; Ascensão and Mira 2007), and 29 individual Western bobtail lizards (*Tiliqua rugosa*) made >200 crossings through underpasses in Australia (Chambers and Bencini *in press*). The culverts in Portugal were 8–37 m long (Ascensão and Mira 2007), while the underpasses in Australia were 23–88 m long (Chambers and Bencini *in press*). These distances are comparable to those required to cross I-10 in the study area,



indicating that it is possible that the species in this study might use the culverts and underpasses in the region. Although genetic connectivity could be maintained if even a few individuals successfully crossed the road via these structures, we currently have no data to assess this likelihood of culvert use.

A more likely explanation for our results is that the large road is a barrier, but we were not able to detect any changes to the genetic population structure for either species. As discussed in the previous section, the population sizes (census estimates and effective) are large and there are no barriers between the populations near the roads and those in the surrounding desert shrubland. Large population sizes reduce the rate at which genetic drift can increase differentiation (Wright 1931). The number of generations since the roads were built also influences the ability to detect changes (Holderegger and Di Giulio 2010). Both species have relatively short generation times, about one year for side-blotched lizards (Tinkle 1967) and 1–2 years for whiptails (Turner et al. 1969). Although the exact dates that the roads were paved was difficult to determine, I-10 replaced U.S. State Route 80 in the 1970s, and U.S. 80 was paved in the 1930s (<http://www.steveriner.com/nmhighways/us-interstates.htm>, accessed 5 July 2015). New Mexico 9 follows an old railroad that was built in the 1900s and removed in the 1960s ([http://www.abandonedrails.com/South\\_Line](http://www.abandonedrails.com/South_Line), accessed 5 July 2015), becoming a dirt road, and then was paved in the 1990s (C. Lieb, pers. comm.). Thus the populations have been fragmented by a paved road in the current location of I-10 for >50 generations of side-blotched lizards and >25–50 generations of whiptails, and by NM-9 for >20 generations of side-blotched lizards and >10–20 generations of whiptails. Although some studies have found genetic differentiation in a similar number of generations (e.g., Lesbarreres et al. 2006, Delaney et al. 2010), simulation studies found conflicting results regarding the number of generations required to detect changes.

Landguth et al. (2010) and Blair et al. (2012) showed detectable changes to genetic structure within 10 generations after the creation of a barrier, but found that changes take longer to appear with shorter dispersal distances. Simulations by Safner et al. (2011b) found much longer time required, on the order of >500 generations. They used larger population sizes ( $N = 10,000$ ), and allowed the simulated population to reach equilibrium before adding the barriers. Blair et al. (2012) suggested that the larger population size and the equilibrium status prior to the barrier are not likely in natural populations, and are thus the results of Safner et al. (2011b) were not realistic. However, our study system might be similar to the conditions used by Safner et al. (2011b). The population sizes are large, and other than a likely shift from grasslands to shrublands >100 years ago that coincided with the introduction of cattle grazing (Brown and Archer 1999), the region has likely experienced only minor anthropogenic disruptions. Taken together, the short dispersal distances, large population sizes, and possibly genetic equilibrium prior to the creation of the roads increase the number of generations required before any changes to genetic structure become apparent.

Finally, it is possible that our markers did not have sufficient power to detect what was likely weak genetic structure. For the side-blotched lizards, three of the loci were monoallelic for most of the populations within the study region, and the overall diversity was lower than in the Arizona and Nevada outgroups. The probability of identity (calculated in GenAlEx 6.5, Peakall and Smouse 2012) within the study region populations was  $1.5 \times 10^{-4}$ , while for just the Arizona and Nevada populations it was  $3.8 \times 10^{-8}$ . Lower  $P_{(ID)}$  generally indicate increased power of the markers (Waits et al. 2001, Latch et al. 2011). However, Delaney et al. (2010) did detect population differentiation attributable to roads using the same loci. Their estimates of allelic richness were similar to ours, but their expected heterozygosities were higher, potentially increasing the power of the markers in their study region. The microsatellite loci we used for the

marbled whiptails were developed for other species in the genus (Crawford et al. 2008). Only eight of the 13 loci amplified reliably, and of those eight, two were monoallelic, one had likely point mutations that made it difficult to assign the correct allele length, and one had evidence of null alleles. The  $P_{(ID)}$  with the locus with possible null alleles was  $1.6 \times 10^{-4}$ , similar to that for the side-blotched lizards. Other studies found  $P_{(ID)}$  several orders of magnitude lower than our study (e.g., Waits et al. 2001, Stow and Sunnucks 2004, Latch et al. 2011). The use of additional and more loci would likely increase our ability to detect changes (Landguth et al. 2012). Development of additional markers, either microsatellite or SNPs, would be beneficial for these species, given their wide-spread range throughout the southwestern United States and long history of use in ecological and evolutionary studies.

### **3.5.3 Comparisons with outgroup populations**

The populations of side-blotched lizards in the study area had lower genetic diversity than those from AZ and NV, but higher than those from OR and WA. Oregon and Washington are at the northernmost extent of the range for side-blotched lizards (Degenhardt et al. 1996), and peripheral populations often have lower genetic diversity than core populations (Hutchison 2003, Howes and Loughheed 2008, Blevins et al. 2011, Peterman et al. 2013). This lower genetic diversity could be due to founder effects following range expansion post-glaciation (Ballinger and Tinkle 1972). A range-wide phylogeny by Corl et al. (2010) found that *Uta stansburiana stejnegeri*, the previously designated subspecies in New Mexico and Texas (Ballinger and Tinkle 1972), was a highly divergent and monophyletic lineage. Their results are concordant with ours regarding the significant divergence of the populations from the study region and TX from the other populations. However, it is not clear why the genetic diversity would be lower in the study region and TX than in AZ and NV. The study region is located where the ancient Lake Cabeza de Vaca existed until

the mid-Pleistocene (700,000 – 350,000 years before present), and the lake was hypothesized to have affected the distribution and differentiation of terrestrial vertebrates (Axtell 1978). More recent cycles of glaciation caused desert habitat to expand and contract, with the *U. s. stejnegeri* range becoming restricted to central Mexico during glacial maxima (Ballinger and Tinkle 1972). A more recent change is the shift from grasslands to shrublands in the Chihuahuan Desert following the introduction of cattle grazing in the 1800s (Brown and Archer 1999). Side-blotched lizards are less abundant in grasslands (Jones 1981), and their presence across the study region might reflect relatively recent founder effects.

We had only two outgroup populations with which to compare the marbled whiptails. There were no significant differences in the diversity metrics, and only 24% of the pairwise population differentiation tests were significant (Table 3.4). The clustering analysis did indicate that the TX populations were distinct from the study region populations, although with some small percent of admixture (Fig. 3.3c). To our knowledge, range-wide genetic analyses have not yet been conducted. The relatively low levels of genetic differentiation seen between the study region populations and the TX populations (about 260 km apart) likely contributed to the difficulty in detecting changes caused by the roads in the study. As discussed above, if there is little prior genetic structure, even the more sensitive individual-based genetic clustering methods will not be informative (Holderegger and Di Giulio 2010, Simmons et al. 2010).

### **3.6 CONCLUSIONS**

We expected to find differences in the population genetic response to roads in these two lizard species, given their different life histories and foraging behaviors. However, the roads in our study affected neither the genetic diversity nor differentiation for either species. Because the large and continuous populations of both species decreased our ability to detect changes in genetic

structure, it is difficult to determine if the larger road is acting as a barrier to lizard movement. Many of the studies that examine the effects of roads on the population genetics of different taxa work in fragmented habitats (e.g., Delaney et al. 2010, Munguia-Vega et al. 2013, Munshi-South et al. 2013), where population sizes are generally lower and genetic drift is stronger. In our study region, the average effective population sizes were fairly similar among the populations. It is likely that what we designated populations (the individuals from a particular trapping distance) are actually part of a larger population. This result is particularly likely for the whiptails, which had  $N_e$  estimates around 1000 individuals and 9.7% of the movements detected were between trapping distances. The side-blotched lizards had smaller estimates of  $N_e$ , around 370 individuals, and only 1.8% of the detected movements were between trapping distances. For both species the estimates of  $N_e$  were considerable larger than our estimates of population abundance (discussed above and in Chapter 2). Although it is possible to detect changes in more continuous habitat (Robinson et al. 2012), it is more likely when roads have strong barrier effects. For example, only the interstate highway was found to be a barrier to red-backed salamanders (*Plethodon cinereus*), while several smaller roads caused no detectable increase in differentiation (Marsh et al. 2008). The population near the interstate highway was also thought to have a lower effective population size, again increasing the rate of genetic drift.

Roads are thought to be a unique barrier in the landscape because they are generally a small percentage of overall land use, but are often highly resistant to movement (Forman et al. 2003). Assessing whether or not this assumption is correct in different regions and with particular species is important for developing mitigation plans (van der Grift et al. 2012). When studies do not find significant road effects, the difficulty in determining the particular reason (e.g., lack of marker power, insufficient time since road construction, or the roads not acting as a barrier for that species)

highlights the need to include additional methods. We used mark-recapture methods (Chapter 2) along with the genetics to determine that lizards do cross NM-9, but we were not able to determine if they cross I-10. Further work tracking individual movements would help to determine if lizards are crossing that road.

In addition, working in regions with naturally patchy populations that decrease overall population sizes would enhance our ability to detect changes. An ideal setting would be a region with several isolated patches bisected by roads of varying size and traffic volumes. Such a design is similar to that of Delaney et al. (2010), who found significant differentiation in three species of lizards, and of Munguia-Vega et al. (2013), who found that roads were partial barriers to a single species of lizard. In such situations, roads can be one part of the overall landscape that can isolate populations and reduce long-term population persistence. However, in locations similar to our study region, with large continuous populations, it appears that roads have little short-term impact on population persistence.

### **3.7 ACKNOWLEDGEMENTS**

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### 3.8 TABLES

Table 3.1. Diversity statistics for side-blotched lizards.

Road	Population	Number of individuals	Number alleles per locus	Allelic richness	H <sub>O</sub>	H <sub>E</sub>	N <sub>e</sub>	Relatedness: Q&G	L&R	W
I-10	3N_close	38	3.78	3.21	0.39	0.37	422	-0.030	0.001	-0.010
	3S_close	26	4.11	3.36	0.35	0.36	342	0.016	0.015	-0.058
	3S_far	27	3.78	3.13	0.37	0.34	380	0.055	0.014	0.063
	4S_close	26	3.67	3.07	0.27	0.31	219	0.133*	0.045***	0.040
	4N_close	28	3.67	3.04	0.37	0.35	380	0.026	0.018*	0.091
	4N_far	24	3.33	3.03	0.36	0.37	360	-0.020	0.018	-0.026
	CN2	26	3.78	3.14	0.35	0.35	342	0.028	0.024*	-0.010
NM-9	6S_close	28	3.78	3.21	0.36	0.37	360	-0.007	0.012	-0.052
	6N_close	26	4.00	3.25	0.38	0.36	400	0.015	0.018	0.059
	6N_mid	27	4.67	3.76	0.39	0.39	422	-0.070	-0.007	-0.109
	8N_close	26	4.11	3.40	0.41	0.38	468	-0.046	0.017	0.014
	8S_close	26	3.22	2.83	0.36	0.33	360	0.086	0.035***	0.158
	8S_mid	21	3.67	3.16	0.39	0.35	422	0.034	0.013	0.093
	CN3	22	4.00	3.34	0.35	0.36	342	-0.005	-0.009	-0.017
Outgroups	OG_TX	16	2.56	2.33	0.24	0.31	183			
	OG_AZ	16	6.22	5.61	0.64	0.63	1679			
	OG_NV	16	5.89	5.14	0.62	0.56	1481			
	OG_OR	18	1.33	1.26	0.06	0.06	33			
	OG_WA	15	1.22	1.16	0.06	0.17	34			

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , significantly higher relatedness than expected based on 1000 bootstrap iterations

H<sub>O</sub> = observed heterozygosity, H<sub>E</sub> = expected heterozygosity, N<sub>e</sub> = effective population size

Q&G = Queller and Goodnight (1989), L&R = Lynch and Ritland (1999), W = Wang (2002)

Population names detailed in Supplementary table S3.1



Table 3.2. Diversity statistics for marbled whiptails.

Road	Population	Number of individuals	Number alleles per locus	Allelic richness	H <sub>O</sub>	H <sub>E</sub>	N <sub>e</sub>	Relatedness:		
								Q&G	L&R	W
I-10	3N_close	28	6.2	5.27	0.62	0.55	1481	0.011	0.006	0.055
	3S_close	28	6.2	5.25	0.5	0.55	750	-0.001	-0.006	-0.014
	3S_far	28	6.2	5.36	0.5	0.54	750	0.020	-0.005	-0.019
	4S_close	28	6.2	5.23	0.53	0.54	882	0.024	0.015*	0.008
	4N_close	28	5.2	4.48	0.54	0.53	931	0.074**	0.050***	0.057
	4N_far	26	5.6	4.94	0.58	0.53	1167	0.055*	0.009	0.055
	CN2	23	6.4	5.32	0.58	0.58	1167	-0.040	0.001	-0.014
NM-9	6S_close	23	5.6	4.97	0.55	0.55	985	0.001	0.005	0.005
	6N_close	28	6.4	5.42	0.59	0.55	1237	0.018	0.023**	0.021
	6N_mid	19	6.2	5.27	0.6	0.56	1313	0.026	0.020	0.028
	8N_close	20	5.2	4.70	0.51	0.55	791	-0.013	0.004***	-0.003
	8S_close	27	5.4	4.82	0.55	0.56	985	-0.002	0.036	0.001
	8S_mid	28	6.2	5.14	0.51	0.55	791	0.013	0.001	0.002
	CN3	28	6.4	5.50	0.57	0.56	1102	-0.009	-0.004	-0.018
Outgroups	OG_TX	19	5.0	4.50	0.46	0.49	607			
	OG_TX2	19	5.8	4.99	0.47	0.48	640			

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , significantly higher relatedness than expected based on 1000 bootstrap iterations

H<sub>O</sub> = observed heterozygosity, H<sub>E</sub> = expected heterozygosity, N<sub>e</sub> = effective population size

Q&G = Queller and Goodnight (1989), L&R = Lynch and Ritland (1999), W = Wang (2002)

Population names detailed in Supplementary table S3.1

Table 3.3. Pairwise genetic distances for side-blotched lizards. Jost's D is above the diagonal,  $F_{ST}$  below the diagonal. Values where the 95% confidence interval based on 1000 bootstrap iterations do not overlap 0 are indicated by \*.

	3N_close	3S_close	3S_far	4S_close	4N_close	4N_far	CN2	6S_close	6N_close	6N_mid	8N_close	8S_close	8S_mid	CN3	OG_TX	OG_AZ	OG_NV	OG_OR	OG_WA
3N_close		0	0	0.005	0.001	0	0	0	0	0	0	0.001	0	0	0.03*	0.351*	0.349*	0.582*	0.437*
3S_close	0.002		0	0.005	0.003	0.004	0	0.002	0.002	0.002	0.009	0.006	0.005	0.005	0.020	0.305*	0.34*	0.557*	0.432*
3S_far	-0.001	-0.004		0.001	0	0.002	0.001	0	-0.003	0.003	0.004	0.002	0.001	0.002	0.030*	0.328*	0.355*	0.539*	0.424*
4S_close	0.014	0.025	0.011		0	0.002	0.012*	0.008	0	0.005	0.006	0.001	0.003	0.002	0.039*	0.374*	0.416*	0.548*	0.415*
4N_close	0.004	0.009	-0.001	0.003		0	0.005	0.002	0	0.005	0.004	0.006	0.004	0.004	0.041*	0.364*	0.408*	0.529*	0.428*
4N_far	-0.002	0.014	0.009	0.010	-0.004		0	0.004	0	0.006	0.002	0.003	0.003	0.002	0.048*	0.373*	0.411*	0.558*	0.449*
CN2	0.001	0.003	0.006	0.026	0.014	0.002		0.003	0.003	0.001	0.005	0.004	0.001	0	0.039*	0.368*	0.360*	0.577*	0.455*
6S_close	-0.003	0.008	0.003	0.021	0.011	0.019	0.011		0.002	0.002	0	0.003	0.003	0	0.019	0.357*	0.358*	0.603*	0.433*
6N_close	0.001	0.011	-0.007	0.001	-0.004	0.002	0.012	0.009		0.002	0.004	0.003	0	0	0.035*	0.348*	0.377*	0.520*	0.419*
6N_mid	0.005	0.014	0.013	0.026	0.024	0.014	0.013	0.008	0.01		0.003	0.001	0	-0.001	0.026*	0.363*	0.322*	0.551*	0.403*
8N_close	0.001	0.025*	0.012	0.018	0.016	0.014	0.018	-0.002	0.014	0.007		0	0	0	0.027*	0.379*	0.364*	0.595*	0.429*
8S_close	0.009	0.034*	0.020	0.009	0.019	0.016	0.028	0.012	0.011	0.007	0		-0.003	-0.003	0.022*	0.378*	0.372*	0.552*	0.416*
8S_mid	0.003	0.025	0.008	0.013	0.016	0.012	0.012	0.008	0.004	0.002	-0.005	-0.006		-0.001	0.026*	0.376*	0.343*	0.549*	0.413*
CN3	0.004	0.023	0.012	0.008	0.013	0.011	0.018	0	0	-0.005	0.001	-0.007	-0.003		0.022	0.35*	0.345*	0.560*	0.410*
OG_TX	0.111*	0.085*	0.108*	0.134*	0.112*	0.138*	0.127*	0.084*	0.115*	0.077*	0.111*	0.121*	0.129*	0.087*		0.351*	0.412*	0.548*	0.396*
OG_AZ	0.374*	0.366*	0.376*	0.397*	0.368*	0.358*	0.379*	0.361*	0.361*	0.334*	0.355*	0.379*	0.365*	0.345*	0.346*		0.058*	0.266*	0.234*
OG_NV	0.441*	0.439*	0.448*	0.469*	0.445*	0.432*	0.446*	0.429*	0.436*	0.407*	0.424*	0.446*	0.432*	0.419*	0.430*	0.055*		0.250*	0.261*
OG_OR	0.672*	0.698*	0.703*	0.731*	0.691*	0.695*	0.705*	0.691*	0.690*	0.667*	0.690*	0.714*	0.716*	0.703*	0.752*	0.372*	0.438*		0.016*
OG_WA	0.614*	0.643*	0.651*	0.68*	0.641*	0.643*	0.654*	0.626*	0.648*	0.605*	0.626*	0.661*	0.666*	0.640*	0.693*	0.414*	0.435*	0.647*	

Table 3.4. Pairwise genetic distances for marbled whiptails. Jost's D is above the diagonal,  $F_{ST}$  below the diagonal. Values where the 95% confidence interval based on 1000 bootstrap iterations do not overlap 0 are indicated by \*.

	3N_close	3S_close	3S_far	4S_close	4N_close	4N_far	CN2	6S_close	6N_close	6N_mid	8N_close	8S_close	8S_mid	CN3	OG_TX	OG_TX2
3N_close		0	-0.009	0.003	0.017	0.000	0.001	0.009	0.007	0.032	1E-04	0.026	-0.001	0	0.016	0.013
3S_close	-0.003		-0.003	-0.005	0.006	1E-04	0.003	0.000	0.006	0.008	0.001	0.002	-0.001	0.003	0.017	0.014
3S_far	-0.006	-0.005		-0.001	0.019	-0.008	0.000	0.004	0.019	0.003	0.003	0.022	0.003	-0.004	0.032	0.016
4S_close	-1E-04	-0.007	-0.004		0.022	0.003	0.013	0.005	0.021	0.005	0.001	0.020	0.002	0.008	0.020	0.013
4N_close	0.014	0.004	0.015	0.016		0.018	0.006	0.040*	0.027	0.006	0.011	0.044*	0.033*	0.048*	0.031	0.032
4N_far	0.003	0.003	-0.006	0.008	0.026		1E-04	0.003	0.030	0.011	0.009	0.036	0.010	0.001	0.022	0.003
CN2	3E-04	3E-04	4E-04	0.010	0.008	0.005		0.001	0.010	0.023	0.007	0.022	0.010	0	0.017	0.022
6S_close	0.006	0.001	0.006	0.011	0.030	0.012	0.002		0	0.004	-0.013	0	0.000	0	0.039	0.051*
6N_close	0.008	0.009	0.022	0.019	0.028*	0.037*	0.009	-0.002		0.035	0.010	0.013	0	0.000	0.029	0.014
6N_mid	0.028	0.019	0.022	0.026	0.031	0.030	0.021	0.002	0.025		0.003	0.023	0.013	0.016	0.086*	0.099*
8N_close	-0.005	-0.005	-0.004	-0.004	0.012	0.007	0.006	-0.012	0.005	-0.001		0.004	-0.019	0.002	0.031	0.034
8S_close	0.018	0.004	0.016	0.013	0.037*	0.028	0.024	-0.001	0.011	0.015	0.001		0.000	0.005	0.050*	0.064*
8S_mid	-0.002	-0.003	0.001	0.000	0.025	0.008	0.008	-0.008	0.004	0.007	-0.015	0.001		-0.001	0.042	0.024
CN3	0.000	0.002	-0.006	0.006	0.034*	0.006	0.005	-0.007	0.003	0.014	-0.004	0.003	-0.005		0.036	0.045*
OG_TX	0.015	0.011	0.024	0.019	0.033*	0.023	0.011	0.033	0.028	0.080*	0.026	0.051*	0.030	0.034		-0.001
OG_TX2	0.014	0.010	0.014	0.017	0.027	0.007	0.015	0.041*	0.041*	0.076*	0.027	0.057*	0.024	0.034	-0.010	

Table 3.5. Isolation by distance and partial Mantel test results for side-blotched lizards and marbled whiptails. A) Isolation by distance was measured using the transformed genetic distance ( $F_{ST} / 1 - F_{ST}$ ) as suggested by Rousset (1997) and the ln-transformed geographic distance. Significance was assessed using 10,000 bootstrap iterations. B) Partial Mantel tests were used to assess the importance of roads as a barrier while partialling out the geographic distance, using 10,000 bootstrap iterations to assess significance.

A) Simple Mantel								
	Side-blotched lizards				Marbled whiptails			
	$F_{ST}$		Jost's $D$		$F_{ST}$		Jost's $D$	
	Mantel's $r$	Significance	Mantel's $r$	Significance	Mantel's $r$	Significance	Mantel's $r$	Significance
All populations	0.671	9.9E-05	0.699	9.9E-05	0.468	1.0E-04	0.427	3.0E-04
Study region only	0.415	4.0E-04	0.412	0.001	0.299	6.0E-04	0.241	0.003
I-10 only <sup>1</sup>	0.398	0.031	0.274	0.115	-0.231	0.929	-0.124	0.760
NM-9 only <sup>1</sup>	0.172	0.153	0.309	0.051	0.018	0.431	0.022	0.439

B) Partial Mantel, genetic distance relative to side of the road								
	$F_{ST}$		Jost's $D$		$F_{ST}$		Jost's $D$	
	Mantel's $r$	Significance	Mantel's $r$	Significance	Mantel's $r$	Significance	Mantel's $r$	Significance
Study region only	-0.105	0.786	-0.105	0.778	0.137	0.223	0.214	0.092
I-10 only <sup>1</sup>	-0.105	0.778	-0.092	0.615	-0.065	0.668	-0.071	0.646
NM-9 only <sup>1</sup>	-0.166	0.833	-0.233	0.894	-0.194	0.878	-0.206	0.917

<sup>1</sup>Only 5040 permutations possible with 7 populations

### 3.9 FIGURES

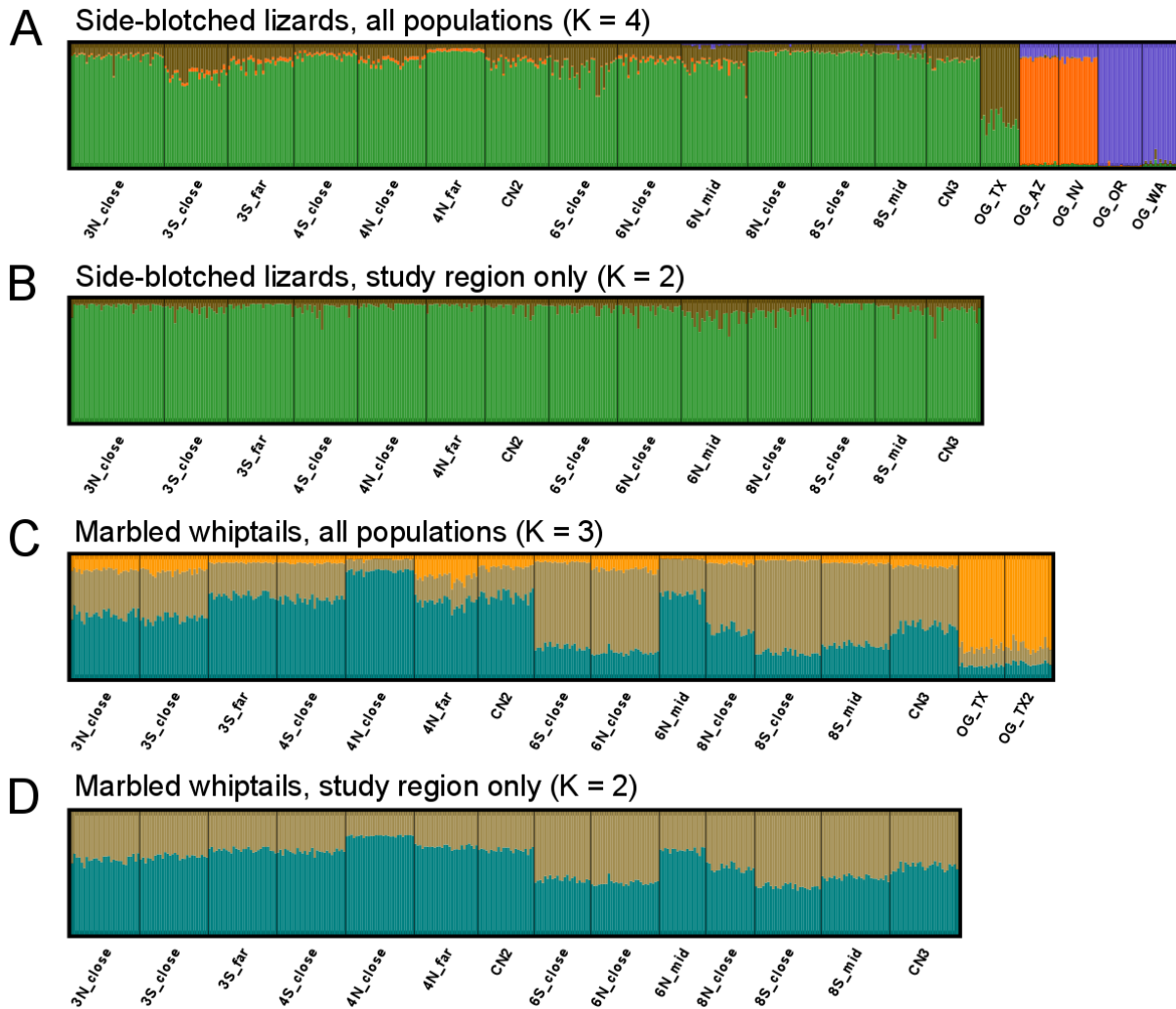


Fig. 3.1. Bayesian clustering results for side-blotched lizards and marbled whiptails. Each column represents one individual and the color represents the percentage each individual is assigned to a genetic cluster ( $K$ ). The results for all populations (A and C) are for the most likely value of  $K$ . For the plots of the study region populations (B and D), the Evanno method indicated  $K = 2$ , but the results of the bar plots show that  $K = 1$  is more likely.

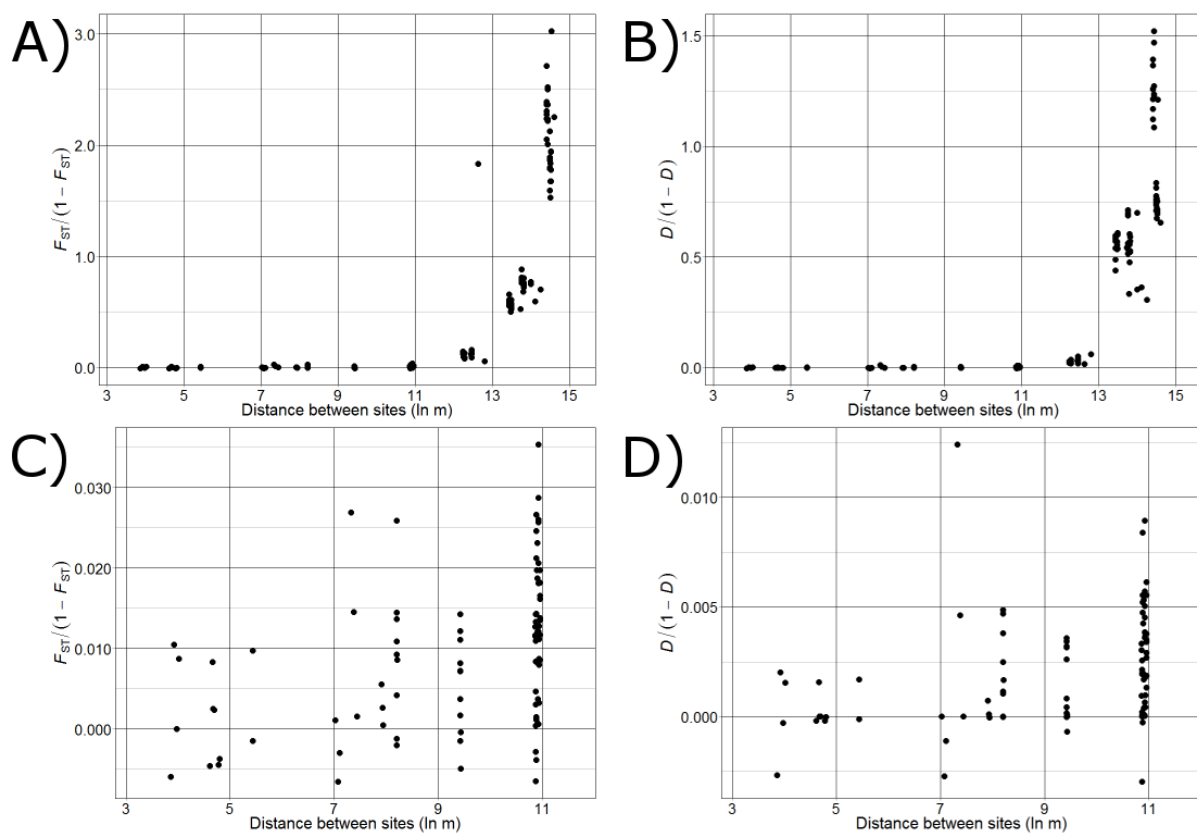


Fig. 3.2. Isolation by distance plots for side-blotched lizards. There was significant isolation by distance with all populations for A)  $F_{ST}$  and B) Jost's  $D$ , and for just the populations in the study region for C)  $F_{ST}$  and D) Jost's  $D$ . Note the difference scales on the y-axes for all plots, and the x-axes for A and B vs. C and D.

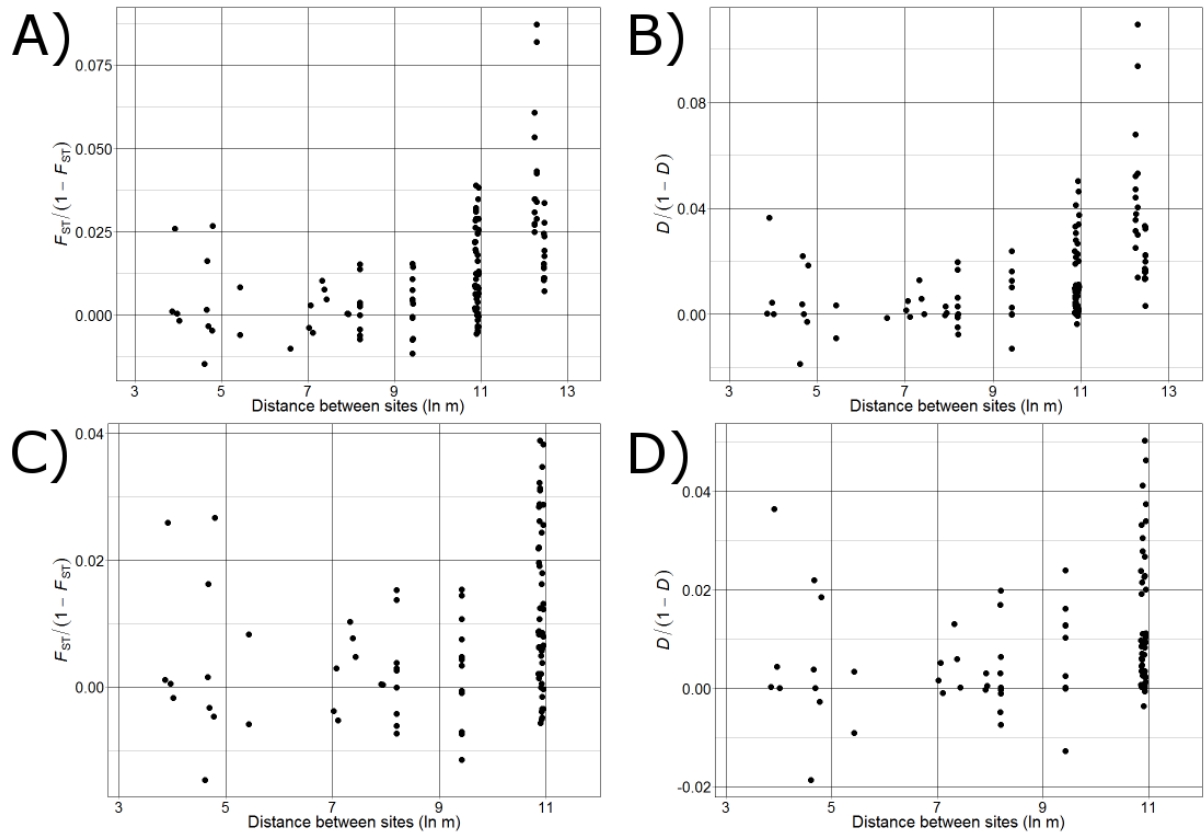


Fig. 3.3. Isolation by distance plots for marbled whiptails. There was significant isolation by distance with all populations for A)  $F_{ST}$  and B) Jost's  $D$ , and for just the populations in the study region for C)  $F_{ST}$  and D) Jost's  $D$ . Note the difference scales on the y-axes for all plots, and the x-axes for A and B vs. C and D.

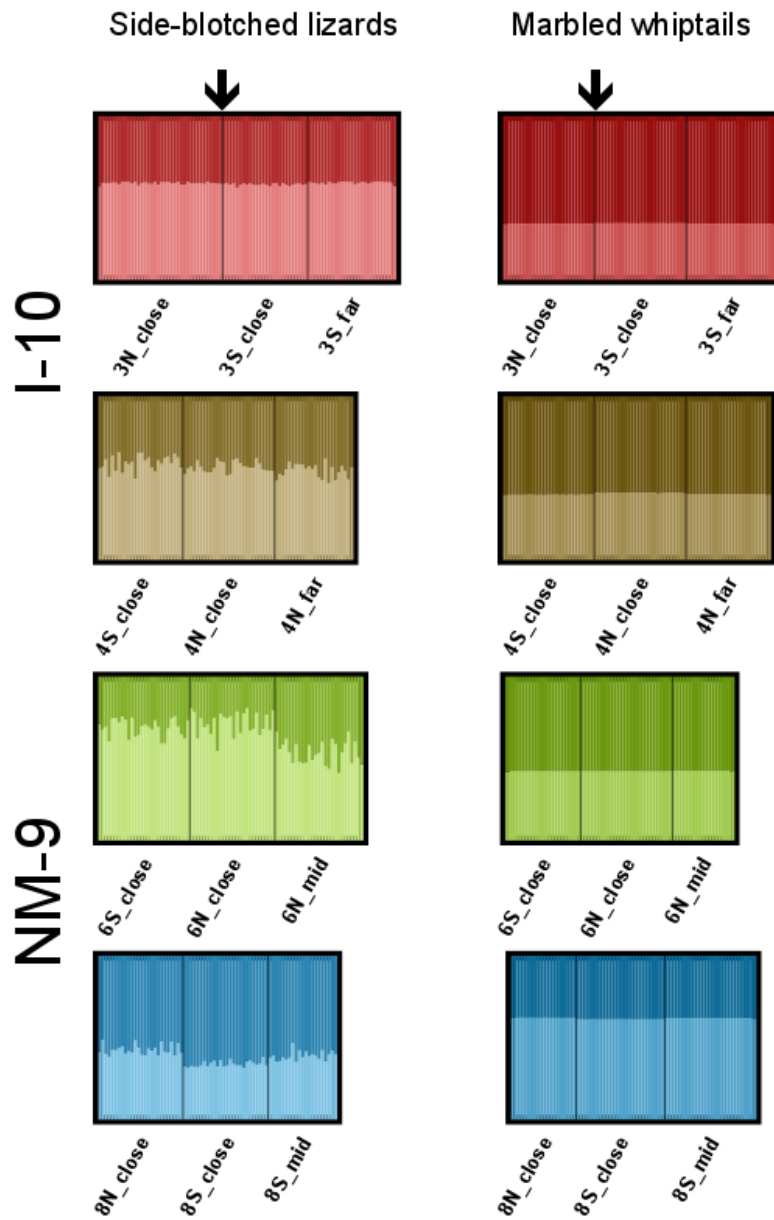


Fig. 3.4. Bayesian clustering results for barrier detection at each site. Each site was analyzed separately setting  $K = 2$ . If a road is a barrier, the individuals on opposite sides (N and S) should be assigned to different clusters. The arrows indicate the relative location of the road.



### 3.10 SUPPLEMENTARY DATA

Table S3.1. Sample location and distance to the road. The number in the population name refers to the particular study site and the N and S refer to the side of the road. CN2 and CN3 are the control sites. Five potential sites and two control sites were mapped for each road and were numbered sequentially. Only three sites and one control site were included per road for the trapping study (Chapter 2), but the original numbers were retained for archival purposes. Lizards from study sites 1 and 9 were used for the population demography portion of the study (Chapter 2), but not for the genetics portion.

Road	Population name	Latitude	Longitude	Distance to road (m)
I-10	3N_close	32.25275	-107.09807	24
	3S_close	32.25178	-107.09788	30
	3S_far	32.25072	-107.09772	149
	4S_close	32.24737	-107.13617	28
	4N_close	32.24832	-107.13635	27
	4N_far	32.24939	-107.13661	149
	CN2	32.23918	-107.12324	1236
NM-9	6S_close	31.79410	-106.95729	14
	6N_close	31.79460	-106.95728	32
	6N_mid	31.79505	-106.95733	81
	8N_close	31.80857	-106.82761	30
	8S_close	31.80810	-106.82752	14
	8S_mid	31.80768	-106.82744	62
	CN3	31.81856	-106.82917	1034
Outgroup	OG_TX <sup>1</sup>	30.77189	-105.01123	NA
	OG_TX <sup>2</sup>	30.77688	-105.01617	NA
	OG_AZ	33.56667	-114.26667	NA
	OG_NV	36.48333	-116.11667	NA
	OG_OR	44.35000	-121.28333	NA
	OG_WA	46.96667	-119.96667	NA

<sup>1</sup>Called Prospect in some figures

<sup>2</sup>Called Ranchhouse in some figures

Table S3.2. PCR conditions and descriptive statistics per locus, averaged over all populations for both species. All PCR reactions were conducted in 10  $\mu$ L using multiplex PCR kits (QIAGEN, Valenica, CA). Loci were grouped into multiplex groups based on similar annealing temperatures and to minimize size overlap of alleles. All PCRs had an initial denaturing at 95°C for 15 minutes, followed by 32 cycles of 94°C for 30 s, the specific T<sub>A</sub> for 90 s, and 72°C for 60 s, followed by a final extension at 60° C for 45 min.

	Locus	multiplex group	T <sub>A</sub> (°C)	Primer conc. <sup>1</sup>	Fluorophore	Total unique alleles	Allele richness per pop.	size range	H <sub>O</sub> per pop.	H <sub>E</sub> per pop.
Side-blotched lizards	SVeg	1	51	0.2 $\mu$ M	NED	5	1.26 (0.10)	104 - 118	0.02 (0.01)	0.02 (0.01)
	MCC	1	51	0.08 $\mu$ M	6-FAM	6	1.37 (0.22)	143 - 155	0.06 (0.03)	0.05 (0.03)
	BRtt	1	51	0.2 $\mu$ M	VIC	12	2.89 (0.40)	160 - 182	0.32 (0.05)	0.29 (0.05)
	SMcL	1	51	0.3 $\mu$ M	NED	15	6.47 (0.49)	193 - 233	0.71 (0.07)	0.69 (0.05)
	SPhill	1	51	0.3 $\mu$ M	PET	13	6.84 (0.55)	196 - 224	0.71 (0.06)	0.69 (0.05)
	PLkn	2	56	0.15 $\mu$ M	VIC	13	7.00 (0.52)	177 - 203	0.63 (0.05)	0.64 (0.04)
	10000M's	2	56	0.1 $\mu$ M	6-FAM	7	1.37 (0.24)	182 - 224	0.04 (0.03)	0.05 (0.04)
	IGs	2	56	0.1 $\mu$ M	PET	9	3.00 (0.40)	292 - 308	0.29 (0.05)	0.31 (0.05)
	NGff	2	56	0.1 $\mu$ M	NED	8	3.32 (0.32)	280 - 304	0.39 (0.05)	0.41 (0.04)
Marbled whiptails	Acos5	1	55	0.3 $\mu$ M	VIC	2	1.06 (0.06)	161 - 167	0 (0)	0.01 (0.01)
	Acos2	1	55	0.3 $\mu$ M	PET	1	1.0 (0)	196	0 (0)	0 (0)
	Ai5013 <sup>2</sup>	1	55	0.3 $\mu$ M	NED	NA	NA	NA	NA	NA
	Ai5062	1	55	0.3 $\mu$ M	6-FAM	13	7.94 (0.28)	229 - 281	0.81 (0.02)	0.77 (0.01)
	Acos3	2	55	0.2 $\mu$ M	PET	4	2.31 (0.12)	213 - 221	0.42 (0.04)	0.49 (0.01)
	Ai5071	2	55	0.16 $\mu$ M	VIC	1	1.0 (0)	239	0 (0)	0 (0)
	Ai5043	2	55	0.16 $\mu$ M	NED	4	3.00 (0.13)	199 - 208	0.53 (0.03)	0.53 (0.02)
	Ai5037	2	55	0.2 $\mu$ M	6-FAM	24	15.13 (0.45)	212 - 304	0.95 (0.01)	0.91 (0.003)

<sup>1</sup>Primer concentration for whiptails are given for the reverse direction primer. The forward direction and the fluorescently-tagged "tail" were each at half the concentration of the reverse primer.

<sup>2</sup>Alleles for this locus amplified consistently, but the repeat numbers were not consistent with the published repeat size. The repeat was 4 bp, but some alleles were 1, 2, and 3 bp apart. We could not determine the correct allele sizes, so excluded this locus from all analyses.

## **Chapter 4: Synthesis and Conclusions**

### **4.1 SYNTHESIS**

This study has shown that, despite differences in life history and movement behaviors, neither lizard species was negatively impacted by either road analyzed. Behavioral avoidance of open areas by both species is the likely reason that abundance and survival were not decreased near the roads. Neither species completely avoided crossing the smaller road, with more whiptails than side-blotched lizards detected crossing, as expected. It is unclear if these species also behaviorally avoid vehicles (Jaeger et al. 2005). Although no individuals were detected crossing the heavily-traveled interstate (as expected if they avoid vehicles), the reliance on trapping animals limited the overall ability to assess movement rates. In one of the only studies of lizard behavior pertinent to road type, Brehme et al. (2013) concluded that both lizard species studied did show vehicle avoidance because no lizards crossed a heavily traveled road, but did cross a lightly traveled road of similar dimensions. They used fluorescent powder to track individual movements, but did have problems with some lizard species, including side-blotched lizards, due to the smooth scales not holding the powder as well as the fur of small mammals did. Similar studies, perhaps using different methods (discussed below), would help our understanding of the relationship between road size, traffic volume, and lizard behavior. Given the consistency of the abundance and survival estimates for both species, it seems unlikely that the short-term population persistence of either species is at risk under current conditions.

One long-term concern is that behavioral avoidance of roads will create genetic isolation, eventually leading to lower genetic diversity, inbreeding depression, and overall lower probability of population persistence. In our study region, these negative genetic impacts will likely take much longer to become apparent unless there are significant changes to landscape use. The study region is relatively unique in the United States in that the road density is low and the habitat is mostly

undisturbed (Riitters and Wickham 2003, Gardner et al. 2007). Large populations in unfragmented habitats have reduced rates of genetic drift and less inbreeding (Allendorf et al. 2013). The slow rate of genetic change makes current genetic methods unable to detect shifts in population structure for many generations (Safner et al. 2011b). Even the individual based assignment methods like STRUCTURE require some degree of genetic distinctiveness in source populations to identify genetic clusters. When the formation of the putative barriers dividing large populations are relatively recent, the genetic structure will not be informative for assessing the strength of the barrier for many generations (Safner et al. 2011b). However, the same features that make the genetic changes slow to appear also provide longer periods in which to address the potential problems created by the barriers. Such timeframes can allow for prioritization of mitigation efforts to regions with higher levels of fragmentation and/or smaller population sizes. It can also change the mitigation strategy. If the populations are currently large, mitigation can focus on methods to keep animals out of roadways, such as fencing or vegetation management of the verge (e.g., Grilo et al. 2014). More expensive mitigation efforts designed to restore connectivity can be postponed or redirected to other regions.

This study is one of the few to combine population demography and population genetics to fully assess the impacts of roads. The combination of data collected here allowed a more complete picture of the status of both species, particularly with regards to the genetic findings. Without estimates of abundance and movement rates, finding similar levels of genetic diversity and a lack of genetic differentiation across the study region would have been more difficult to explain. More road studies are beginning to include demography and genetics, although most are using the demography to estimate dispersal rather than population size (e.g., Riley et al. 2006, Stevens et al. 2006, Safner et al. 2011a). Other population and landscape genetic studies focused

on habitat fragmentation are using a combination of techniques, often gaining better insights than a single technique would allow (e.g., Templeton et al. 2011, Lamy et al. 2012, Neuwald and Templeton 2013, Tobler et al. 2013). One reason for not using multiple complementary methods is the increased difficulty in logistics and study design. Assessing demographic trends often requires multiple surveys at the same locations, while genetic sampling is best conducted over a broader spatial scale and can usually be completed in a single collection event (e.g., Schwartz and McKelvey 2009, Anderson et al. 2010, Robinson et al. 2012). Alternative methods for obtaining inferences of population demographics in shorter time periods include distance sampling (Buckland 2004) and occupancy modeling (MacKenzie and Kendall 2002, MacKenzie et al. 2006), but these methods do not allow for estimates of age (at least for small animals like lizards), movement, or individual survival. They also do not require capturing animals, so collecting tissue samples for genetic analysis would require different approaches. Non-invasive genetic sampling using hair snags or fecal DNA can be used to both estimate animal abundance and population genetics (e.g., Mills et al. 2000, Waits and Paetkau 2005). These techniques are commonly used with mammals that are difficult to otherwise survey, but it is difficult to envision how such methods might be applied to most reptiles other than snakes, where shed skins are more obvious in the environment and can be a source of genetic material (Fetzner Jr 1999). The inherent complexities of integrating demographics and genetics make studies such as the one reported here relatively rare.

#### **4.2 FUTURE DIRECTIONS**

The results of this study demonstrate that these two species of lizards are not negatively affected by these two roads. How generalizable these results are to other locations and species is not yet known. The two roads in the study represent two relatively opposite ends of the size and

traffic volume spectrum. The low volume road was very lightly traveled, and although lizards did show some reduction in crossing frequency, some proportion did still cross the road. The high volume road had approximately 30X the traffic volume as the low volume road, although still much lower than the roads in southern California studied by Delaney et al. (2010). Studying how lizards respond to roads with intermediate traffic volumes would help to determine a potential threshold volume when enough vehicles pass to increase mortality risks close to 100%. At that point the road will become a complete barrier, either from mortality or behavioral avoidance. If the lizards do show traffic avoidance as suggested by Brehme et al. (2013), studying the responses to a wider range of traffic volumes will help to distinguish between mortality and avoidance at different volumes.

Overall, understanding how animals behave upon encountering a road is a critical and understudied part of road ecology. There have been mixed results in the reptile species studied to date. Some snakes have shown partial road avoidance (Shine et al. 2004, Andrews and Gibbons 2005, Kovar et al. 2014, Siers et al. 2014), while others have not (Row et al. 2007). Even with partial avoidance, enough snakes use roads that road-cruising is a common method for sampling snakes, and enough are killed to warrant concern (Andrews et al. 2008). A similar result was found for turtles, with evidence that at least some species show partial road avoidance (Shepard et al. 2008, Proulx et al. 2014), but with freshwater turtles in particular suffering high enough rates of road mortality to shift the sex ratios to be male biased (Gibbs and Steen 2005, Steen et al. 2006). Additional studies of lizard behavior will be difficult for small species like the ones in this study. Radio-tracking lizards has been done for at least two decades (Ellis-Quinn and Simony 1991), and transmitter weights have decreased to a size that allow smaller lizards to be tracked. A recent study tracked adult southwestern fence lizards (*Sceloporus cowlesi*) using transmitters weighing

0.35 g, which was about 5% of the lizard body mass (Refsnider et al. *in press*). These lizards are slightly larger than side-blotched lizards, but much smaller than whiptails. Thus whiptails could potentially be radio-tracked to assess their movement patterns, but side-blotched lizards would be more challenging. Nevertheless, transmitters continue to decrease in mass, and are currently used to track larger insects (Kissling et al. 2014). Tracking side-blotched lizards is thus not an impossibility. However, radio-tracking is a relatively expensive endeavor, particularly when smaller transmitters must be used. Other options for measuring movement behaviors include the fluorescent powder used by Brehme et al. (2013) or more intensive surveillance of marked individuals (e.g., Peterson and Whitford 1987).

It is also important to determine how landscape configuration might affect the impacts of roads. As discussed above, the study region was mostly undisturbed save for the presence of the two roads. In regions with higher road densities each habitat patch would be smaller, and would support smaller populations. Smaller populations are at greater risk of extirpation from demographic effects (Lande 1993), and from decreased genetic diversity due to increased genetic drift and inbreeding (Frankham 2005). Many of the previous road ecology studies that found genetic changes were in regions with higher levels of fragmentation and smaller patch sizes (e.g., Epps et al. 2005, Keller et al. 2005, Delaney et al. 2010, Major et al. 2014). Changes should occur more rapidly in such situations, but these studies confounded the effects of habitat fragmentation overall with effects specific to roads. The studies that have found reduced gene flow across roads sampled lizard populations in patches of different sizes and degrees of isolation (Delaney et al. 2010, Munguia-Vega et al. 2013). Importantly, studies examining the effects of habitat fragmentation (not road related) on lizards have found increased genetic differentiation in isolated patches, especially for species that use habitat features that are patchy in the landscape like rock

outcrops (e.g., Stow et al. 2001, Berry et al. 2005, Levy et al. 2013, Neuwald and Templeton 2013). To extend the results of the current study, a similar sampling approach to these habitat fragmentation studies should be used. Side-blotched lizards and marbled whiptails (and other members of the *tigris* complex) are widespread across variety of habitats in the southwestern U.S. (Pianka 1970, Parker and Pianka 1975), and thus should be found in a wide variety of remnant habitat patches of different sizes and degrees of isolation. As such they would be excellent model organisms for examining the effects of roads across a wide range of road sizes and patchy habitat types.

From a conservation perspective, the cause of the fragmentation is perhaps less important than determining the effects and mitigating them. If fragmentation is due to urbanization or agriculture, restoring connectivity between remaining populations might be more difficult because of larger expanses of non-habitat (also called the “matrix” in fragmentation studies). However, most roads are relatively narrow, at least in comparison with agricultural fields or cities, so restoring connectivity is more feasible. Mitigation efforts are usually either wildlife overpasses or underpasses, and these are being implemented globally (Clevenger 2012). Although initially structures were built and not monitored for their effectiveness, studies are beginning to determine how well different structures work at restoring demographic and genetic connectivity (van der Ree et al. 2009, van der Grift et al. 2012, Sawaya et al. 2013, Sawaya et al. 2014). Although the results of the current study do not suggest that mitigation measures are required for side-blotched lizards or marbled whiptails in the study region, a better understanding of how to best design mitigation measures for smaller animals could be useful in regions with greater levels of fragmentation.

Finally, future studies should expand to other species of lizards, in particular larger lizards, more mobile lizards, or those that use open habitat more often. Studies on mammals (Rytwinski



and Fahrig 2011) and snakes (Dodd et al. 1989, Andrews and Gibbons 2005) found that larger animals were more likely to suffer negative road effects. How larger lizards are affected by roads is largely unknown. The movement patterns of blue-tongued lizards (*Tiliqua scincoides*) in suburban Australia indicated that they actively avoided roads (Koenig et al. 2001). However, Gila monsters (*Heloderma suspectum*) in Phoenix, AZ, crossed lightly used two-lane roads and used refuges within 2 m of roads (Kwiatkowski et al. 2008). Neither study addressed potential road mortality. The larger lizards in the study region of the current study were much less abundant than either side-blotched lizards or marbled whiptails, making it impossible make any inferences about how they interact with roads. For example, only 36 desert spiny lizards (*Sceloporus magister*), 18 Texas horned lizards (*Phrynosoma cornutum*), and 15 long-nosed leopard lizards (*Gambelia wislizenii*) were trapped out of a total of >3600 unique lizards. More vagile species of frogs (Carr and Fahrig 2001), snakes (Jochimsen et al. 2014), and turtles (Reid and Peery 2014) experience greater negative road effects. Including a wider variety of lizard species of a variety of sizes and with different movement behaviors would help to determine if there are generalizable trends across not only reptiles, but other taxa as well. Although I compared two species with different sizes, life histories, and movement behaviors in this study, perhaps they were too similar (relative to range of sizes represented in mammals) to show differential responses to the roads.

#### **4.3 CONCLUSIONS**

The study region was selected because the roads were the only form of major disturbance. This situation allowed for an assessment of the direct impacts of the roads instead of measuring larger patterns of habitat fragmentation with roads only part of the overall landscape. I was able to determine that lizards in this region are currently unaffected by the two roads, either

demographically or genetically. However, the desert southwest is expected to undergo changes from both climate change and continued increases in the human population.

Climate change models predict that the southwest will become warmer and drier during this century (Seager et al. 2007, Diffenbaugh et al. 2008, Seager and Vecchi 2010, Garfin et al. 2013). Decreased soil moisture and drought conditions could increase to levels that are unprecedented during the past 1000 years (Cook et al. 2015). The precipitation in 2011 was below average in the study region, and I observed decreases in annual and perennial plant growth, and the associated decrease in the insect community. The side-blotched lizard abundance decreased in response, and the marbled whiptail juveniles did not hatch at the usual time. Whiptail hatchlings first appeared in the sites in July and August in 2009 and 2010, but did not appear until September in 2011. It is unclear if this late appearance was due to a delay in egg laying or an overall decrease in egg laying. The precipitation remained below average in 2012 before returning to average in 2013 (National Weather Service, [http://www.srh.noaa.gov/epz/?n=santateresa\\_monthly\\_precip](http://www.srh.noaa.gov/epz/?n=santateresa_monthly_precip), accessed 14 July 2015). Relatively brief fluctuations are common in the desert (Cook et al. 2015), and I expect that the populations of both species have rebounded since 2011–2012 (although I have not conducted field surveys since then). The longer periods of drought expected this century will present an additional stress to the lizard populations. In the large and relatively undisturbed habitat where I conducted this study, it is probable that the lizards will persist. In regions with increased fragmentation and smaller patch sizes, the smaller populations are more likely to be extirpated (Lande 1993). If those patches are isolated, for example by roads, agriculture, or urbanization, those patches may not be recolonized.

Although there is uncertainty regarding the shift of biomes in the southwest as climate change progress, the Chihuahuan Desert shrubland is expected to remain in the same general

region, with possible expansion altitudinally (Rehfeldt et al. 2012). Thus the lizards in the region will likely not face the gradual shift in distribution that species living in most sensitive biomes face (Parmesan 2006). Species in biomes that are projected to shift in latitude or altitude might encounter roads during their migration, and could be prevented from tracking their habitat requirements. Reptiles in general may be particularly sensitive to shifting biomes because of their low mobility. Habitat specialists like the Coachella Valley fringe-toed lizard (*Uma inornata*), a dune-restricted habitat specialist, was projected to persist during increased temperature and drought if their habitat was not fragmented (Barrows et al. 2010). However, the Coachella Valley is highly fragmented, and the fringe-toed lizards persist in small isolated populations that are more likely to experience population declines during droughts. Highlighting the risk that projected climate change presents, Barrows et al. (2010) documented the extirpation of several small lizard populations during an extended drought from 2000–2004. Although many lizards bask to reach their specific physiologically active body temperature, high temperatures can cause them to exceed the critical thermal maximum if they do not retreat to cooler refuges (Sinervo et al. 2010). As temperatures increase, the length of time that lizards must spend in their refuges increases, and they have less time to forage and reproduce, which can decrease population growth rates. Sinervo et al. (2010) found that 12% of local populations of 48 *Sceloporus* species in Mexico present in 1975 had been extirpated by 2009. Extirpated populations were correlated with higher rates of temperature changes during the winter–spring breeding seasons. Sinervo et al. (2010) estimated that 58% of Mexican *Sceloporus* species and 20% of all lizard species could be extinct by 2080. The most susceptible species were the habitat specialists, particularly those in higher montane environments. Munguia-Vega et al. (2013) suggested that habitat fragmentation and increased temperatures have synergistic effects on the persistence of isolated populations of black-tailed

brush lizards (*Urosaurus nigricaudus*). The loss of vegetation cover due to habitat conversion reduced shade between remnant habitat patches, and the small body size and limited dispersal abilities of the lizards could prevent movement between patches as temperatures continue to increase (Munguia-Vega et al. 2013). The side-blotched lizards and marbled whiptails in the study region are habitat generalists and are not likely to experience the same problems as the montane Mexican lizards if the habitat remains unfragmented.

The Southwest has been the fastest-growing region in the U.S. since the 1970s, and the population is projected to increase by 33% by 2030 (Theobald et al. 2013). In Arizona and New Mexico alone, the population is projected to increase from 8.5 million in 2010 to 12.3 million people by 2030, an increase of 46% (Theobald et al. 2013). Traffic volumes will certainly increase, and new roads and expansion of current roads is likely. As side-blotched lizards and marbled whiptails show some reduction in road crossing, additional roads will create smaller and more isolated populations. These populations would likely diverge from each other more rapidly, potentially followed by lower genetic diversity and greater risk of local extirpations. Given the option between increasing road density or traffic volumes, increased traffic volumes are expected to have less impacts (Rhodes et al. 2014). Completely isolated but larger populations should resist genetic changes better than small populations with some amount of genetic connectivity, and for some species the population-wide mortality risks could increase with additional roads (Rhodes et al. 2014). New roads or road expansion projects should include mitigation structures like underpasses or overpasses to reduce the potential of negative impacts.

In addition to being one of the first studies to assess road impacts on lizards in the desert Southwest, this study provides baseline demographic data for future studies. In a review of the effects of habitat change on amphibians and reptiles, Gardner et al. (2007) found that many studies

suffered from a lack of large enough patches of remaining habitat to serve as controls. While most of the reviewed studies did not have access to control sites larger than 1000 ha, the shrubland in the study area is  $>1000 \text{ km}^2$  with minimal disturbances currently. Comparisons of demographic and genetic patterns of populations in small habitat patches with those of the populations in the study area would greatly enhance our understanding of how habitat fragmentation affects lizards. In addition, long-term monitoring of lizard populations throughout climate fluctuations like the drought year at the end of this study will improve predictions of responses to ongoing climate change. Although long-term studies are difficult to fund and maintain, the large impact that climate fluctuations have on lizard populations (Pianka 1970, Parker and Pianka 1975) requires sufficient data to determine overall trends.

This study is one of the first to examine the effects that roads have on lizards in the Southwestern U.S., and one of the few to combine demographic and genetic data to obtain a better understanding of the short- and long-term impacts of roads. Although the two study species were selected for their differences in life history and movement patterns, they are too similar to show different responses to roads in this study. The study was designed to isolate the effects of roads from other forms of habitat fragmentation, and demonstrated that large populations in continuous habitat are minimally impacted, particularly for species that show some degree of road avoidance behavior. Additional studies with a variety of lizard species and differing degrees of road density will hopefully enhance our ability to generalize about road effects and help guide efforts to mitigate those effects.

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## Appendix

### A. Literature review of molecular road ecology studies since 2010 that reported genetic diversity results.

Species	Genetic marker	Number of loci	Results	Number of individuals	Sites or Populations	Time since road construction	Study
Northern dusky salamander ( <i>Desmognathus fuscus</i> )	SSRs	5	lower in most isolated population	20–60 per site	5	not reported	Munshi-South et al. (2013)
Wood frog ( <i>Lithobates sylvaticus</i> )	SSRs	8	none detected	1489	65 ponds in 5 clusters	20 wood frog generations have passed since major road construction	Gabrielsen et al. (2013)
Smooth newt ( <i>Lissotriton vulgaris</i> )	SSRs	6	none detected	266	10	not reported	Sotiropoulos et al. (2013)
Macedonian crested newt ( <i>Triturus macedonicus</i> )	SSRs	7	none detected	439	10	not reported	Sotiropoulos et al. (2013)
Timber rattlesnake ( <i>Crotalus horridus</i> )	SSRs	9	decreased allelic richness	504	19	80–90 years	Clark et al. (2010)
Painted turtle ( <i>Chrysemys picta</i> )	SSRs, mtDNA	5 SSR, 450 bp CO1	mtDNA diversity lower, no impact on nuclear diversity	220	7	not reported	Laporte et al. (2013)
Side-blotched lizard ( <i>Uta stansburiana</i> )	SSRs	8	none detected	7–24 per pop	12	increased fragmentation for 30 years	Delaney et al. (2010)

Species	Genetic marker	Number of loci	Results	Number of individuals	Sites or Populations	Time since road construction	Study
Western fence lizard ( <i>Sceloporus occidentalis</i> )	SSRs	8	none detected	5–29 per pop	12	increased fragmentation for 30 years	Delaney et al. (2010)
Western skink ( <i>Plestiodon skiltonianus</i> )	SSRs	6	none detected	14–18 per pop	12	increased fragmentation for 30 years	Delaney et al. (2010)
Desert tortoise ( <i>Gopherus agassizii</i> )	SSRs	16	none detected	859	spread across study region	not reported	Latch et al. (2011)
Black-tailed brush lizard ( <i>Urosaurus nigricaudus</i> )	SSRs	10	none detected	280	11	50 years as 2 lane, 6 years as four lane	Munguia-Vega et al. (2013)
Blanding's turtle ( <i>Emydoidea blandingii</i> )	SSRs	14	decreased, regression models with road density performed best	371	11	not reported	Reid and Peery (2014)
Painted turtle ( <i>Chrysemys picta</i> )	SSRs	7	none detected	461	15	not reported	Reid and Peery (2014)
Snapping turtle ( <i>Chelydra serpentina</i> )	SSRs	9	none detected	208	12	not reported	Reid and Peery (2014)
Florida Scrub lizard ( <i>Sceloporus woodi</i> )	SSRs, mtDNA	5 SSR, 301 bp Cyt b	none detected	139	7	80 years, ca. 62 generations	Tucker et al. (2014)
Prairie rattlesnake ( <i>Crotalus viridis</i> )	SSRs	8	none detected	215	4	40–60 years	Weyer et al. (2014)
Ornate box turtle ( <i>Terrapene ornata</i> )	SSRs, mtDNA	12 SSR, 578 bp mtDNA control region	none detected	80	2	80 years	Cureton II et al. (2014)

Species	Genetic marker	Number of loci	Results	Number of individuals	Sites or Populations	Time since road construction	Study
Eastern box turtle ( <i>Terrapene c. carolina</i> )	SSRs	8	none detected	163	3	50–75 years	Marsack and Swanson (2009)
Wrentit ( <i>Chamaea fasciata</i> )	SSRs	7	lower in smaller patches	2–15 per pop	12	increased fragmentation for 30 years	Delaney et al. (2010)
White-fronted chat ( <i>Epthianura albifrons</i> )	SSRs	18	lower in isolated populations	78	5	not reported, but populations were continuous in the early 1900s	Major et al. (2014)
Tree sparrow ( <i>Passer montanus</i> )	SSRs	7	none detected	602	14 urban, 5 rural	20	Zhang et al. (2013)
Black-tailed prairie dogs ( <i>Cynomys ludovicianus</i> )	SSRs	11	none detected	510	9	not reported	Sackett et al. (2012)
Muskrat ( <i>Ondatra zibethicus</i> )	SSRs	9	none detected	97	3	not reported	Laurence et al. (2013)
Swamp rats ( <i>Rattus lutreolus</i> )	SSRs	11	none detected	179	8	3–5 generations	Stephens et al. (2013)
New England cottontail ( <i>Sylvilagus transitionalis</i> )	SSRs	11	none detected between sites, but low overall	157	54 patches, 4 regions	not reported	Fenderson et al. (2014)
Roe deer ( <i>Capreolus capreolus</i> )	SSRs	11	group in between two highways had lower allelic richness	176	NA	30–45 years	Hepenstrick et al. (2012)
Bobcat ( <i>Lynx rufus</i> )	SSRs	16	coastal subgroup had lower diversity	106	4	12–25 generations	Lee et al. (2012)

Species	Genetic marker	Number of loci	Results	Number of individuals	Sites or Populations	Time since road construction	Study
Bobcat ( <i>Lynx rufus</i> )	SSRs	9 neutral, 7 immune-linked	not significant, but most isolated population had lowest values	365 for neutral, 299 for immune	4	60 years for 101, 50 years for 405	Serieys et al. (2015)
Koala ( <i>Phascolartos cinereus</i> )	SSRs	6	none detected	1106	8	not reported	Dudaniec et al. (2013)
Black bear ( <i>Ursus americanus</i> )	SSRs	11	none detected	138	2	not reported	Coster and Kovach (2012)
Mongolian gazelle ( <i>Procapra gutturosa</i> )	mtDNA	control region, bp length not stated	none detected	103	4	40 years	Okada et al. (2012)
Roe deer ( <i>Capreolus capreolus</i> )	SSRs	10	none detected	536	10	not stated, but pop. expansion only for about 10 generations	Breyne et al. (2014)
European Pine marten ( <i>Martes martes</i> )	SSRs	15	none detected	101	spread across study region	not reported	Ruiz-González et al. (2014)
Black bear ( <i>Ursus americanus</i> )	SSRs	20	none detected	101	20 crossing structures plus other locations	50 years, but passages installed for ca. 30 years	Sawaya et al. (2014)
Grizzly bear ( <i>Ursus arctos</i> )	SSRs	20	none detected	113	20 crossing structures plus other locations	50 years, but passages installed for ca. 30 years	Sawaya et al. (2014)

<b>Species</b>	<b>Genetic marker</b>	<b>Number of loci</b>	<b>Results</b>	<b>Number of individuals</b>	<b>Sites or Populations</b>	<b>Time since road construction</b>	<b>Study</b>
Moose ( <i>Alces alces</i> )	SSRs	10	none detected	89	2	65 years since built, 25 since widening and exclusion fencing installed	Wilson et al. (2015)

SSRs = microsatellites

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## Vita

Kevin Floyd earned both his Bachelor of Science degree in Zoology and Master of Science degree in Ecology from the University of California, Davis. His thesis, “Spatial Demography of a Genetically-Mapped Population of a Desert Shrub (*Sarcobatus vermiculatus*)” combined the use of DNA fingerprinting and demographic modeling to investigate the effects of competition in structuring desert shrub populations. Kevin entered the Environmental Science and Engineering Ph.D. program at the University of Texas at El Paso (UTEP) in 2006, where his research continued to combine genetic and demographic data to gain better understanding of population ecology. His dissertation research focused on how lizards were impacted by roads in the southwestern United States. During his dissertation work, he published two articles, one as lead author, and has presented at numerous local and national meetings. Kevin is also deeply committed to education, and has taught many classes at both UTEP and the El Paso Community College. He has mentored several undergraduates in both field and laboratory work. Kevin has received several grants while at UTEP, including from T&E, Inc, Sigma Xi, and the UTEP Graduate School. His career goals are to continue teaching and involving undergraduates in research focused on how the urban environment impacts wildlife and sustainability.

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