



*Final Report*

**Assessment and Prediction of the  
Effects of Highways on Population  
Ecological and Genetic Properties  
of Selected Faunal Groups: The  
Consequences of Breakup and Isolation**

Prepared By

Alan E. Stiven & Christopher P. Brooks

University of North Carolina at Chapel Hill  
Department of Biology  
CB# 3280, Coker Hall  
Raleigh, NC 27599-3280

June 2005

**Technical Report Documentation Page**

1. Report No. <b>FHWA/NC/2003-01</b>		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle <b>Assessment and Prediction of the Effects of Highways on Population Ecological and Genetic Properties of Selected Faunal Groups: The Consequences of Breakup and Isolation</b>				5. Report Date <b>June 2005</b>	
				6. Performing Organization Code	
7. Author(s) <b>Alan E. Stiven &amp; Christopher P. Brooks</b>				8. Performing Organization Report No.	
9. Performing Organization Name and Address <b>The University of North Carolina at Chapel Hill Department of Biology CB# 3280, Coker Hall Chapel Hill, NC 27599-3280</b>				10. Work Unit No. (TRAIS)	
				11. Contract or Grant No.	
12. Sponsoring Agency Name and Address <b>U.S. Department of Transportation Research and Special Programs Administration 400 7<sup>th</sup> Street, SW Washington, DC 20590-0001</b>				13. Type of Report and Period Covered <b>Final Report July 2000 – December 2002</b>	
				14. Sponsoring Agency Code <b>2001-14</b>	
15. Supplementary Notes Supported by a grant from the U.S. Department of Transportation and the North Carolina Department of Transportation, through the Center for Transportation and the Environment, NC State University.					
16. Abstract <p>The focus of this study was to identify features of amphibian species and roads that might serve as predictors of future sensitivity to habitat fragmentation. To accomplish this task 134 sites were sampled primarily in the piedmont region of North Carolina using both active and passive methods (Fig. 1). In those sites animals were collected throughout the study period (Aug 2000 – Dec 2002). A significant drought (rainfall deficit &gt; 14 inches) event spanned the entire study period. The low number of animals is likely a result of the high mortalities and low activity levels displayed by amphibians during drought conditions. Here we provide an extensive review of the literature dealing with roads as barriers to amphibian movement. Patterns derived from the literature provide a framework for the recommendations made in this report. Also included is a manuscript (in press) that describes a model dealing with the nature of habitat fragmentation and barriers. This paper was the framework for the interpretation of the genetic analysis and is the focus of ongoing study in our laboratory.</p>					
17. Key Words habitat fragmentation, landscape connectivity, small vertebrate, road effects, gene flow, genetic isolation			18. Distribution Statement		
19. Security Classif. (of this report) unclassified		20. Security Classif. (of this page) unclassified		21. No. of Pages 60	22. Price

## **Disclaimer**

“The contents of this report reflect the views of the author(s), who are responsible for the facts and the accuracy of the data presented herein. This document is disseminated under the sponsorship of the U.S. Department of Transportation and North Carolina Department of Transportation in the interest of information exchange. This report does not constitute a standard, specification, or regulation. The US Government assumes no liability for the contents or use thereof.”

## **Acknowledgements**

Support for this research was provided by the Center for Transportation and the Environment in cooperation with the U.S. Department of Transportation and North Carolina Department of Transportation through the Institute for Transportation Research and Education, North Carolina State University. Thanks to Tim Savidge, Hal Bain, Derry Schmidt, Roger Rochelle and the entire advisory board for this work. Thanks also to Dean Urban, Peter White, Bob Peet, Aaron Moody, Dana Moseley, Cindy Skema, Eric Chen, J. Richard Miller, Susan Brooks, Cody Crenshaw, Justin Crenshaw, and Brad Lamphere for helpful discussions and assistance with field work.

## **Summary**

More than 100 sites were sampled in the piedmont and mountain region of North Carolina in an effort to identify amphibian life history and road characters that correlate with genetic isolation. A significant drought (more than a 14 inch deficit in rainfall) over the study period prevented the collection of sufficient tissue for population genetic analysis. We present a summary of the genetic data collected during the study along with a literature review of road effects on isolation. We also present a technique developed during the study period that can be used to develop a low-cost assessment of the degree of connectivity between populations prior to road construction (and thus a baseline with which to assess changes in functional connectivity after the road is built). Based on the literature, we recommend the installation of a number of mitigating structures when constructing, altering or repairing roads. These include, but are not limited to, the installation of wildlife underpasses every 1-3 km in conjunction with wider roadside verges to reduce the reflective effect of the road on wildlife.

## Table of Contents

Title Page	1
Documentation Page (DOT 1700.7) & Abstract	2
Disclaimer	3
Acknowledgements	3
Summary	3
Table of Contents	4
<b>Introduction</b>	<b>5</b>
<b><u>Chapter One</u> – Description of Work Completed</b>	<b>7</b>
Introduction & Background Information	8
Materials & Methods	8
Results	12
<b><u>Chapter Two</u> – Roads as Barriers to Amphibian Movement</b>	<b>14</b>
Introduction	15
Landscape Effects	16
Organism Effects	19
<b><u>Chapter Three</u> – Mitigation Techniques &amp; Recommendations</b>	<b>23</b>
Mitigation Techniques & Recommendations	
Table A. Specific Recommendations	25
<b><u>Chapter Four</u> – A Scalar Analysis of Landscape Conductivity</b>	<b>26</b>
“A Scalar Analysis of Landscape Connectivity BY CHRISTOPHER BROOKS, 2003, OIKOS 102:433-439	
<b>Tables &amp; Figures</b>	<b>40</b>
<b>Cited References</b>	<b>52</b>

## INTRODUCTION

Roads are an integral part of the economy in the U.S., allowing for the relatively inexpensive transport of goods across the continent. Unlike some other forms of human influence, roads represent a relatively permanent change in the structure of the landscape. Interest in the ecological impacts of such changes has expanded among scientists and transportation planners as the need to mitigate impacts to endangered or threatened species has increased (see NCHRP 2002). Much of the early research on road ecology in the U.S. has emphasized population-level impacts on large mammals, but the effects may be more severe for small vertebrate species where the scale of the road effect zone is large (Forman et al. 1997, Forman 2000) and the spatial scale of perception is relatively small. This interaction of scales can result in strong barrier effects that isolate populations and increase the potential for local extinctions (Gibbs & Shriver 2002).

Barrier effects occur in one of two ways: as a filtering barrier or as a reflective barrier. Species-road interactions that can be described as filtering are those in which there is little or no behavioral avoidance of the roads. The impact of filtering is simply the direct mortality (road kill, desiccation, etc.) associated with road crossing. Reflective interactions are those that are associated with a behavioral avoidance of roads and/or road verges. Such interactions have led to the proposal of a road effect zone that can extend 100m or more beyond the boundaries of the road (Forman et al. 1997, Forman 2000). The relative effect of each barrier type may differ among species, and also between different life stages of a single species (e.g., deMaynadier & Hunter 2000). Additionally, they may be related to the location of the road with respect to topography and vegetation, traffic

volume, and maintenance procedures on the road (Clevenger et al. 2003, Gibbs 1998b, deMaynadier & Hunter 2000).

What follows is the description of a project intended to describe the details of these interactions through the use of population genetic estimates of movement for amphibian populations throughout the Piedmont region of North Carolina. As a result of the severe drought that persisted over the entire study period the surface activity of target species was severely reduced, while adult and juvenile mortality were likely to be significantly increased in local populations (*sensu* Pechmann et al. 1991). The description of work completed is followed by a literature review of road effects on small vertebrate species along with a list of general recommendations for mitigating road effects on local amphibian populations, and an article published during the funding period that describes the impacts of human alterations of the landscape (i.e., roads) on landscape connectivity.

## **CHAPTER ONE:**

### **DESCRIPTION OF WORK COMPLETED**

## INTRODUCTION

The growth of the highway system in the Piedmont of the eastern United States has created the need to understand and predict the ecological and genetic consequences of this growth. New highway construction frequently bisects wildlife habitats creating a potential barrier to dispersal (gene flow) between the remaining habitat fragments (deMaynadier & Hunter 1999, 2000; Inbar & Mayer 1999; Forman & Alexander 1998; Gibbs 1998a, 1998b, 1998c; Hitchings & Beebee 1997). This increased isolation frequently leads to changes in ecological processes including higher demographic stochasticity resulting in a higher probability of local extinction (Reh & Seitz 1990, Lande 1998, Stoms 2000 and see Stamps *et al.* 1987). The association of a species' dispersal ability and fragmentation resistance have been examined on a few occasions (e.g., deVries *et al.* 1996, Gibbs 1998b) and with conflicting results. However, with the exception of some avian studies (Whitcomb *et al.* 1981, Hansen & Urban 1992) and a study of the brown apple moth (Gu & Danthanarayana 1992), the importance of life history to fragmentation resistance has been largely ignored. Establishing relationships between genetic isolation due to road fragmentation and aspects of an animal's life history would improve our understanding of fragmentation effects, allow prediction of the effects of future road construction projects on local populations and allow evaluation of potential mitigation procedures.

## MATERIALS & METHODS

### STUDY SPECIES

Study species were chosen to determine the predictive power of selected life history characteristics for habitat fragmentation by roads. Species were from three major



amphibian groups: Mole Salamanders (Genus Ambystoma), Lungless Salamanders (Family Plethodontidae), and Chorus Frogs (Genera Acris and Pseudacris). The general life history characters of these taxa are shown in Table 1. Species from within each of these taxa were considered appropriate study species though few individuals were present during the study period.

### **FIELD COLLECTION METHODS**

Over the course of this project two separate study areas were proposed and sampled. The first and most extensively sampled area was within the piedmont region of North Carolina. Within this area 134 sites were identified and sampled for the presence of breeding populations of each study species. These sites were chosen using three procedures. First, a majority of sites were identified using the collections at the North Carolina State Museum of Natural Sciences. The sites chosen for sampling were then a subset of those that were on public lands and were collected within the past 20 years. A second method was to search floodplains on public lands throughout the study region. A number of sites were also chosen at random within Duke Forest and Eno River State Park. The second study area sampled was in the mountain region of North Carolina, primarily within Pisgah National Forest. The Pisgah National Forest office failed to supply permits for collection within the national forest during the study period and therefore collections were restricted to private lands near Moses Cone National Park.

Field sampling occurred from January to November 2000 and again from January to November 2001. Most of the sampling, however, was conducted between May and August of each year. Collection of animals was conducted using both passive and active

methods of sampling. When reliable breeding sites were identified methods included the use of pitfall-driftfence traps (Fig. 2) and coverboards as well as time-constrained searches. Pitfall traps were opened throughout the breeding season and checked a minimum of every two days. Coverboards were checked weekly and time-constrained searches occurred on a single date for each additional site. Time-constrained searches were 60 minute searches of sites in which all large cover objects within an approximately 400 m<sup>2</sup> area were moved and searched and remaining time was spent searching through the leaf litter. Individuals that were captured were identified to species and transported back to the lab for processing and genetic analysis.

#### **GEOGRAPHIC INFORMATION SYSTEMS AND HABITAT MODELING**

In order to identify populations and evaluate the gene flow between patches habitat models were to be developed based on location data where individuals were captured. By using a Mahalanobis distance statistic the mean vector of environmental conditions where an organism is present can be identified. By using a raster grid each cell can then be assigned a probability for the presence of that species based on a Chi-square distribution. Habitat was to be defined as all cells with greater than or equal to an 80% probability of occurrence.

Such a model was not constructed due to the low number of individuals of each species that were collected during the drought. Alternative models were explored using museum collections and published literature on habitat requirements, especially Wilson (1995). Models from museum collections were quickly abandoned because of the temporal sensitivity of habitat information (see Chapter 4) in modeling requirements.

Models resulting from literature accounts were at too coarse of a resolution to allow use for identifying individual populations in the landscape. As a result development of these models was also abandoned.

## **DEVELOPMENT OF GENETIC ANALYSIS TECHNIQUES**

In anticipation of the genetic analysis of subpopulations from large-scale collections of specimens of several target amphibian and other species from different study sites as outlined in the original proposal, we attempted to resolve as many genetic loci as possible using preliminary sample collections of the target species. While the 2-year drought significantly impacted even our preliminary sampling, we were able to collect a sufficient number of specimens of a variety of amphibia for use in the development of genetic techniques. Sufficient numbers of protein enzyme loci were resolved for the target species to have permitted appropriate measures of genetic distance among the habitat treatments as proposed in the original grant. The amphibian species (sample numbers in brackets) included Acris crepitans (37), Ambystoma opacum (3), Desmognathus monticola (12), Desmognathus fuscus (10), and Eurycea bislineata cirrigera (9).

Sample specimens were transported from field sites in the piedmont and southern Appalachians in cooled containers and maintained at 10-15° C until sacrificed. Individuals were anesthetized in chlorobutanol before they were measured and sacrificed. Samples of equal portions of body wall musculature and viscera-liver were homogenized in approximately equal volume of grinding buffer (0.1 M Tris, 0.001 M EDTA, and 5 x 10<sup>-5</sup> M NADP, ph 7.0). Homogenization was carried out by either glass grinding

techniques or a sonifer cell destruction technique. Supernatants of these tissues were extracted by centrifugation at 4°C for 30 min at 12,000 g. Supernatants were then stored in a deep freezer at -80°C.

Two protein electrophoretic resolving media were utilized to find the best resolving medium for a variety of presumptive genetic loci (isoenzymes). The first was a starch medium derived by mixing Electrostarch and Sigma starch in a 3:1 ratio. The second medium was cellulose acetate that is purchased as small plates from ready for use from Helena Laboratories.

Tables 1-6 show a summary of the extensive data representing the attempts at resolving many of the protein loci for future use in the above species. Since most of the loci could be resolved using the less time-consuming cellulose acetate procedure, although it is somewhat more expensive than the starch gel procedure, we had elected to utilize this procedure for the anticipated extensive collections of these target species from our study plots representing the various highway treatments outlined in our original design.

## **RESULTS**

As stated in the Introduction the impacts of the drought that persisted throughout the study period and had severe implications for sampling. Pechmann et al. (1991), among others, have demonstrated the effects of drought on both juvenile and adult survivorship. This study was chosen as an example because of its close proximity to the study area. At the Savannah River Ecology Lab (SREL) the authors measured the impact of drought on the survivorship of both juvenile and adult salamanders. The authors found

that when November-March rainfall dropped below 400 mm there was significant mortality among both breeding adults and juveniles. During the study period the precipitation levels during that period were always below 400 mm (376 mm in 2000-01 and 353 mm in 2001-02 (National Weather Service)) and were greatly increased by snowfall events in each year. As a result few individuals were captured across the study area and those that were captured came from localized lowland areas that were the last to dry. Data for streamflow in the Eno River during the period shows a similar pattern of reduced streamflow (Fig. 3) that resulted in the drying of local breeding ponds before metamorphosis could occur (Fig. 4). Similar patterns were reported by other herpetologists in both the coastal plains and piedmont of North Carolina (Jeff Beane personal communications).

As a result of the severe impacts of the drought on our sampling design efforts have been focused on using geographic information systems and simulation modeling to answer similar questions about the nature of landscape barriers. This model is currently under construction and is designed to predict life histories that might be sensitive to barrier placement. The model will not be able to predict the impacts of road type, however. The manuscript that follows (Chapter 4) will serve as the theoretical basis for the eventual prediction of habitat fragmentation sensitivity that is an ongoing investigation in our laboratory.

## **CHAPTER TWO**

### **ROADS AS BARRIERS TO AMPHIBIAN MOVEMENT: A REVIEW OF THE LITERATURE**

## INTRODUCTION

Roads have become a ubiquitous feature in the modern landscape because of their importance in economic and social development. Study of the ecological effects of roadways has accelerated in recent years as scientists and engineers have sought to mitigate the potential impacts of roads on local floral and faunal populations (e.g., Trombulak & Frissell 2000, NCHRP 2002, Forman et al. 2003). Much of the work has focused on the direct mortality associated with road kills (Lode 2000, Hels & Buchwald 2001) especially bird and mammal populations (Brody & Pelton 1989, Lode 2000). And a variety of mitigation techniques have been proposed, implemented, and evaluated with varying results (Guyot & Clobert 1997, Clevenger & Waltho 2000, Cuperus et al. 2001). It is likely that the variation in success can be attributed to the species-specific nature of responses to the structures meant to facilitate successful road crossings (Clevenger et al. 2001). Ecological success that is economically feasible in mitigating the effects of roads on local populations will require an understanding of underlying processes that have led to the observed patterns of isolation.

Such potentially predictive patterns exist in the way in which roads act as barriers to local movements. In some cases individuals will readily attempt to cross roads and are subjected to some rate of mortality associated with crossing (Clevenger et al. 2001, Gibbs 1998b, Guyot & Clobert 1997, deMaynadier & Hunter 2000, Clarke et al. 1998, Hels & Buchwald 2001). This lack of behavioral avoidance and subsequent mortality causes a barrier that can be referred to as a filtering barrier (deMaynadier & Hunter 2000). In other systems there is evidence that individuals avoid roads altogether and thus few individuals are subject to direct mortality (deMaynadier & Hunter 2000, Baur & Baur 1990, Brody &

Pelton 1989, Hels & Buchwald 2001). The presence of such behavioral avoidance results in what is termed a reflective barrier. Although these responses lie at the extremes of a continuum (Fig. 5) the ecological consequences and strategies for mitigation will differ greatly for each. Our focus here is to describe the nature of roads as barriers and to discuss the ecological consequences of those patterns. The ultimate goal is to provide a cogent analysis of roads as barriers to movement that provides cross-system guidance towards the development of effective mitigation techniques. We will provide an outline of road and landscape characteristics that produce the observed barrier effect. A discussion of life history characters and their influence on the barrier effect will then be followed by a discussion of potential mitigation techniques and suggestions. Although road effects can result in various additional consequences we will focus solely on roads as barriers with special reference to amphibians and other small vertebrates.

#### **LANDSCAPE EFFECTS: ROAD CHARACTERISTICS & THE ROAD EFFECT ZONE**

The impact of roads in ecosystems can be classified as either direct (e.g., mortality from road kill, changes in local temperature, etc.) or indirect (e.g., alteration of habitat quality, barrier to movement, etc.). In general, direct effects are those that tend to create filtering barriers while indirect effects are more likely to cause behavioral avoidance or reflectance. As a result, the characteristics of a road, including methods of maintenance and its context in the landscape, can have an impact on the degree of filtering or reflectance attributed to it.

Roads interact with the surrounding landscape to produce a zone of influence that extends beyond the spatial extent of the road. The presence of physical, chemical and biological alterations in the landscape that extend beyond the road surface have been



described in detail (Forman et al. 1997, Forman 1998, 2000). This road effect zone can extend more than 100 m from the edge of the road itself (see Fig. 2, Forman 2000) and some evidence suggests that it may extend as much as 1.5 km (Carr & Fahrig 2001). Where the zone extends further from the road species may be more likely to exhibit behavioral avoidance.

The context of the road in the landscape can alter the shape and extent of the road effect zone and determine where individuals are most likely to cross. On raised sections of roads, some road effects can be propagated downhill of a road (see Forman et al. 2003). Clevenger et al. (2003) found that there was a lower incidence of roadkill along raised sections of road compared to other sections suggesting a behavioral avoidance of these elevated sections of road. Similarly, the authors found that the incidence of road kills (or a lack of behavioral avoidance) was greatest when vegetative cover occurred near the road edge. Roads that have a larger total distance between adjacent forest edges may, in turn, create a more reflective barrier. Oxley et al. (1974) found that the number of mammals crossing Canadian roads was reduced when the clearance (distance between forested edges) exceeded 19 meters. This effect appeared to be irrespective of body size, road type or traffic density. However, both Spotted (Ambystoma maculatum) and Marbled Salamanders (Ambystoma opacum) will cross large, open areas in order to reach breeding pools but will avoid roads (Gibbs 1998b) suggesting that some other characteristic, like chemical treatment of the road or road verge, may be causing the avoidance. A variety of chemical treatments occur on roads that may alter the behavioral responses of dispersers. An examination of amphibian crossings on two forest roads in Maine demonstrated that the number of crossing movements for Spotted Salamanders,

Blue-Spotted Salamanders (*Ambystoma laterale*), and Red-Spotted Newts were reduced on a 12 m wide road treated with calcium chloride versus an untreated 5 m wide road (deMaynadier & Hunter 2000). Calcium chloride is a common agent used to control dust and erosion but would result in a serious desiccation risk for slow moving amphibians that require moist microclimates.

Additional reflectance can be attributed to the traffic density on a road. High traffic density leads to a great deal of traffic noise in the landscape. This noise is a part of the road effect zone that can alter the distribution of songbirds (Forman et al. 2003) and cause behavioral avoidance by a variety of animal species including black bear (Brody & Pelton 1989), coyote, marten, weasel, hare, and squirrel (Clevenger et al. 2001).

Although behavioral avoidance of roads due to traffic noise may be common among birds and mammals, little evidence for such avoidance is present among salamander species. Additionally, there is no direct evidence that anurans avoid traffic noise, but Fahrig et al. (1995) did find reduced calling near roads while Carr & Fahrig (2001) found a negative relationship between leopard frog (*Rana pipiens*) density and traffic density within 1.5 km of high traffic roads.

Despite the potential for reflectance in the road effect zone individuals that reach the road are then subject to direct mortality risks. The primary risk of road crossing is generally thought to be automobile traffic that seems to serve as the final filter to dispersers. The density of traffic on the road then determines the mortality risk, or pore size, of the filter. In fact, Lode (2000) found that mortality increases exponentially as traffic density increases. Some direct estimates suggest that mortality due to road traffic is high among the amphibia. Heine (1987 as cited in Fahrig et al. 1995) estimated that 26

cars per hour was enough to reduce the survivorship of crossing toads to 0%, while Wymann (1991) estimates the mortality of red-spotted newts and redback salamanders crossing a paved rural road at 50-100%.

### **ORGANISM EFFECTS: SCALE OF PERCEPTION, SENSITIVITY AND LIFE HISTORY**

Ultimately, the aspects of landscape structure described above serve as the template that interacts with a species' perception and behavior to create what we observe as a variably permeable road effect zone in the landscape. When dispersing individuals encounter the road effect zone in the landscape it is the interplay between the characteristics of the road effect zone, the individual's scale of perception and its sensitivity to the landscape alterations that determine the ultimate effect of the barrier. As a result, the barrier effect depends on the specific characteristics of both the road effect zone and the dispersing organism.

Tradeoffs are common in the evolution of life histories and such tradeoffs ultimately affect the scale and magnitude of gene flow, and thus landscape connectivity. Gene flow is a result of an organism's dispersal ability (vagility) interacting with environmental cues and constraints in the landscape. The most common tradeoffs are between increased energy towards reproduction (and thus population growth locally) or towards dispersal ability. Organisms that have good dispersal abilities often have lower individual fecundities (Frank 1996, Roff & Fairbairn 1991). The physiological mechanism for this tradeoff has even been demonstrated directly in one species of cricket (Zhao & Zera 2002). Because there is risk associated with dispersal and the tradeoff is between energy for local reproduction versus dispersal, local conditions must favor leaving the patch in order for dispersal to persist as an evolutionarily stable strategy. The

presence of temporal variability in habitat quality locally is sufficient to favor dispersal as an evolutionary stable strategy in the landscape (Schmidt et al. 2000) and such temporal variation is ubiquitous in all landscapes (see Chapter 4).

While the dispersal ability and number of dispersers provide the raw materials of gene flow, the sensitivity of individual species to landscape structure determines the eventual patterns of gene flow in the landscape. The sensitivity of an organism to landscape features such as the road effect zone is related to the strength of the behavioral response to that feature. Ultimately, an organism's sensitivity to the road effect zone may be related to how long an individual would be in it (mode of locomotion, speed of movement) and the perceived cost of exposure to it (risk of direct mortality). Thus, the most sensitive species are likely to be those that move slowly on the ground and have strict environmental tolerances, such as amphibians.

Among amphibians, frogs and mole salamanders seem to be less likely to see roads as behavioral barriers than do lungless salamanders and newts. Frogs and ambystomatid salamanders have generally been found to traverse both paved (Clevenger et al. 2001, 2003, Hels & Buchwald 2001, Gibbs 1998b) and gravel roads (deMaynadier & Hunter 2000) more often than lungless salamanders and newts (deMaynadier & Hunter 2000, Gibbs 1998b, Hels & Buchwald 2001). Differences between groups can likely be attributed to differences in life history strategies. General life history differences between the taxa are illustrated in Table 2.

In an examination of the behavioral responses of several amphibian species to forest logging roads deMaynadier & Hunter (2000) found that while frogs would cross the gravel roads most salamanders avoided them. In particular, Ambystomatid

salamanders would cross the more narrow, untreated gravel roads and would avoid the wider, treated roads. Other species, including newts, would avoid roads entirely. Gibbs (1998b) found that species of Ambystomatid salamanders would cross large, open areas in order to reach breeding sites but displayed a behavioral avoidance of forest-road edges. The authors also found that pickerel frogs did not display an avoidance of forest edges but did prefer intermittent streambeds. These findings suggest that species of mass breeding amphibians (i.e., mole salamanders and frogs) may be more likely to traverse open areas, including roads, more than species with smaller clutches. The conflicting results with regard to ambystomatid salamanders may result from traffic mortality (sensu Gibbs 1998b) or an avoidance of the desiccating agent calcium chloride (sensu deMaynadier & Hunter 2000). Species of frogs may be able to traverse the same roads simply because of more rapid movement and a higher tolerance of desiccation.

At least some forest-specialists may also see the openness of the road verge is sufficient to create a reflective barrier to movement. Gibbs (1998b) and deMaynadier & Hunter (2000) both found that red-spotted newt (Notophthalmus viridescens) individuals avoid forest edges entirely, regardless of the presence or absence of a road. Hels & Buchwald (2001) also noted an avoidance of roads by forest-specialists. Such an avoidance may be directly due to the scale of perception of an organism – or the ability of the organism to assess the quality of habitat at some distance.

The scale of perception of an organism may be among the most critical characteristics to understanding the ultimate impact of roads on dispersal. Species that can perceive the road or a change in habitat quality as a result of the road (road effect zone) at greater distances have a greater potential to alter their behaviors. Simultaneously,

a large scale of perception may allow an individual to perceive habitat quality beyond the barrier as well, thus motivating it to cross the barrier. Though it is difficult to assess (and depends on the character of what is being perceived, e.g., sound, chemical alteration, open canopy, etc.) there is some data that suggests an importance of body size to perception.

For amphibians the temporal nature of movements may also play a role in the impact of roads to dispersal. Mass breeding amphibian species typically have a stage of juvenile dispersal to the more terrestrial adult habitat. Such mass movements are often associated with nighttime rainfall events (Clevenger et al. 2003), and are therefore somewhat predictable events. Where amphibians are common members of the ecological community they are also common among the road-killed fauna and many anecdotal accounts of mortality during these mass breeding movements exist (e.g., Clevenger et al. 2001b). In light of the potentially lower reflectance of roads to mass breeding species as opposed to less explosive breeders mitigation needs might be more temporally constrained.

## **CHAPTER THREE**

### **Mitigation Techniques & Recommendations**

## **Mitigation Techniques & Recommendations**

Although the suite of road effects on local populations have been described (Trombulak & Frissell 2000, NCHRP 2002, Forman 2003) and mitigation techniques for barrier effects examined (e.g., Clevenger & Waltho 2000) underlying patterns of response across taxa have not been examined. We have described the nature of roads as reflective and filtering barriers to amphibian dispersal in the landscape and described patterns evident from the literature. When discussing roads as barriers, however, we will consider mitigation of reflectance separate from the mitigation of filtering effects.

As we described in the preceding sections, the realized impacts of the barrier effect due to roads differs based on the degree or reflectance of the road. Although the filtering effects of roads (road kills) have been examined more extensively the reflectance of a road is likely more critical to population structure locally and regionally. Especially among amphibians species where the filtering effect is likely to be restricted to species with already high juvenile mortality rates. Although amphibian species that rely on lower rates of dispersal may exhibit a greater degree of genetic substructure in the landscape the additional barrier effect of roads (especially reflecting effects) may prevent critical aspects of regional persistence. By increasing the isolation of patches in these taxa roads prevent the recolonization of locally extinct patches and prevent the use of sink habitats to 'store' offspring during good years (Schmidt et al. 2000).

Although filtering effects are likely to have different ecological consequences than reflectance the impacts can be severe locally. Because dispersal is more likely to occur in large numbers and over short periods of time in populations of explosive breeders the number of road kills is usually very large (e.g., Clevenger et al. 2000b) and



can impose a significant mortality risk on juveniles. The result can be local declines and extinctions in populations where traffic density is sufficient to cause a large filtering effect. These findings lead us to the recommendations found in Table A below.

**Table A.** A list of specific recommendations for mitigation of isolation caused by North Carolina roads. Techniques for reducing reflectance are intended to reduce behavioral avoidance and must be associated with mitigation for filtration as well.

Barrier Type	Specific Alteration	Mitigation Technique(s)
<b>Reflective Barrier</b>		
Traffic	Increased noise	- reduce the number of raised road sections - maintain areas of more narrow road verges every 1-3 Km
Wide Road Verge	Open Canopy Creation of Sink Habitats	- maintain areas of more narrow road verges every 1-3 Km
Chemical Treatment	Widening of verge  Creation of more desiccating environment	- see above  - Maintain areas nearby narrower road verges untreated & create a more discriminate policy towards chemical treatments.
<b>Filtering Barrier</b>		
Traffic	Exponential increase in mortality risk.	- Provide an array of sizes of culverts and 'underpass' crossing structures every 1-3 Km (near narrow verges). - Installation of fences and crossing structures nearby known breeding sites.

## **CHAPTER FOUR**

**A SCALAR ANALYSIS OF LANDSCAPE CONNECTIVITY  
BY CHRISTOPHER BROOKS, 2003 OIKOS**

## Introduction

Landscape connectivity, the degree to which a landscape facilitates or impedes the movement of individuals (Taylor *et al.* 1993), is a critical property in the persistence of spatially structured populations (Metzger & Décamps 1997). Because connectivity depends on both the spatial structure of the landscape and how individuals interact with that spatial structure (Wiens *et al.* 1997) it is both a landscape- and species-specific characteristic. While models based on percolation theory have been used extensively to examine connectivity (e.g., Gardner & O'Neill 1991, With 1997, With & King 1997, 1999) empirical studies have focused on patterns of movement based on microcosm experiments (e.g., deVries *et al.* 1996, Wiens *et al.* 1997, Golden & Crist 2000) that may be difficult to extrapolate to larger scales (Wiens 1997).

Landscape connectivity can be described as consisting of a structural component, which describes the shape, size, and location of features in the landscape; and a biological component, which consists of both the response of individuals to landscape features (behavior) and the patterns of gene flow that result from those individual responses. Traditional measures of connectivity in landscape ecology have focused on structural components such as nearest-neighbor distances, patch area, edge to area ratio, etc. (*sensu* O'Neill *et al.* 1988, Turner 1989). In population ecology, connectivity is typically measured with biological criteria, including mark-recapture and measures of genetic structure (e.g., Korona 1991, Giles & Goudet 1997). Studies that have attempted to relate dispersal movements to landscape structure have typically been based in one of three basic designs. Those based on microcosm experiments (e.g., Wiens *et al.* 1997), have attempted to scale individual behavior in response to landscape features up to population-

level responses. Data from such experiments are difficult to extrapolate across scales due to non-linear responses to heterogeneity (see Ludwig *et al.* 2000, Figure 3). Spatially explicit population models (e.g., With & Crist 1996) are necessarily complex and may not be good predictors beyond a single level of spatio-temporal resolution (Conroy *et al.*, 1995). Empirical studies of connectivity have focused primarily on highly vagile species like birds and mammals (see Andr en 1994) and the potential utility of corridors (e.g., Haddad 1999a, 1999b, Ims & Andreassen 1999). However, measures of landscape connectivity that can be compared across systems and that have an intuitive biological interpretation have not emerged (see Tischendorf & Fahrig 2000a, b). I argue that such an index of connectivity can emerge only from the explicit comparison of the temporal and spatial scales of the structural and biological components of connectivity.

In this paper I explore the potential for an analysis of scale to explain the observed inconsistencies in isolation effects across systems. I first review the current methods for quantifying both structural and biological components of landscape connectivity and introduce methods for quantifying the scales of both landscape structure and gene flow. I discuss the concept of the Unit Pattern (Watt 1947) and its potential utility as a conceptual framework for a scalar analysis of landscape connectivity. Finally, I present some specific predictions that emerge from the analysis and some potential avenues for testing those predictions.

## **STRUCTURAL CONNECTIVITY**

Numerous methods have been devised to describe the spatial structure in landscapes (see O'Neill *et al.* 1988, Turner *et al.* 1991 for reviews). Among these are

measures used to quantify the connectivity of habitat patches in the landscape such as patch cohesion (Schumaker 1996), proximity index (Whitcomb *et al.* 1981), average nearest-neighbor distance, and fractal dimension (O'Neill *et al.* 1988). Each index describes an aspect of the spatial configuration of habitat (and is therefore not completely biologically neutral) but lacks information as to its ecological importance to specific populations. Although new measures of structural connectivity are being developed (e.g., Jaeger 2000), there remains no index that allows cross system or landscape comparisons (Davidson 1998).

Empirical studies of structural influences have focused on the utility of corridors (Andreassen *et al.* 1996a, 1996b; Hjermmann & Ims 1996; Andreassen *et al.* 1998; Haddad 1999a, b; Haddad & Baum 1999; Haddad *et al.* 2000), and the effect of edges on emigration rates (Stamps *et al.* 1987), species richness (Golden & Crist 2000), and abundance (Gibbs 1998a, 1998b; Demaynadier & Hunter 1998). Although examining the effects of landscape structure on individual behavior patterns may provide a more complete picture of metapopulation structure (Wiens *et al.* 1997), the difficulty in collecting data would make the assessment of even a handful of species impractical. A more efficient approach may be to relate the scale of landscape structure to cumulative measures of the scale of individual responses.

### *Measuring the Scale of Landscape Structure*

The structural scale of landscape connectivity can be assessed through a combination of graph theory (Harary 1969), percolation theory (Stauffer & Aharony 1985) and GIS technology. Percolation theory is the study of connectivity in

stochastically generated structures (Stauffer and Aharony 1985), and has been the basis of neutral models in landscape ecology (Gardner *et al.* 1987). Neutral landscape models predict a non-linear, threshold response of a landscape to habitat fragmentation (Gardner & O'Neill 1991, With 1997, With & King 1997). Above the threshold, habitat destruction simply results in a loss of suitable habitat, but at the threshold even small losses of habitat result in the rapid breakup of the landscape into disconnected clusters (With & King 1999). Percolation-based models also predict threshold responses of species and communities to changes in habitat structure (With & Crist 1995, Metzger & Decamps 1997, With & King 1999).

Percolation theory is related to graph theory through bond percolation, a special case of percolation (Keitt *et al.* 1997). Graphs are commonly used to maximize connectivity in road, telephone, and computer networks (Gross & Yellen 1999). These graphs are composed of points (nodes) and lines (edges) that are used to represent patches and connections between them, respectively. Both edges and nodes can be given weights that can represent structural characteristics such as nearest-neighbor distance or biological characteristics such as the cost of moving between patches (*sensu* Bunn *et al.* 2000).

By utilizing bond percolation in conjunction with the graphical representation of a landscape Keitt *et al.* (1997) developed an edge thresholding technique that can be used to quantify the scale of landscape structure. Edge thresholding involves the creation of a graph with nearest-neighbor distance as edge weights. The method involves varying the maximum value for the edge weight (nearest-neighbor distance) between any pair of connected nodes (habitat patches) in a stepwise manner and calculating the average size

of connected clusters at each iteration (a measure of structural connectivity). The percolation threshold or scale of structural connectivity can be determined for a species in a given landscape by plotting the average size of connected clusters vs. each iteration of edge threshold and determining the inflexion point of the curve (Fig. 1) (see Keitt *et al.* 1997, Bunn *et al.* 2000). The distance value for that inflexion point can then be interpreted as the scale of structural connectivity for that species in that landscape. Although the structural scale is, itself, species-specific (because of the classification of habitat) the structural scale of habitat alone provides little information as to how a species interacts with the landscape. Recognition that each species will respond differently to the spatial structure (e.g., Lehmkuhl & Ruggiero 1991) makes the integrated interpretation of both structural and biological components of connectivity necessary.

### **BIOLOGICAL (GENETIC) CONNECTIVITY**

Biological connectivity involves the actual movement of individuals and their genes between populations in the landscape. Taylor *et al.* (1993) suggested that measures of the probability of movement between patches be used to quantify connectivity. Fahrig & Paloheimo (1988) and Heinen & Merriam (1990) each utilized these measures in simulation models but accurate field estimates of interpatch movements are difficult, and typically impractical to obtain in empirical studies. Tischendorf & Fahrig (2000a) suggest that fine-scale responses to landscape features are required to measure connectivity. Such an effort would be intractable in empirical studies. A more appropriate measure of actual connectivity might be gene flow in the landscape. Gene flow provides a cumulative

estimate of movement between patches of suitable habitat that includes connectivity-related responses of individuals to landscape structure.

### *Measuring the Scale of Gene Flow*

One method for the analysis of spatial scale in gene flow data is the use of autocorrelation analysis. Autocorrelation analysis has been used extensively to examine pollination within plant populations (e.g., Chung & Chung 1999a, 1999b; Chung & Park 2000; Chung *et al.* 2000; Mizuyazaki & Isagi 2000). But these studies have typically focused on gene flow within patches and use single loci or alleles to measure the scale of gene flow. The use of autocorrelation analysis on individual alleles or loci is subject to the effects of mutation and drift and may, therefore, produce erroneous measurements of scale (Chung *et al.* 2000).

A Mantel test provides a test of significant autocorrelation between two matrices of dissimilarity or distance (Sokal & Rolf 1994). By calculating Nei's (1972) genetic distance for each pair of populations and by measuring the actual distance between them we can produce matrices for genetic and geographic distances. Such a measure avoids the potential bias associated with single allele or locus measures of autocorrelation and provides a more robust measure of the scale of gene flow. A Mantel correlogram (Legendre & Fortin 1989) can subsequently be used to explore the scale of autocorrelation between geographic and genetic distances. Using this technique we can then interpret the scale(s) at which autocorrelation breaks down as the scale of gene flow in a landscape.



## LANDSCAPE CONNECTIVITY AND THE UNIT PATTERN

Heterogeneity has likely existed in the landscape throughout the evolution of the species we observe today. Such temporal and spatial variation in habitat quality can alter dispersal rates (Fretwell & Lucas 1970, Johnson & Gaines 1990, Lidicker & Stenseth 1992, McPeck & Holt 1992, Boulinier & Lemel 1996, Lemel *et al.* 1997, Paradis 1998) and have profound effects on dispersal dynamics and habitat selection (Burger 1982, Boulinier 1996). As a result, the spatial scale of that heterogeneity has likely resulted in a spatial scale of gene flow that maintains connectivity between habitat patches. Spatial scale is the more relevant measure (as opposed to measures of spatial arrangement) because processes such as succession and disturbance cause shifts in the spatial arrangement of landscape features through time. It is the alteration of the temporal scale of disturbance, or the addition of new disturbances that often results in the isolation of populations.

Concern over isolation effects (see Saunders *et al.* 1991 for review) has provided the impetus for studies of connectivity in landscapes, specifically in the utility of direct connections such as corridors (e.g., Andreassen *et al.* 1996a, 1996b; Hjermann & Ims 1996; Andreassen *et al.* 1998; Haddad 1999a, 1999b; Haddad *et al.* 2000; Haddad & Baum 1999). Tischendorf & Fahrig (2000a) review the use of “landscape connectivity” in 33 studies and found that few studies adhered to the duality in the definition of landscape connectivity. Tischendorf & Fahrig (2000a) argue that in order to include both structural and biological components of landscape connectivity, both fine scale responses of individuals to landscape features and the configuration of those features in the landscape must be determined explicitly. The authors suggest that connectivity be measured based

on immigration rates into equally sized areas to prevent the conclusion that fragmentation increases connectivity. Such a measurement of connectivity in empirical studies would quickly become intractable for large landscapes or for more than one species.

Measurement of gene flow between patches of suitable habitat can provide estimates of immigration that account for individual responses to heterogeneity and focus on rates of inter-patch movement.

#### *THE UNIT PATTERN AND THE SCALE OF GENE FLOW*

In order to compare structural and biological measures of connectivity we must first have a framework in which to view the interaction of processes in time and space. The unit pattern, proposed by Watt (1947), provides such a framework. The unit pattern is the scale, or window size, at which each seral stage of a forest community is represented. According to Watt, although their arrangement in space may shift, each seral stage is represented in the unit pattern in an amount proportional to the time spent in that stage. Habitat for a species, because it is, in part, determined by the spatial arrangement of successional stages, would also be present in constant (or nearly constant) amounts, while its location would shift through time. The result is the evolution of dispersal strategies that respond to the scale (both spatial and temporal) of structure of habitat in the landscape. Rates of dispersal are not neutral characters (Travis & Dytham 1998) and the evolutionarily stable strategy (ESS) for dispersal in spatially-explicit models is the strategy that results in spatially homogeneous fitness levels (McPeck & Holt 1992, Lemel *et al.* 1997). As a result dispersal strategies should evolve at the same (or larger) spatial scale as the structural scale of habitat in the landscape.

Anthropogenic disturbances that fragment habitat and alter the structural scale of that habitat in the landscape (Doak *et al.* 1992) do so by producing a series of more isolated segments of habitat in the landscape (Jaeger 2000). Such a change in the structural scale of habitat would likely occur on a time scale too rapid to allow a response in gene flow. As a result, gene flow in the landscape would be constricted. Because of the larger temporal scale of measures of gene flow (e.g., allozyme, minisatellite, etc.) changes in the measurement of the scale of gene flow in response to the changes in landscape structure would also occur more slowly allowing identification of potentially fragmented populations. Using the unit pattern as a framework we can integrate the methods for measuring the scale of gene flow (Mantel correlograms) and habitat structure (edge thresholding) to examine the landscape- and population-specific potential for future isolation effects in a species.

### *Interpreting Patterns*

Edge thresholding is based on remotely sensed images, so habitat is defined based on a 'snapshot' in time. Measurement of the scale of gene flow, in contrast, encompasses a much larger time scale than does edge thresholding (the exact scale depends on the organizational level of variability being analyzed – allozyme or DNA). Measures of variability in population genetic structure account for the accumulated genetic variance from a variety of processes (e.g., selection, drift, and gene flow) over several generations. Because of the difference in the temporal scale of gene flow and habitat structure we must consider both current and historical landscape structure when comparing and interpreting differences in their spatial scales.

In order to interpret any newly devised hypothesis it is important to examine your expectations under ‘neutral’ or ‘null’ conditions. Here it would be appropriate to determine our expectations for an undisturbed landscape, a landscape devoid of any anthropogenic influence. When analyzing an undisturbed landscape we assume that the life histories of each species have evolved in the context of the unit pattern. Because of the spatial and temporal variability in landscape heterogeneity we would expect the spatial scale of gene flow for each species ( $t$ ) to be equal to or larger than the scale of habitat structure ( $\mu_{crit}$ ) in the landscape ( $t \geq \mu_{crit}$  in Fig. 1). Temporal and spatial variability in landscape heterogeneity favors non-null rates of dispersal between populations (Kuno 1981) that are, themselves, variable in space and time (Lemel *et al.* 1997). Although individuals that disperse may suffer survivorship costs during dispersal and future reproductive costs (see Belichon *et al.* 1996), dispersal is only selected when there is an overall gain in fitness (Lemel *et al.* 1997). In undisturbed landscapes differences between the temporal scales of gene flow and landscape structure are relatively unimportant to interpretation. In undisturbed landscapes, the scale of gene flow ‘fits’ the scale of habitat structure in the landscape (which is essentially constant) and so the point in time at which we measure structural scale is unimportant. However, it is the difference in the temporal scales at which gene flow and habitat structure act that is the focus of the analysis in real landscapes.

In real landscapes any measurement of the scale of gene flow or habitat structure would result in one of two possible outcomes: either the scale of gene flow is greater than the scale of habitat structure or it is less than the scale of habitat structure. If the spatial scale of gene flow is larger than (or equal to) the scale of habitat structure (if  $t = \mu'_{crit}$  in

Fig. 1), then the scale of gene flow in the historical landscape was either larger than the historical scale of structure in the unit pattern, or that population has had sufficient time to recover from any isolation effects. If the observed patterns are due to historical patterns of gene flow and habitat structure ( $\mu'_{crit} = t > \mu_{crit}$  in Fig. 1), then the potential for isolation effects exist (if those populations are responding to a scale of heterogeneity larger than the measured scale of habitat structure). The second possible outcome would be for the scale of gene flow to be less than the scale of habitat structure in the landscape ( $t \ll \mu'_{crit}$  in Fig. 1). In this case it is likely that the focal species has yet to respond to changes in the scale of habitat structure. Species that show this pattern would be expected to be the most susceptible to isolation effects in the future.

### *Model Predictions*

By examining patterns of landscape connectivity through explicit treatment of scale some testable predictions about the relationship between habitat structure and gene flow emerge. If temporal and spatial heterogeneity in the landscape cause selection for positive rates of dispersal (Kuno 1981) then they must also result in a scale of dispersal that is non-zero as well. To date there has been no empirical evidence or simulation studies that have examined the evolution of the scale or spatial extent of dispersal in a landscape. In the context of the unit pattern of Watt (1947) the spatial scale of gene flow is predicted to be comparable or larger than the structural scale of heterogeneity in the landscape. Likewise, landscape fragmentation is predicted to disproportionately affect species for which the scale of gene flow is closest to the scale of landscape structure (i.e., those for which  $t \approx \mu_{crit}$ ).

A test of these predictions could consist of the acquisition of habitat and genetic data for a range of species in landscapes with low levels of human disturbance. Determining the scale of gene flow for each species and comparing that scale to the structural scale of habitat would allow an analysis of the relationship between  $t$  and  $\mu_{crit}$  for each. If  $t$  is equal to or significantly greater than  $\mu_{crit}$ , support for the hypothesis is provided. Small, isolated populations may also experience a further reduction in the scale of gene flow through genetic drift and inbreeding, enhancing the observed differences between  $t$  and  $\mu_{crit}$ . If species could be found in both fragmented and unfragmented landscapes then the analysis could be conducted as a comparison.

Another prediction is that species with a scale of gene flow smaller than the scale of landscape structure would be more susceptible to isolation effects such as a loss of genetic variability. In order to test this prediction, population characteristics such as variance in population size, genetic variability, and other variables associated with the potential effects of isolation could be examined for a single species in several landscapes differing in the time since isolation. In each landscape the difference between the scale of landscape structure and gene flow could be compared to potential effects of isolation to evaluate the prediction.

Simulation models could also be devised to evaluate the robustness of each of these predictions. Expansion of models that examine the interaction of temporal and spatial scales in the evolution of dispersal (e.g., Boulinier & Lemel 1996; Paradis 1998) to include the spatial scale of gene flow could be especially informative. Observing the development of the spatial scale of gene flow in undisturbed landscapes and changes in

the spatial scale of gene flow due to subsequent alteration of habitat structure in such models could provide qualitative tests of the predictions.

### **PERSPECTIVE**

Wiens (1997) recognized that the synthesis of metapopulation study and landscape ecology had been slow to occur, in part due to the lack of theory dealing with spatial patterning in landscape ecology. This paper represents an attempt to integrate explicit measures of scale with existing ecological knowledge in order to develop a theoretical framework for the ecological interpretation of observed spatial patterns. Nekola & White (1999) developed a comparable synthesis of spatial changes community similarity, called the distance-decay of similarity, in spruce-fir forests of North America. In analyzing data from more than 800 plots they found that the rate of change of distance-decay of similarity was negatively related to a species' dispersal ability and positively related to the geographic isolation of plots. Advancement of theoretical development in landscape ecology depends on the development of testable hypotheses and predictions of pattern and process.

The explicit treatment and interpretation of scalar effects on ecological patterns and processes is in its infancy. But the issue of addressing pattern and scale is among the central issues of ecology (Levin 1992). Analysis of how temporal and spatial scales interact to produce observed patterns may provide the key to understanding the ecological significance of pattern and scale. In addressing this challenge unique approaches must be developed that enhance our understanding of ecological dynamics and the influence of human disturbance on ecological pattern and process.

**Table 1.** General life history differences between selected amphibian taxa that may can be related to habitat fragmentation sensitivity.

Taxonomic Group	Explosive Breeder	Aquatic Larvae	Desiccation Sensitivity	Clutch Size
Ambystoma salamanders	Y	Y	LOW	LARGE
Desmognathine salamanders	N	Y	HIGH	SMALL
Acris & Pseudacris frogs	Y	Y	LOW	LARGE
Plethodontine salamanders	N	S	HIGH	SMALL



**Table 2.** Protein enzyme loci resolved for *Acris crepitans*. Data include the enzyme locus, the medium used (S for starch, C for cellulose acetate), the tray buffer, and whether the locus was polymorphic (P) or monomorphic (M).

Locus	Medium	Tray Buffer	Genetic Properties
AP (la)	C	0.01M Citrate Phosphate pH 6.4	M
	S	Lithium Hydroxide	M
AP (lgg)	C	0.01M Citrate Phosphate pH 6.4	P
	C	0.05M Tris Maleate pH 7.8	P
AP (pp)	C	0.01M Citrate Phosphate pH 6.4	M
IDH	C	0.01M Citrate Phosphate pH 6.4	M
MDH	C	0.01M Citrate Phosphate pH 6.4	M
ME	C	0.015M Tris EDTA Borate MgCl <sub>2</sub>	Locus A - P Locus B - M
	C	0.05M Tris Maleate pH 7.8	M
MPI	C	0.02M Phosphate pH 7.0	M
PGM	C	0.05M Tris Maleate pH 7.8	P
	C	0.02M Phosphate pH 7.0	P
	S	Lithium Hydroxide	P
GOT	S	Tris-Citrate pH 8.0	P

**Table 3.** Protein enzyme loci resolved for Ambystoma opacum. Data include the enzyme locus, the medium used (S for starch, C for cellulose acetate), the tray buffer, and whether the locus was polymorphic (P) or monomorphic (M).

Locus	Medium	Tray Buffer	Genetic Properties
AP (la)	C	0.01M Citrate Phosphate pH 6.4	M
AP (lgg)	C	0.05M Citrate Phosphate pH 6.4	P
AP (pp)	C	0.01M Citrate Phosphate pH 6.4	M
G3PDH	C	0.05M Tris Maleate pH 7.8	M
IDH	C	0.05M Tris Maleate pH 7.8	M
MDH	C	0.05M Tris Maleate pH 7.8	M
ME	C	0.05M Tris Maleate pH 7.8	M

**Table 4.** Protein enzyme loci resolved for Desmognathus monticola. Data include the enzyme locus, the medium used (S for starch, C for cellulose acetate), the tray buffer, and whether the locus was polymorphic (P) or monomorphic (M).

Locus	Medium	Tray Buffer	Genetic Properties
AP (la)	C	TEMB pH 7.8	M
AP (lgg)	C	0.0116M Phosphate pH 7.0	P
AP (pp)	C	Tris-Glycine pH 8.5	M
PGDH	C	TEMB pH 7.8	M
PGI	C	TEMB pH 7.8	P
	C	0.0116M Phosphate pH 7.0	P
GA3PDH	C	0.0116M Phosphate pH 7.0	M
G3PDH	C	0.0116M Phosphate pH 7.0	M
IDH	C	0.01M Citrate Phosphate pH 6.4	M
LDH	C	TEMB pH 7.8	M
FUMH	C	0.01M Citrate Phosphate pH 6.4	M
GLCDH	C	TEMB pH 7.8	P

**Table 5.** Protein enzyme loci resolved for Eurycea bislineata cirrigera. Data include the enzyme locus, the medium used (S for starch, C for cellulose acetate), the tray buffer, and whether the locus was polymorphic (P) or monomorphic (M).

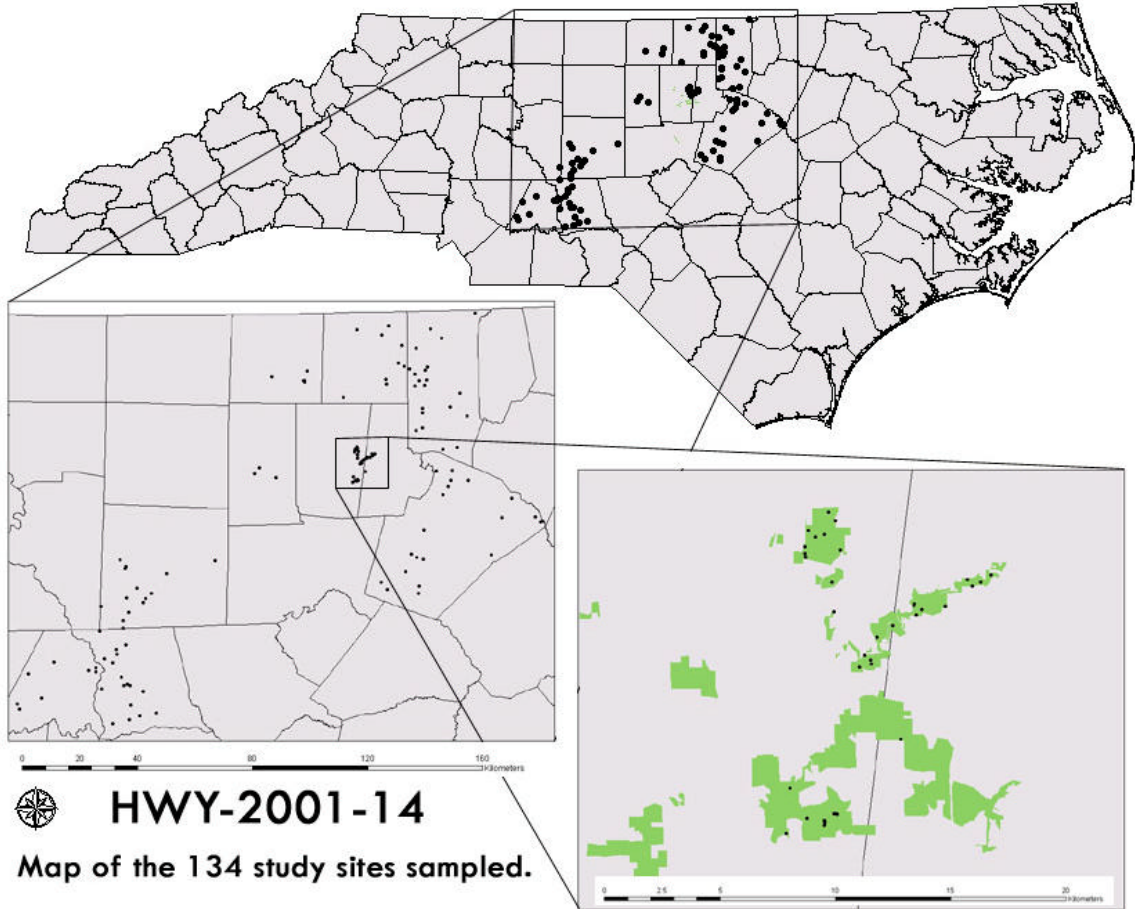
Locus	Medium	Tray Buffer	Genetic Properties
AP (Ia)	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
AP (Igg)	C	Tris-Glycine pH 8.5	P
		0.0116M Phosphate pH 7.0	P
AK	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
AAT	C	Tris-Glycine pH 8.5	P
		0.0116M Phosphate pH 7.0	P
G6PDH	C	Tris-Glycine pH 8.5	P
		0.0116M Phosphate pH 7.0	P
PGI	C	Tris-Glycine pH 8.5	P
		0.0116M Phosphate pH 7.0	P
IDH	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
LDH	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
MDH	C	Tris-Glycine pH 8.5	P
		0.0116M Phosphate pH 7.0	P
ME	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
PGDH	C	Tris-Glycine pH 8.5	P

**Table 6.** Protein enzyme loci resolved for Desmognathus fuscus. Data include

the enzyme locus, the medium used (S for starch, C for cellulose acetate), the tray buffer, and whether the locus was polymorphic (P) or monomorphic (M).

Locus	Medium	Tray Buffer	Genetic Properties
AP (lgg)	C	0.01M Citrate Phosphate pH 6.4	P
		0.0116M Phosphate pH 7.0	P
AP (pp)	C	Tris-Glycine pH 8.5	M
		0.0116M Phosphate pH 7.0	M
AAT	C	0.01M Citrate Phosphate pH 6.4	P
PGDH		0.0116M Phosphate pH 7.0	P
PGM	C	0.0116M Phosphate pH 7.0	M
AAT		0.01M Citrate Phosphate pH 6.4	M
LDH	C	0.0116M Phosphate pH 7.0	P
FUMH		Tris-Glycine pH 8.5	M
G6PDH	C	0.01M Citrate Phosphate pH 6.4	P
MDH		0.0116M Phosphate pH 7.0	M
ME	C	0.01M Citrate Phosphate pH 6.4	P

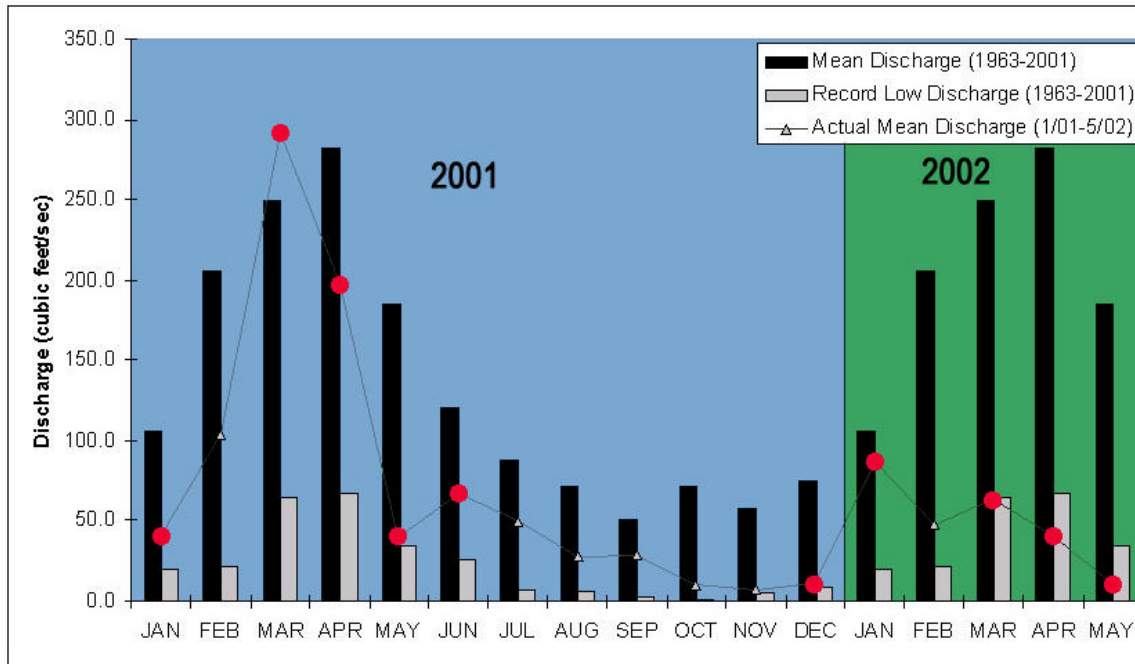
**Figure 1.** Map of the sampling sites throughout the piedmont region of North Carolina.



**Figure 2.** Photographs of the primary passive sampling method, pitfall-driftfence traps. The insets show the traps along the fence – one with a captured Ambystoma maculatum.



**Figure 3.** Streamflow data for the Eno River, Durham, North Carolina from January 2001 to May 2002. Circular markers denote the occurrence of a daily record low flow during the month.

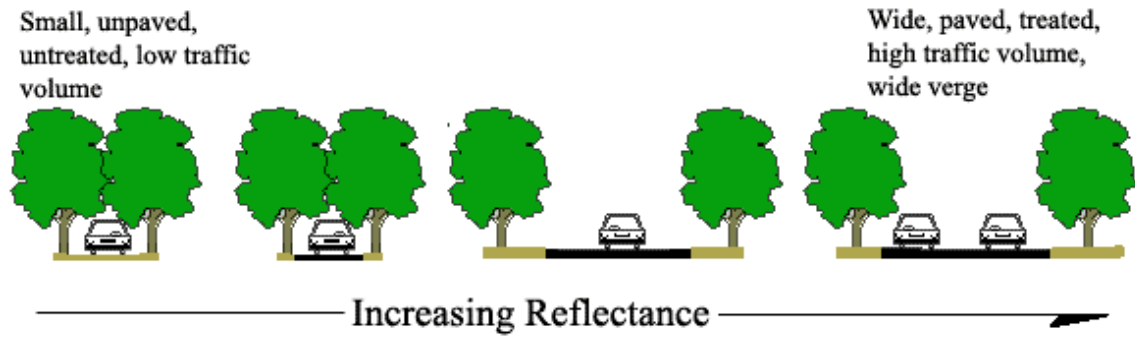




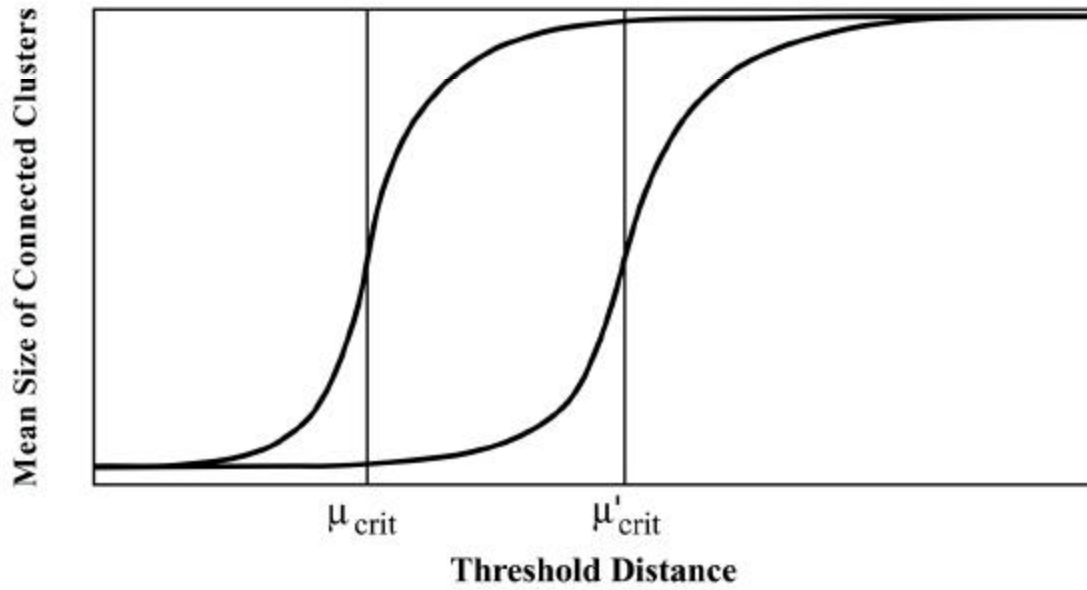
**Figure 4.** Photos from two breeding ponds in the study area. A. photo of a temporary pond in the Eno River State Park taken January 2001. Note the egg masses present in the pond. B. Photo from a temporary pond in Duke Forest taken March 2001. Arrows show the presence of desiccated egg masses due to drought conditions.



**Figure 5.** Illustration of the continuum of barrier effect from filtration to reflectance. Note that the degree of filtration will also occur along the continuum for species that display no behavioral avoidance.



**Figure 6.** Example of edge thresholding analysis in which the scale of gene flow can be compared to the structural scale of habitat. Habitat is connected for organisms that have gene flow scales greater than the structural scale of habitat. Notice that anthropogenic fragmentation alters the scale of habitat structure such that organisms for which habitat was connected before fragmentation may now suffer effects of isolation.



## Literature Cited

Andreassen, H. P., Halle, S. and Ims, R. A. 1996a. Optimal design of movement corridors in root voles – not too wide and not too narrow. – *J. Appl. Ecol.* 33: 63-70.

Andreassen, H. P., Ims, R. A. and Steinset, O. K. 1996b. Discontinuous habitat corridors: the effects on male root vole movements. – *J. Appl. Ecol.* 33: 555-560.

Andreassen, H. P., Hetzberg, K. and Ims, R. A. 1998. Space use responses to habitat fragmentation and connectivity in the root vole *Microtus oeconomus*. – *Ecology* 79: 123-135.

Andren, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. – *Oikos* 71: 355-366.

Baur, A & Baur. 1990. Are roads barriers to dispersal in the land snail *Arianta arbustorum*? *Can. J. Zool.* 68: 613-617.

Belichon, S., Clobert, J. and Massot, M. 1996. What differences in fitness components between philopatric and dispersing individuals? – *Acta Oecologica* 16: 503-517.

Boulinier, T. 1996. On breeding performance, colony growth and habitat selection in Buffnecked Ibis (*Theristicus caudatus*). – *Condor* 98: 440-441.

Boulinier, T. and Lemel, J.-Y. 1996. Spatial and temporal variations of factors affecting breeding habitat quality in colonial birds: some consequences for dispersal and habitat selection. – *Acta Oecologica* 17: 531-552.

Brody, A.J. & Pelton, M.R. 1989. Effects of roads on Black Bear movements in western North Carolina. *Wildl. Soc. Bull.* 17: 5-10.

Bunn, A. G., Urban, D. L. and Keitt, T. H. 2000. Landscape connectivity: a conservation application of graph theory. – *J. Env. Mgt.* 59: 265-278.

Burger, J. 1982. The role of reproductive success in colony-site selection and abandonment in Black Skimmers (*Rynchops niger*). – *Auk* 99: 109-115.

Carr, W. & Fahrig, L. 2001. Effect of road traffic on two amphibian species of differing vagility. *Cons Biol.* 15(4): 1071-8.

Chung, M. G. and Chung, M. Y. 1999b. Spatial genetic structure of clonal and sexual reproduction in a population of *Abeliophyllum distichum* (Oleaceae), an endangered monotypic genus. – *Genes & Gen. Sys.* 74: 9-14.

- Chung, M. G. and Park, C. W. 2000. Notes on spatial genetic structure in a hybrid population between *Aconitum japonicum* subsp *napiforme* and *A. jaluense* (Ranunculaceae). – *Ann. Bot. Fenn.* 37: 243-247.
- Chung, M. G., Chung, J. M., Chung, M. Y. and Epperson, B. K. 2000. Spatial distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of *Rhus javanica* (Anacardiaceae). – *Heredity* 84: 178-185.
- Chung, M. Y. and Chung, M. G. 1999a. Notes on spatial genetic structure in populations of *Cymbidium goeringii* (Orchidaceae). – *Ann. Bot. Fenn.* 36: 161-164.
- Clarke, GP, PCL White & S Harris. 1998. Effects of roads on badger *Meles meles* populations in south-west England. *Biolog. Conserv.* 86: 117-124.
- Clevenger, A. P. & Waltho, N. 2000. Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. *Cons. Biol.* 14: 47-56.
- Clevenger, A. P., Chruszcz, B. & Gunson, K. 2001a. Drainage culverts as habitat linkages and factors affecting passage by mammals. *J. Appl. Ecol.* 38: 1340-49.
- Clevenger, A. P.; MacIvor, M.; MacIvor, D.; Chruszcz, B. & Gunson, K. 2001b. Tiger salamander *Ambystoma tigrinum*, movements and mortality on the Trans-Canada Highway in southwestern Alberta. *Can. Field. Nat.* 115: 199-204.
- Clevenger, A. P., Chruszcz, B. & Gunson, K. 2003. Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. *Biol. Conserv.* 109: 15-26.
- Conroy, M.J., Cohen, Y., James, F.C., Matsinos, Y.G. and Maurer, B.A. 1995. Parameter estimation, reliability, and model improvement for spatially explicit models of animal populations. *Ecol. Appl.* 5: 3-11.
- Cuperus, R.; Kalsbeek, M.; Haes, H & Canters, K. J. 2001. Preparation and implementation of seven ecological compensation plans for Dutch highways. *Environ. Mgt.* 29(6): 736-49.
- Davidson, C. 1998. Issues in measuring landscape fragmentation. – *Wild. Soc. Bull.* 26: 32-37.
- deMaynadier, P. G. and Hunter, M. L. 1998. Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. – *Cons. Biol.* 12: 340-352.
- deMaynadier, PG and ML Hunter. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. *J. Wild. Mgt.* 63: 441-450.
- deMaynadier, PG and ML Hunter. 2000. Road effects on amphibian movements in a forested landscape. *Nat. Areas J.* 20: 56-65.

deVries H. H., denBoer, P. J. and van Dijk, Th. S. 1996. Ground beetle species in heathland fragments in relation to survival, dispersal, and habitat preference. –*Oecologia* 107: 332-342.

Doak, D. F., Marino, P. C. and Karieva, P. M. 1992. Spatial scale mediates the influence of habitat fragmentation on dispersal success: implications for conservation. – *Theor. Pop. Biol.* 41: 315-336.

Fahrig, L. and Paloheimo, J. 1988. Determinants of local population size in patchy habitats. – *Theor. Pop. Biol.* 34: 129-212.

Forman, RTT & LE Alexander. 1998. Roads and their major ecological effects. *Ann. Rev. Ecol. System.* 29: 207-231.

Forman, R. T. T. 1998. Road ecology: a solution for the giant embracing us. *Landscape Ecol.* 13: iii-v.

Forman, R.T.T. 2000. Estimate of the area affected ecologically by the road system in the United States. *Cons. Biol.* 14: 31-5.

Forman, R.T.T.; Friedman, D.S.; Fitzhenry, D.; Martin, J.D.; Chen E.S. & Alexander L.E. 1997. Ecological effects of roads: toward three summary indices and an overview for North America. Pp. 40-54 in: *Habitat Fragmentation and Infrastructure* (K. Canters, ed.). Public Works & Water Mgt., Delft, Netherlands.

Forman, R.T.T.; Sperling, D.; Bissonette, J.A.; Clevenger, A.P.; Cutshall, C.D.; Dale, V.H.; Fahrig, L.; France, R.; Goldman, C.R.; Heanue, K.; Jones, J.A.; Swanson, F.J.; Turrentine, T. & Winter, T.C. 2003. *Road Ecology: Science and Solutions*. – Island Press.

Frank, S.A. 1996. Models of parasite virulence. *Q. Rev. Biol.* 71: 37-78.

Fretwell, S. D. and Lucas, H. J. 1970. On territorial behavior and other factors influencing habitat distribution in birds. – *Acta Biotheor.* 19: 16-36.

Gardner, R. H. and O'Neill, R. V. 1991. Pattern, process and predictability: the use of neutral models for landscape analysis.– In: Turner, M. G. and Gardner, R. H. (eds.), *Quantitative methods in landscape ecology* Springer-Verlag, pp 289-307.

Gardner, R. H., Milne, B. T., Turner, M. G. and O'Neill, R. V. 1987. Neutral models for the analysis of broad-scale landscape pattern. – *Landscape Ecol.* 1: 19-28.

Gibbs, J. P. & Shriver, G. 2002. Estimating the effects of road mortality on turtle populations. *Cons. Biol.* 16: 1647-52.

- Gibbs, J. P. 1998a. Distribution of woodland amphibians along a forest fragmentation gradient. – *Landsc. Ecol.* 13: 263-268.
- Gibbs, J. P. 1998b. Amphibian movements in response to forest edges, roads, and streambeds in southern New England. – *J. Wildl. Mgt.* 62: 584-589.
- Gibbs, J. P. 1998c. Genetic structure of the redback salamander *Plethodon cinereus* populations in continuous and fragmented forests. *Biol. Conservat.* 86: 77-81.
- Giles, B. E. and Goudet, J. 1997. A case study of genetic structure in a plant metapopulation. – In: Gilpin, M. and Hanski, I. (eds.), *Metapopulation Biology: Ecology, Genetics, and Evolution*. Academic Press, pp. 429-454.
- Golden, D. M. and Crist, T. O. 2000. Experimental effects of habitat fragmentation on rove beetles and ants: patch area or edge. – *Oikos* 90: 525-538.
- Gross, J. and Yellen, J. 1999. *Graph Theory and Its Applications*. – CRC Press.
- Gu, H & W Danthanarayana. 1992. Quantitative genetic analysis of dispersal in *Epiphyas postvittana*. II. Genetic covariations between flight capacity and life history traits. *Heredity*. 68: 61-69.
- Guyot, G & J Clobert. 1997. Conservation measures for a population of Hermann's Tortoise *Testudo hermanni* in southern France bisected by a major highway. *Biolog. Conserv.* 79: 251-256.
- Haddad, N. M. 1999a. Corridor and distance effects on interpatch movements: a landscape experiment with butterflies. – *Ecol. Appl.* 9: 612-622.
- Haddad, N. M. 1999b. Corridor use predicted from behaviors at habitat boundaries. – *Am. Nat.* 153: 215-227.
- Haddad, N. M. and Baum, K. A. 1999. An experimental test of corridor effects on butterfly densities. – *Ecol. Appl.* 9: 623-633.
- Haddad, N. M., Rosenberg, D. K. and Noon, B. R. 2000. On experimentation and the study of corridors: response to Beir and Noss. – *Cons. Biol.* 14: 1543-1545.
- Hansen, AJ & DL Urban. 1992. Avian response to landscape pattern: The role of species' life histories. *Landsc. Ecol.* 7: 163-180.
- Harary, F. 1969. *Graph Theory*. – Addison Wesley.
- Heinen, K. and Merriam, G. 1990. The elements of connectivity where corridor quality is variable. – *Landsc. Ecol.* 4: 157-170.

- Hels, T. & Buchwald, E. 2001. The effect of road kills on amphibian populations. *Biol. Conserv.* 99: 331-40.
- Hitchings, SP & TJC Beebee. 1997. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* 79: 117-127.
- Hjermann, D. O. and Ims, R. A. 1996. Landscape ecology of the wart-biter *Decticus verrucivorus* in a patchy landscape. – *J. Anim. Ecol.* 65: 768-780.
- Ims, R. A. and Andreassen, H. P. 1999. Effects of experimental habitat fragmentation and connectivity on root vole demography. – *J. Anim. Ecol.* 68: 839-852.
- Inbar, M & RT Mayer. 1999. Spatio-temporal trends in armadillo diurnal activity and road-kills in central Florida. *Wildlife Soc. Bull.* 27: 865-872.
- Jaeger, J. A. G. 2000. Landscape division, splitting index, and effective mesh size: new measures of landscape fragmentation. – *Landsc. Ecol.* 15: 115-130.
- Johnson, M. L. and Gaines, M. S. 1990. Evolution of dispersal: theoretical models and empirical tests using birds and mammals. – *Ann. Rev. Ecol. Syst.* 21: 449-480.
- Keitt, T. H., Urban, D. L. and Milne, B. T. 1997. Detecting critical scales in fragmented landscapes. – *Cons. Ecol.* 1 (online) URL: <http://www.consecol.org/Journal/vol1/iss1/art4/>
- Korona, R. 1991. Genetic basis of behavioral strategies. Dispersal of female flour beetles, *Tribolium confusum*, in a laboratory system. – *Oikos* 62: 265-270.
- Kuno, E. 1981. Dispersal and persistence of populations in unstable habitats: a theoretical note. – *Oecologia* 49: 123-126.
- Lande, R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. *Res. Popul. Ecol.* 40: 259-269.
- Legendre, P. and Fortin, M.- J. 1989. Spatial pattern and ecological analysis. – *Vegetatio* 80: 107-138.
- Lehmkuhl, J. F. and Ruggiero, L. F. 1991. Forest fragmentation in the Pacific Northwest and its potential effects on wildlife. – In: Ruggiero, L. F., Aubry, K. B., Carey, A. B. and Huff, M. H. (tech. coord.), *Wildlife and Vegetation of Unmanaged Douglas-Fir Forests*. GTR-PNW-285. U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station, pp 34-36.



Lemel J- Y., Belichon, S., Clobert, J. and Hochberg, M. E. 1997. The evolution of dispersal in a two-patch system: some consequences of differences between migrants and residents. – *Evol. Ecol.* 11: 613-629.

Levin, S. A. 1992. The problem of pattern and scale in ecology. – *Ecology* 73: 1943-1967.

Lidicker, W. Z. and Stenseth, N. C. 1992. To disperse or not to disperse: who does it and why? – In: Stenseth, N. C. and Lidicker, W. Z. (eds.), *Animal Dispersal*. Chapman & Hall, pp. 21-34.

Lode, T. 2000. Effect of a motorway on mortality and isolation of wildlife populations. *Ambio*. 29(3): 163-6.

Ludwig, J.A., Wiens, J.A. and Tongway, D.J. 2000. A scaling rule for landscape patches and how it applies to conserving soil resources in savannas. *Ecosystems* 3: 84-97.

McPeck, M. A. and Holt, R. D. 1992. The evolution of dispersal in spatially and temporally varying environments. – *Am. Nat.* 140: 1010-1027.

Metzger, J.- P. and Decamps, H. 1997. The structural connectivity threshold: an hypothesis in conservation biology at the landscape scale. – *Acta Oecologica* 18: 1-12.

Mizuyasaki, Y. and Isagi, Y. 2000. Pollen flow and the intrapopulation genetic structure of *Heloniopsis prientalis* on the forest floor as determined using microsatellite markers. – *Theor. Appl. Genetics* 101: 718-723.

National Weather Service Climate Data.  
<http://www.erh.noaa.gov/er/rah/climate/index.html#lcd>

NCHRP 2002. Interaction between roadways and wildlife ecology. NCHRP Synthesis 305.

Nei, M. 1972. Genetic distance between populations. – *Am. Nat.* 106: 283-292.

Nekola, J. C. and White, P. S. 1999. The distance decay of similarity in biogeography and ecology. – *J. Biogeog.* 26: 867-878.

O'Neill, R. V., Krummel, J. R., Gardner, R. H., Sugihara, G., Jackson, B., DeAngelis, D. L., Milne, B. T., Turner, M. G., Zygmunt, B., Christensen, S. W., Dale, V. H. and Graham, R. L. 1988. Indices of landscape pattern. – *Landsc. Ecol.* 1: 153-162.

Oxley, D. J.; Fenton, M. B. & Carmody, G. R. 1974. The effects of roads on populations of small mammals. *J. Appl. Ecol.* 11: 51-9.

- Paradis, E. 1998. Interactions between spatial and temporal scales in the evolution of dispersal rate. – *Evol. Ecol.* 12: 235-244.
- Pechmann, J.H.K.; Scott, D.E.; Semlitsch, R.D.; Caldwell, J.P.; Vitt, L.J. & Gibbons, J.W. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* 253: 892-5.
- Reh, W & A Seitz. 1990. The influence of land use on the genetic structure of populations of the common frog, *Rana temporaria*. *Biol. Conservat.* 54: 239-249.
- Roff, D.A. & Fairbairn, D.J. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the insecta. *Am Zool.* 31: 243-51.
- Saunders, D. A., Hobbs, R. J. and Margules, C. R. 1991. Biological consequences of ecosystem fragmentation: A review. – *Conserv. Biol.* 5: 18-32.
- Schmidt, K. A.; Earnhardt, J. M.; Brown, J. S. & Holt, R. D. 2000. Habitat selection under temporal heterogeneity: exorcizing the ghost of competition past. *Ecology* 81: 2622-30.
- Schumaker, N. H. 1996. Using landscape indices to predict habitat connectivity. – *Ecology* 77: 1210-1225.
- Sokal, R. R. and Rolf, F. J. 1994. *Biometry : The Principles and Practice of Statistics in Biological Research.* – W. H. Freeman & Co.
- Stamps, J. A., Buechner, M. and Krishnan, V. V. 1987. The effects of edge permeability and habitat geometry on emigration from patches of habitat. – *Am. Nat.* 129: 533-552.
- Stauffer, D. and Aharony, A. 1985. *Introduction to percolation theory.* – Taylor and Francis.
- Stoms, DM. 2000. GAP management status and regional indicators of threats to biodiversity. *Landscape Ecol.* 15: 21-33.
- Taylor, P. D., Fahrig, L., Henein, K. and Merriam, G. 1993. Connectivity is a vital element of landscape structure. – *Oikos* 68: 571-573.
- Tischendorf, L. and Fahrig, L. 2000a. On the usage and measurement of landscape connectivity. – *Oikos* 90: 7-19.
- Tischendorf, L. and Fahrig, L. 2000b. How should we measure landscape connectivity? – *Landsc. Ecol.* 15: 633-641.
- Travis, J. M. J. and Dytham, C. 1998. The evolution of dispersal in a metapopulation: a spatially explicit, individual-based model. – *Proc. R. Soc. Lond. B* 265: 17-23.

- Trombulak, S. C. & Frissell, C. A. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Cons. Biol.* 14: 18-30.
- Turner, M. G. 1989. Landscape ecology: the effect of pattern on process. – *Ann. Rev. Ecol. Syst.* 20: 171-197.
- Turner, S. J., O'Neill, R. V., Conley, W., Conley, M. R. and Humphries, H. C. 1991. Pattern and scale: statistics for landscape ecology. – In: Turner, M. G. and Gardner, R. H. (eds.), *Quantitative Methods in Landscape Ecology*. Springer-Verlag, pp. 17-49.
- Watt, A. S. 1947. Pattern and process in the plant community. – *J. Ecol.* 35:1-22.
- Whitcomb, R. F., Lynch, J. F., Klimkiewicz, M. K., Robbins, C. S., Whitcomb, B. L. and Bystrak, D. 1981. Effects of forest fragmentation on avifauna of the eastern deciduous forest. – In: Burgess, R. L. and Sharpe, D. M. (eds.), *Forest Island Dynamics in Man-Dominated Landscapes*. Springer-Verlag, pp. 125-205.
- Wiens, J. A. 1997. Metapopulation dynamics and landscape ecology. – In: Gilpin, M. and Hanski, I. (eds.), *Metapopulation Biology: Ecology, Genetics, and Evolution*. Academic Press, pp 43-62.
- Wiens, J. A., Schooley, R. L. and Weeks, R. D. 1997. Patchy landscapes and animal movements: do beetles percolate? – *Oikos* 78: 257-264.
- Wilson, L.A. 1995. *The land manager's guide to the amphibians and reptiles of the south*. Nature Conservancy.
- With, K. A. 1997. The application of neutral landscape models in conservation biology. – *Conserv. Biol.* 11: 1069-1080.
- With, K. A. and Crist, T. O. 1995. Critical thresholds in species' responses to landscape structure. – *Ecology* 76: 2446-2459.
- With, K. A. and Crist, T. O. 1996. Translating across scales: simulating species distributions as the aggregate response of individuals to heterogeneity. – *Ecol. Modelling* 93: 125-137.
- With, K. A. and King, A. W. 1997. The use and misuse of neutral landscape models in ecology. – *Oikos* 79: 219-229.
- With, K. A. and King, A. W. 1999. Extinction thresholds for species in fractal landscapes. – *Conserv. Biol.* 13: 314-326.

Wymann, R.L. 1991. Multiple threats to wildlife: climate change, acid precipitation, and habitat fragmentation. pp. 134-55 in: *Global Climate Change and Life on Earth* (R.L. Wyman, ed.). Chapman & Hall.

Zhao, Z.W. & Zera, A.J. 2002. Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proc. Nat. Acad. Sci.* 99(26): 16829-34.