5-1-2009

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Determination of Dispersal Patterns of the Small-mouthed Salamander 
(*Ambystoma texanum*) in Eagle Creek Park (Indianapolis, IN)

A Thesis

Presented to the Department of Biological Sciences

College of Liberal Arts and Sciences

and

The Honors Program

of

Butler University

In Partial Fulfillment

of the Requirements for Graduation Honors

Stacey Diane Summitt

May 1, 2009
Abstract

Urbanization and the increasing threat of habitat fragmentation are contributing to the significant declines in amphibian populations world-wide. Ambystomatid species are particularly susceptible to habitat fragmentation because they migrate yearly across upland forests to their breeding sites. Habitat changes could be altering the genetic structure in these species; however few studies have focused on this topic and more generally, examined the dispersal patterns of Ambystomatids. In 2003, Clark, Cripe, and Stachniw conducted a study on “Metapopulation Structure of Ambystoma texanum in Eagle Creek Park (Indianapolis, IN) and the Potential for Gene Flow.” They hypothesized that distance limits the dispersal of populations and creates genetically isolated populations. In the present study, I use an integrative approach including landscape ecology and microsatellite analysis in order to test their hypothesis of dispersal patterns and gene flow in small-mouthed salamander populations. I located breeding sites of *A. texanum* at Eagle Creek Park marked by Van Deman (1998) using GPS, and I described new breeding sites. I located 8 of the 19 sites marked by Van Deman (1998), all of which were still active breeding sites, and I observed evidence of breeding in two sites previously described by Van Deman (1998) as non-breeding. I also located 18 new ephemeral sites, and 15 contained evidence of *A. texanum* breeding. In addition, I collected larvae from 8 of the breeding sites, with the average sample size being 17 individuals from each pond and used PCR to amplify microsatellite markers for *A. texanum* for each of these samples. Only one marker, Atex 143, produced successful trials. I determined the allelic composition of 4 ponds and the preliminary results infer that distance limits gene flow in *A. texanum*. Information from this study provides a valuable starting point for the use of microsatellite markers in determining dispersal patterns of *A. texanum*. In addition, knowledge of the landscape ecology and
hydrology of Eagle Creek Park sets the stage for comparison and long-term monitoring of the
*A. texanum*, demonstrating the impact of urbanization on these wetland populations.

**Introduction**
Declines in amphibian populations world-wide have been associated with multiple
anthropogenic causes (Walston and Mullin, 2008); however, current studies suggest that the
leading cause of these declines is due to habitat loss and fragmentation (Semlitsch, 2000;
Hamer and McDonnell, 2008). Fragmentation of the landscape is particularly detrimental to
amphibian species because it increases the proportion of potentially unsuitable habitat in the
landscape (Graeter et al., 2008) as well as the risk of predation (McDonough and Paton,
2006). Furthermore, habitat fragmentation may impede or prevent dispersal (McDonough
and Paton, 2006). Disruption of dispersal is highly problematic, especially for juveniles, as it
is the basis for the maintenance of amphibian population dynamics via increasing the genetic
diversity of metapopulations and promoting recolonizations of sink populations (Semlitsch,
2000).

Urbanization and rural-city fringes are the most prominent driving forces of habitat
reported that 88% of threatened amphibians are affected by habitat fragmentation due to
urbanization. Despite this, there are few studies on the effects of urbanization on amphibian
habitat and on pond-breeding amphibians (Hamer and McDonnell, 2008; Marsh and
Trenham, 2001). In addition to habitat changes, fragmentation of the landscape due to
human expansion can also change the genetic structure of a population, possibly creating
isolated populations that are susceptible to stochastic fluctuations and local extinctions
Arens, 2007). Little is known about this subject, however, and Gibbs (1998) suggested that a key area of study for amphibian ecology should focus on the landscape ecology and genetics of urban parklands connected by riparian forests because they are used as suitable habitat corridors for amphibians.

Ambystomatid salamanders are especially vulnerable to habitat fragmentation as data suggests that these species migrate through adjacent upland forests to reach suitable breeding sites (McDonough and Paton, 2007). This study focuses on Ambystoma texanum, one of the seven Ambystomatid species that inhabit Indiana. A. texanum, or the small-mouthed salamander, is commonly found in midwestern and central southern states (Petranka, 1998). They are explosive breeders that breed early in the year, emerging in late winter or early spring and migrating to shallow ephemeral, or temporary ponds. Despite the large amount of information known about the life history of the small-mouthed salamander, there has been only one study to my knowledge conducted on the migration and dispersal of A. texanum (Williams, 1973) and only few have been published for other species in the genus Ambystoma (Madison, 1997; Trenham et al., 2001; Gamble et al., 2007). Harding (1997) suggested that A. texanum stays relatively closer to breeding sites in summer and late winter than other species and is not capable of long distance migration. This assumption is supported by Williams (1973) who observed that the average migration distance for A. texanum is 52.4 m, with a maximum of 160.8m. On the other hand, Trenham et al. (2001) found that Californian tiger salamanders, A. californiense, are probably capable of long distance migration between 500 to 670 m, but not exceeding 700 m. Long distance migration and dispersal has also been documented in marbled salamanders, A. opacum (Gamble et al., 2007) and spotted salamanders, A. maculatum (Madison, 1997).
Similarities between other *Ambystoma* species and *A. texanum* suggest that the small-mouthed salamander may also be capable of longer distance dispersal patterns. Mark-recapture methods used for classic migration studies such as in Williams (1973) have a high probability of missing long range movements, especially in amphibians which are often difficult to locate during terrestrial overwintering (Kinkead et al., 2007). Technological advances have greatly improved the resolution of dispersal processes and provided insight into evolutionary ecology and population structure. Although gene flow and dispersal are not synonymous, the two are generally correlated (Bohonak, 1999). As such, geographical information system (GIS) mapping of habitat variables could be used in conjunction with genetic markers in order to better examine the influence of specific habitat features on genetic structure (Spear et al., 2005) as well as to determine dispersal patterns by analyzing the degree of gene flow that occurs between populations. One of the most versatile types of genetic markers for these types of ecological questions is microsatellites. Microsatellites are high frequency, tandem repeats of one to six nucleotides in the genome that vary greatly in the number of units within a population. Microsatellite loci are non-coding, meaning that they are not subject to selection yet still follow the rules of Mendelian inheritance (Selkow and Toonen, 2006). Microsatellite markers have become increasingly popular because they are easy to differentiate inexpensively using gel electrophoresis and allow for the use of small tissue samples. Specific to ecology, microsatellites have the ability to provide contemporary, fine-scale estimates of migration, distinguishing between high rates of migration and panmixia (Selkow and Toonen, 2006).

An integrative approach involving landscape ecology and population genetics can help to answer not only questions involving migration and dispersal distances in *A. texanum*,
but also to characterize the population structure of this species in a regional area and identify possible effects of habitat fragmentation. In recent years, there has been an increasing popularity in using metapopulation theory to study amphibian ecology. A metapopulation is a “set of local populations within some larger area, where typically migration from one local population to at least some other patches is possible” (Hanski and Gilpin, 1997). This structure provides a basis for conserving species in patchy, fragmented habitats (Marsh and Trenham, 2000) as one male migrant and one female migrant are capable of rescuing local populations (or recolonizing) (Trenham et al., 2001). A single migrant per generation is also capable of preventing significant changes in the genetic variation of the population (Mills and Allendorf, 1996). Gene flow occurs in populations which are able to migrate between breeding sites, and the genetic composition of local populations would be similar to one another. If distance limits dispersal or habitat fragmentation provides barriers against dispersal, however, gene flow would not occur and populations would be become isolated, genetically diverging from each other due to local adaptation and genetic drift.

Accurately quantifying dispersal rates and determining the degree to which landscape features, such as habitat fragmentation, facilitate or hinder these movements is of high research priority (Ricketts, 2001). An integrative approach has already been employed for a several amphibian species (Arens et al., 2007; Gamble et al., 2007; Kinkead et al., 2007; Purrenhage et al., 2009). Clark et al. (2003) used metapopulation theory in order to describe the population structure of *A. texanum* in Eagle Creek Park, Indianapolis, Indiana and hypothesized about the potential for gene flow. Using the migration distance of Williams (1973) for *A. texanum*, they reported 4, and possibly 5, isolated breeding populations within the park based on the distance between the ponds.
This study aims to test the hypothesis of Clark et al. (2003) that distance limits gene flow between breeding populations of *A. texanum* in Eagle Creek Park. My objectives were to 1) use a global positioning system (GPS) and geographic information system (GIS) technology to map the current landscape features and breeding sites of the small-mouthed salamander at Eagle Creek Park 2) use microsatellite analysis to determine gene flow and analyze population structure and 3) quantify dispersal rates for *A. texanum* based on distance between breeding populations and the assumption that isolated populations exist.

This study would be the first modern approach at determining dispersal patterns and population structure in *A. texanum*. Currently, the lack of information on migration and dispersal patterns of *A. texanum* is alarming considering that the small-smouthed salamander is at risk due to wetland destruction throughout its range (Ryan, 2007). In addition, vulnerability of this species and other *Ambystoma* to habitat fragmentation is particularly high, especially in urban and suburban regions. Results of this study will help to further complete the life history of small-mouthed salamanders by providing information on their dispersal patterns. This study will also aid in understanding habitat requirements of *A. texanum* in urban settings in order to better plan conservation efforts needed for salamander populations.

**Methods:**

**Study Site:**

Field work for this study was conducted at Eagle Creek Park in Indianapolis, Indiana from February-May 2008. Eagle Creek is the largest municipal park in Indianapolis and is the
sixth largest city-owned park in the United States. It consists of over 3,900 acres of wooded terrain and 1,300 acres of water, encompassing two different State Nature Preserves within its boundaries. Eagle Creek is home to 260 bird species, 400 species of flower plants, 101 species of trees and a variety of other woodland species including many anurans and salamanders (Eagle Creek Park Foundation, 2009). Eagle Creek Park provides a significant breeding habitat in wetlands and ephemeral ponds for these amphibians. The entire park is also crossed with approximately 10 miles of well-maintained and regularly-used trails.

*Landscape Mapping and larval collection:*

From February-March 2008, an initial survey of wetland areas and ephemeral ponds at Eagle Creek Park was conducted. I used a GPS unit to mark the center of each site, and I visually inspected each site for signs of *Ambystoma texanum* breeding. My criteria for classifying sites as an ephemeral pond were that the site was inundated for a week or more and had a minimum of 10 cm of water at its center point. I described sites as breeding areas if one or more egg masses were observed. Larvae were collected and removed from 8 of the observed breeding sites from April-May 2008, and the average sample size was 17 larvae per pond.

*Tissue Preparation, DNA isolation and Microsatellite amplification:*

Whole larvae were placed in individual 1.5 ml centrifuge tube, preserved in 95% ethanol and frozen. I isolated DNA from tissue samples using a Qiagen DNA®easy Blood & Tissue Kit (2006) according to the manufacturer’s instructions, and ten microsatellites (SIGMA) developed for *A. texanum* by Williams and DeWeeney (2004) were initially screened. I used the following protocol to prepare a 10µL final sample for a standard polymerase reaction (PCR): 1µL DNA, 1µL primer, 1µL e2tak buffer, 1µL DNTP, 0.1µL e2Tak DNA
polymerase, 1µL dimethyl sulfide and 3.9µL of water. PCR conditions followed Williams and DeWeeney (2004) on a iCycler Version 4.006 and electrophoresis was used to run products with negative controls on 1% agarose gels. I added 5µL of ethidium bromide to each gel. I used a ChemiImager 5500 and Flouro5000 software to image gels. Of the ten microsatellites, 5 polymorphic loci amplified successfully, and I obtained fluorescently-labeled primers for Atex 63, Atex 89, Atex 102, Atex 141, and Atex 143. I used the same PCR preparation and conditions as before, substituting primers for the fluorescently-labeled primers, and amplified products were sent to MCLab (San Francisco) for DNA fragmentation analysis.

*Landscape Analysis:*
I overlaid GPS way-point coordinates on a map of Eagle Creek Park using ArcMap, and made comparisons between the wetland areas described in Van Deman (1998). I also used a buffer tool in GIS to predict the number of breeding populations present in Eagle Creek Park, given the average migration distances based on Williams (1972) study and using additional buffers (160 m) based on a more liberal estimate of migration distance (an average of six *Ambystoma* species). I constructed a second map and created a buffer based on the migration distance needed to produce one continuous population in Eagle Creek Park.

*Genetic Analysis:*
I analyzed the chromatograms received from MCLab by observing the strength of peaks that corresponded with the appropriate color and base pair range for each microsatellite marker. I calculated the number of alleles at each locus per individual at each sample site, and I generated an allelic composition per pond by totaling the number of alleles for all individuals within a pond. Allelic composition of each pond was compared in order to determine if
genetic variation existed between individuals from among the different sample sites in Eagle Creek Park.

Results

Landscape Ecology

In this study, I marked 25 breeding sites of *A. texanum* at Eagle Creek Park (Figure 1). I located 8 of the original 19 breeding sites of *A. texanum* found by Van Deman (1998) using GPS, but I was not able to locate the other 11 ponds (Table 1). 56th Street and Sycamore ponds were previously marked as non-breeding sites; however I found *A. texanum* egg masses at both sites. I confirmed that Tiger and Jagger, previously marked permanent and semi-permanent ponds respectively, were non-breeding sites. In addition, I discovered 18 new ephemeral ponds and found that 15 of these were breeding sites for *A. texanum*. Using this information, I constructed two different maps of the structure of *A. texanum population* at Eagle Creek Park. The first map used a liberal estimate of migration distances based on distances from six *Ambystoma* species and demonstrated that a 160m range of dispersal would produce 4 isolated populations (Figure 2). The second map I created shows that there could be one single, connected population if the dispersal range was increased to 250m (Figure 3).

Microsatellite Analysis

Of the five fluorescently labeled primers I obtained, one locus, Atex 143, successfully amplified for 4 of the 8 ponds sampled. Atex 143 is known to contain two alleles between 150-159 base pairs (Williams and DeWeeney, 2004); however, I found that there were three
alleles present in the *A. texanum* population at Eagle Creek Park—150 base pairs, 154 base pairs, and 158 base pairs. I calculated the allelic composition for each breeding site (Figure 4), and genetic variation was observed between the 4 sites. Moss and Mud, contained all three alleles in the population in similar proportions. Amos Ditch was almost completely homozygous for the 158bp allele whereas Water 4 contained only two alleles, 154bp and 158bp. I compared the locations of these 4 breeding sites to the buffered maps with the allelic composition per site, and each of these ponds (or groups of ponds) was located in an area predicted to be a different population by the 160m dispersal range (Figure 5).

**Discussion**

Obtaining habitat and species-specific estimates of dispersal patterns in amphibians is critical to understanding population structure and genetic composition (Gibbs, 1998; Semlitsch, 2000). This study focused on locating breeding sites of *A. texanum* at Eagle Creek Park, Indianapolis, IN and determining their genetic composition in order to infer the dispersal patterns and population structure. Of the original 19 breeding sites located in 1998 by Van Deman, 8 of these sites were present in the spring of 2008. In addition, 15 new breeding sites were located and two previously sites marked as non-breeding contained *A. texanum* egg masses. These findings demonstrate that the breeding of *A. texanum* is variable and breeding site occupancy may change over a short period of time. It emphasizes the significant fluctuation in the rate of local extinctions and suggests that migration and colonization events are especially crucial with changes in the landscape.

Variation in the allelic composition per pond within the population of *A. texanum* at Eagle Creek Park was observed. These results suggest that the population structure of *A.
*texanum* is not consistent with panmixia. There appears to be some isolation on a fairly small geographic scale. This pattern is consistent with my prediction that if migration did not occur between populations, evidence of gene flow would not be present and populations would become genetically isolated. The presence of genetic variation between populations also supports the hypothesis of Clark et al. (2003) which suggested that distance limits gene flow and provides further support for the estimates of *A. texanum* migration distances by Williams (1972). In this population, it is unlikely that *A. texanum* is capable of migration beyond 160m, contrasting the results of similar sized *Ambystoma* species that have been reported to disperse over distances greater than 250m (Madison, 1997; Gamble et al., 2007).

Other explanations exist for the genetic variation and low dispersal distances of *A. texanum* at Eagle Creek Park. Human-induced fragmentation of natural habitats can produce effects in the genetic structure of population over a short amount of time (Gibbs, 1998, Marsh and Trenham, 2001). Habitat fragmentation is a serious threat to amphibian populations, especially in urban areas (Hamer and McDonnell, 2008). The presence of roads could be acting as potential physical barrier to the dispersal of *A. texanum* (Rothermel and Semlitsch, 2002). One of the two main roads in Eagle Creek Park separates Mud and Moss ponds from Water 4 pond and Amos Ditch. Genetic analysis showed variation between the allelic composition of all of these ponds, indicating that only Moss and Mud pond, the two ponds in closest proximity to one another and not separated by a road, are genetically similar. Rothermel and Semlitsch (2002) also suggested that open fields may physically hinder dispersal patterns, and environmental conditions may also hinder behavioral choices of migration (Graeter et al., 2008). Mud and Moss ponds are separated from Amos Ditch by a small plain and an open park maintenance area. Variation between the genetic compositions
of these sites showed the highest amount of divergence, providing positive evidence for the lack of migration between these populations. Amos Ditch did not possess the allele with 154 base pairs, and only contained a single allele that varied from 158 base pairs, unlike Moss and Mud ponds which contained all three alleles. Thus, these landscape features could be promoting low migration distances and the formation of isolated populations.

These findings support the idea that conservation of wetland habitats alone is not a sustainable approach to aid the declining amphibian populations (Skidds et al., 2007). Though wetland habitat is important for the reproduction of amphibians, many studies have demonstrated that habitat connectivity and the conservation of terrestrial upland forests is essential in amphibian conservation (Marsh and Trenham, 2000; Trenham et al., 2000; Skidds et al., 2007; Graeter et al., 2008; Semlitsch, 2008). By focusing on conserving wetland areas when designing urban parks such as Eagle Creek, valuable terrestrial habitat corridors may be lost which *A. texanum* use for key components of their life history, including foraging and over-wintering. Habitat connectivity should be a main focus of future conservation endeavors, especially in suburban parklands as the instance of urbanization and habitat fragmentation increases (Graeter et al., 2008; Hamer and McDonnell, 2008; Walston and Mullin, 2008). The development of habitat corridors is likely to have a large impact on species that persist in highly ephemeral populations or rapidly changing landscapes (Hudgens and Hadden, 2003). These corridors will provide better quality of habitat for *A. texanum* and other amphibian species and promote the maintenance of larger subpopulations (and possibly metapopulations) that will be less vulnerable to stochastic fluctuations and increase the instance of individual dispersers (Trenham et al., 2001; Haddad and Tewksbury, 2005). Haddad et al. (2003) have demonstrated that habitat corridors are effective in facilitating
relatively large dispersal events (>68%) of several taxa including butterflies, small mammals, and bird-dispersed plant and suggest that they could apply to a variety of other species as well as entire communities.

Further work needs to be conducted on the *A. texanum* populations at Eagle Creek Park. Sampling individuals from additional breeding sites, obtaining larger samples sizes of larvae and the amplification of more loci will strengthen the description of the population structure and dispersal patterns demonstrated in this study. On a broader scale, I have identified three areas that require more examination in *A. texanum*. First, in order to accurately calculate dispersal patterns of *A. texanum*, studies need to be conducted in a continuously connected habitat. Information from these studies would also provide a greater understanding on the effects of habitat fragmentation in this species. Second, the relationship between dispersal and the effects of specific landscape characteristics such as open areas, roads and sewage drainage ditches should be experimentally addressed in order evaluate the affect of these factors on one another. These could have significant impacts on the migration ranges of *A. texanum* as well as the location of breeding sites, and more information would benefit future conservation efforts. Third, dispersal patterns of *A. texanum* need to be examined from the context of sex and life-stage. According to Semlitsch (2000), juvenile dispersal away from breeding sites is the most important determinant in the continuance of a population, and McDonough and Paton (2007) found that adult female spotted salamanders disperse nearly twice the distance from breeding sites as males. Both these studies suggest that vital information on the dispersal pattern of *A. texanum* could be linked to life history variables and habitat characteristics, not just habitat characteristics alone which have been the focus of most studies to date.
Acknowledgements

Financial support for this project was provided by the Fairbanks Undergraduate Student Research Program (USRP) and Holcomb Awards Committee (Dr. Christopher M. Hess). I thank Dr. Travis J. Ryan and Dr. Christopher M. Hess for their support and guidance throughout this study; Dr. Carmen M. Salsbury for reviewing this thesis and providing helpful comments; Brent Montgomery for aid in the collection of larvae; and Tyler Hoskins for assistance in the laboratory.
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An initial survey of wetland areas and ephemeral ponds at Eagle Creek Park was conducted from February-March 2008, and all sites were noted for evidence of *Ambystoma texanum* breeding. Twenty-five breeding sites of *A. texanum* were found, including 8 of the original 19 ponds described by Van Deman (1998). Two ponds previously marked as non-breeding contained *A. texanum* egg masses, and 15 new breeding sites were located.
Figure 2
Twenty-five breeding sites of *Amystoma texanum* were located at Eagle Creek Park in the spring of 2008. A liberal estimate of the migration range of *A. texanum* was applied based on distances from six *Ambystoma* species, creating buffer regions of 160m around each site. Overlap of buffered regions predicted that if distance limits gene flow, four isolated populations of *A. texanum* could exist.
In the spring of 2008, twenty-five breeding sites of *Ambystoma texanum* were located at Eagle Creek Park. The structure of amphibian populations is often described using the metapopulation theory which suggests that local populations exist within the context of larger area where migration to at least some patches is possible (Hanski and Gilpin, 1997). In order to classify the population structure of *A. texanum* breeding sites at Eagle Creek Park as a metapopulation, individuals would have to be able to migrate across a range of 250m, approximately 90 meters over Williams (1973) maximum estimated migration distance for *A. texanum*. The scenario predicted above would refute the hypothesis that distance limits gene flow in this population of *A. texanum*. 
Figure 4:
Three alleles containing base pairs 150, 154, and 158 were found at locus Atex 143 in a population of *Ambystoma texanum* at Eagle Creek Park, Indianapolis, Indiana. Allelic composition of each pond was calculated, and samples ranged from 4 to 8 larvae per pond (n=8, n=6, n=7, and n=4 respectively).
The per pond allelic composition of four breeding sites of *Ambystoma texanum* was compared to the location of the breeding site in Eagle Creek Park, Indianapolis, IN. Each of these ponds was located in an area that was described as a different population by the predication of the 160m dispersal range.
Breeding Site | Located | Not Located
--- | --- | ---
Reflecting | X | 
Corner | X | 
Circle | X | 
Pavement | X | 
Maintenance | X | 
Maintenance | X | 
Ditch | X | 
Inner | X | 
Back | X | 
Pit | X | 
Sometimes | X | 
2 Rocks | X | 
Kirtland | X | 
Snail | X | 
Amos Ditch | X | 
Moss | X | 
Mud | X | 
Trio | X | 
Loop | X | 

Table 1
Nineteen breeding sites were of *A. texanum* were located at Eagle Creek Park, Indianapolis, Indiana by Van Deman (1998). From February-March 2008, sites were located, marked, and surveyed for evidence of active breeding. This table shows the positively located breeding sites in comparison with those located by Van Deman. In addition, 15 new breeding sites were located and egg masses were found in two sites previously documented as non-breeding.