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ECOSYSTEM ENGINEERING: BEAVER AND THE
POPULATION STRUCTURE OF COLUMBIA SPOTTED
FROGS IN WESTERN MONTANA

By

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B.A., Whitman College, Walla Walla, WA, 1989

Thesis

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for the degree of

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ECOSYSTEM ENGINEERING: BEAVER AND THE POPULATION STRUCTURE OF COLUMBIA SPOTTED
FROGS IN WESTERN MONTANA

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Beavers (*Castor canadensis*) are considered ecosystem engineers, altering hydrologic regimes, ecosystem processes, and modifying community structure. Effects of beaver on the spatial pattern of lentic habitat and populations using those habitats have not been examined. I used a landscape database and eight microsatellite markers to compare the scale and pattern of lentic sites, their occupancy, and population structure by Columbia spotted frogs (*Rana luteiventris*) between watersheds with and without beaver activity. Across all watersheds frog breeding sites were more clustered than the underlying pattern of lentic habitat. Beaver watersheds had four times as many lentic and breeding sites than non-beaver watersheds. Non-beaver watersheds often had only one frog breeding site. Frog breeding sites were more dispersed within beaver drainages. In addition, frog breeding sites were evenly distributed across the elevational gradient in beaver watersheds while they were centered above the watershed midpoint in non-beaver watersheds. Columbia spotted frog breeding sites were more dispersed within drainages with evidence of beaver presence than would be expected given the configuration of the underlying lentic habitat and have persisted despite being separated by distances larger than its dispersal ability. The genetic divergence seen within watersheds revealed that landscape configuration affected the fine scale population structure of Columbia spotted frogs. Landscape patterns of breeding sites were reflected in the presence and strength of isolation by distance equilibriums and the overall level of population subdivision within watersheds. Watersheds with beaver presence and an average distance of less than five kilometers between breeding sites showed higher levels of connectivity than did non-beaver watersheds with an average distance of more than five kilometers between breeding sites. More importantly, short beaver watersheds had lower levels of genetic divergence between breeding sites than those in long non-beaver watersheds separated by the same distance, even when distances were within the commonly observed dispersal ability of the frogs. Typical beaver watersheds in southwestern Montana with similar habitat configurations are likely composed of a single population, while non-beaver watersheds likely contain a single or a few isolated population/s. Careful consideration of potential population effects for species dependent upon habitat beaver create is required.

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Chapter 1

Introduction

Over the past two decades amphibians have been the focus of increasing concern because of potential population declines around the world (Houlahan et al. 2000). Although amphibian populations naturally undergo wide fluctuations in number and many factors negatively affect amphibian populations, habitat loss and fragmentation are often cited as key factors behind population declines and decreasing overall diversity (e.g., Semlitsch 2002). Although the importance of current land use practices and other anthropogenic activities in recent losses around the world is still unknown, the historic loss of habitat through both changing land use and management activities in temperate regions of North America have affected amphibian populations. For example, losses in amphibian diversity have been tied to the historic draining of wetlands and clearing of forests (Hecnar & M'Closkey 1996), and the introduction of fish to alpine lakes led to population declines of the mountain yellow-legged frog (Knapp & Matthews 2000).

Much of the historical lentic habitat in North America was created by American beaver (*Castor canadensis*) activity. In the upper Mississippi and Missouri river basins, Hey and Philippi (1995) estimated that a pre-trapping population of 40 million beaver could have created 207,000 km² of beaver ponds (an area roughly half the size of Montana). A dramatic decrease in beaver numbers from exploitation resulted in a large change in the landscape, converting a considerable portion of the U.S wetlands to dry land (Naiman et al. 1986). For example, in the upper Mississippi and Missouri river basins, only one percent of the estimated historic area of beaver ponds remains today (Hey & Philippi 1995). Although this habitat was lost rapidly after beaver removal, its rate of creation where beaver have returned has been slow (Johnston & Naiman 1990, Snodgrass 1997).

Beaver wetlands have important effects on water storage and water table levels, biogeochemical cycling such as nitrogen flow and carbon storage, biotic productivity of invertebrate communities, plant and bird biodiversity, and aquatic vertebrate communities in several regions of North America (for reviews see Naiman et al. 1986, Hammerson 1994, Collen & Gibson 2001). In the Intermountain West, alterations to the hydrology and nutrient flow of subalpine and mid-elevation valleys by beaver are important for maintaining the characteristics of aquatic and riparian systems (Jonas 1955, Neff 1957, Munther 1982, Maret 1985, Parker et al. 1985, Dahm 1986). Beaver wetlands also are habitat for many species of amphibians. Disruption of the temporal and spatial

distribution of these critical habitats may fragment amphibian populations dependent on a landscape shaped by beaver disturbance.

Rapid pond drying can result in a decline and the eventual extinction of a local amphibian population (Semlitsch 2002). In southwestern Montana the ephemeral nature of most water bodies (69%; Maxell, unpub. data), small population sizes, and high variability in recruitment may make dispersal of individuals critical for overcoming the effects of habitat fragmentation and for long-term population persistence of pond breeding species.

Metapopulation theory is often invoked in discussions about conservation biology or management of populations at the landscape and regional scale because of its ability to tie population dynamics and landscape processes such as habitat fragmentation together (McCullough 1996). The theory implies that the size, number, and distribution of habitat patches affect the dynamics and long-term persistence of a population (Rieman & Dunham 2000). However, even with the current concerns about habitat fragmentation and the intuitive appeal of metapopulation theory, it is rare to find data that compare movement behavior among landscapes that differ in the amount and configuration of suitable habitat for a species (Wiens 1997). Consequently, little is known about the mechanisms that link changes in habitat pattern with potential short and long-term ecological consequences (McGarigal and Cushman 2002).

The loss of beaver and the associated standing water bodies and wetlands they create may be an important source of habitat loss and fragmentation for lentic breeding amphibians. This research investigated how ecosystem engineering by beaver may be altering the quantity and distribution of breeding habitat for amphibians within watersheds across southwestern Montana and the genetic population structure of one amphibian species, the Columbia spotted frog (*Rana luteiventris*), in these watersheds. I focused on Columbia spotted frogs because of their abundance and because their ecology links them tightly to the lentic habitat created by beaver.

1.1 Columbia spotted frog

1.1.1 Natural history

Columbia spotted frogs have the smooth skin, long legs, and jumping ability typical of a member of the family *Ranidae*, or true frogs. They are highly aquatic and are usually not found far from the edge of lentic or riparian habitat used for foraging. Adults generally over-winter in larger

permanent water bodies or in springs (Turner 1960, Pilliod 2002). Breeding typically occurs soon after snow melt or pond ice out. Females usually deposit eggs in shallow water among emergent vegetation. Most data currently available for spotted frogs from mark recapture and telemetry studies focus on seasonal migrations and short distance dispersal (< 2 km; see Turner 1960, Pilliod 2002, Funk et al. 2005b). For example, mark recapture work on Keeler and Marten Creeks by Funk et al. (2005b), showed most juvenile dispersals covered distances of ≤ 1 km, with low frequency dispersals of ≤ 6 km. Almost all adults in the same area covered distances of ≤ 1 km, while one or two dispersals of ≤ 3 km were recorded (Funk et al. 2005b).

1.1.2 Conservation status

Columbia spotted frogs are common in Pacific Northwest and the Rocky Mountains where they are continuously distributed from eastern Washington, to western Montana and northward to southeast Alaska. Disjunct populations occur on isolated mountains and in arid-land springs in eastern Oregon, northern Nevada and Utah, and southern Idaho. Isolated southern populations in the Great Basin (Idaho, Nevada) are declining due to habitat loss and degradation from dewatering and exotic species (NatureServe 2006). In Montana, within the center of its range, the species is experiencing a loss of habitat from a host of mechanisms commonly cited for amphibian declines in temperate regions including the stocking of historically fishless lakes, loss of habitat due to exotic species like the bullfrog, changing land use (e.g., the draining and filling of wetlands due to development and agricultural uses), the extirpation of beaver, pollution, and the spread of disease (Maxell 2000). Some of these same mechanisms, specifically changing land use and beaver extirpation, have been implicated in declines which led to the protection of two populations at the southern periphery of the species' range (USFWS 2002).

Although range-wide differentiation (Green et al. 1996) and possible patterns of regional isolation have been described (Funk et al. 2005a), the level and importance of current gene flow for local population persistence is still unknown for Columbia spotted frogs (USFWS 2002) and other threatened ranid species in the West. Local population dynamics and ecological connectivity of subpopulations that have not undergone decline need to be investigated if current threats from fragmentation are going to be addressed (Semlitsch 2002). Variation in landscape composition, vital rates, and gene flow in undisturbed landscapes need to be quantified so that their importance to population dynamics can be judged. Studies of local genetic variation using high-resolution

microsatellite markers can help identify fine scale temporal and spatial mechanisms leading to habitat fragmentation and for defining appropriate management units.

1.2 Objectives

Examining dispersal across different landscapes is essential to understand connectivity among amphibian populations and how humans are altering it. Examining genetic divergence within watersheds describes a species' long-term dispersal signature across different breeding habitat distributions, providing critical information about the importance of landscape in determining population structure.

This thesis focused on two main topics involving how landscape influences populations of Columbia spotted frogs in western Montana watersheds. In chapter two, I addressed two main questions: How do spotted frog detection patterns compare to the underlying lentic habitat distribution and their estimated dispersal distances? And, how do lentic habitat and spotted frog detections patterns in beaver and non-beaver watersheds differ? In chapter three, I used among and within watershed patterns of Columbia spotted frog genetic variation to address two questions: How are Columbia spotted frog populations structured? And, how does the configuration of breeding sites within watersheds affect population structure?

1.3 Summary and synthesis

1.3.1 How do spotted frog detection patterns compare to the underlying lentic habitat distribution and their estimated dispersal distances?

Habitat patterns within watersheds were explored using three types of lentic habitat: lentic sites (slow moving or standing bodies of water), potential spotted frog breeding sites (lentic sites with shallow water and emergent vegetation where adult frogs were detected), and spotted frog breeding sites (lentic sites where egg masses, tadpoles, or breeding adults were detected). I compared the scale and pattern of breeding sites to the underlying patterns of lentic habitat using univariate and point pattern statistics. Overall, the landscape structure of Columbia spotted frog breeding sites was more clustered than the underlying pattern of lentic habitat. Since the configurations for lentic sites and

potential breeding sites were similar the availability of breeding habitat does not appear to constrain the distribution of frog breeding sites. Dispersal appears to be more limited than might be predicted based on the availability of suitable habitat, but the data cannot distinguish whether spatial dependence (too few sites at longer distances) or an ecological spatial processes (physical limit to dispersal ability) produced the observed pattern of spotted frog breeding sites. Columbia spotted frog breeding sites were positively spatially autocorrelated up to 7 km given the underlying pattern of lentic sites. Possible mechanisms explaining the scale of clustering seen for spotted frog breeding sites include: lentic sites are too dispersed at longer distances, limited dispersal ability, and demographic stochasticity. Mark-recapture studies and landscape genetics work suggest dispersal of Columbia spotted frogs is common at distances less than 2 km and rare over distances of 5 to 7.5 km (Funk et al. 2005b, Amish Chap. 3). Columbia spotted frog dispersal could explain the higher clustering of breeding sites within the 2.5 to 6 km range than seen in the underlying pattern of lentic habitat.

1.3.2 How do lentic habitat and spotted frog detection patterns in beaver and non-beaver watersheds differ?

Beaver watersheds had higher numbers of all types of lentic habitat (~4x) and much higher spotted frog detection levels than non-beaver watersheds. Columbia spotted frogs and their breeding sites were detected at higher percentages (presence: +28%, breeding: +25%, >1 breeding site: +15%) in beaver than non-beaver watersheds. Although differences in the spatial pattern of lentic habitat were minor between beaver and non-beaver watersheds, major differences in configurations of breeding sites were seen. Breeding sites in beaver watersheds were much more dispersed than in non-beaver watersheds. Distances between different habitat types (lentic sites, potential and breeding sites), and the longest nearest-neighbor distance were significantly longer in beaver watersheds including many that were beyond the estimated dispersal distances for spotted frogs. In contrast, the median number of spotted frog breeding sites in non-beaver watersheds was one. Where multiple breeding sites were detected in non-beaver watersheds, they were tightly clustered in the upper portion of the watershed with shorter (2 - 4 km) median distances between all habitat types well within estimated dispersal distances.

1.3.3 Overall genetic structure

Population structure for Columbia spotted frogs in six western Montana watersheds varied from a single population to five populations for each of the five breeding sites sampled. In general watersheds were characterized by low genetic connectivity between breeding sites with moderate levels of within population genetic diversity. The level of genetic differentiation seen in this study across scales of 1 to 25 km ($F_{ST} = 0.01 - 0.232$) was similar to recent work done on *R. luteiventris* (Funk et al. 2005a) and *R. cascadae* (Monsen and Blouin 2004). Lower values for the same scale ($F_{ST} = 0.04 - 0.09$) were seen for *R. temporaria* (Johansson et al. 2006) across a landscape with less physical relief and set in a matrix more hospitable to movement among sites. Estimated levels of expected heterozygosity were within the range seen in other anuran studies (reviewed Hoffman et al. 2004, Monsen and Blouin 2004).

Across the study area, watersheds structure spotted frog populations. Similar to results in Funk et al. (2005a), basin or watershed groupings of breeding sites explained the highest portion of loci variation (18.1%) after breeding sites (23.9%). Landscape structures associated with watershed boundaries (like ridges) have been seen to be important for structuring populations of Columbia spotted frogs (Funk et al. 2005a) and are well supported for other amphibians (García-Paris et al. 2000, Shaffer et al. 2000, Tallmon et al. 2000, Monsen and Blouin 2004). The strong genetic subdivisions seen in two montane frog species (Monsen and Blouin 2004, Funk et al. 2005a, this study) and known effects of ridges suggest headwater watersheds are well suited for use as conservation and management units.

Patterns of isolation by distance and levels of population subdivision within watersheds were different between ecoregions and are reflected in Columbia spotted frog population structure even though the hierarchical analysis found differences between ecoregion's genetic variation to be non-significant. Differences in effective population size do not appear to be responsible, because expected heterozygosity and the average number of alleles were similar across ecoregions. Geomorphology or patterns of human settlement may have influenced colonization and dispersal histories between regions.

1.3.4 Population structure within watersheds

Within watersheds, both the landscape pattern of sites and the relative location of sites within a watershed affected site levels of genetic subdivision. Bayesian analysis of breeding site allele

frequencies subdivided most watersheds into three or more populations. The range of population subdivision seen agreed with earlier work (1 – 5 populations; Funk et al. 2005a) and suggests fine-scale population structure for Columbia spotted frogs varies widely. General patterns of watershed subdivision fit well with drainage topography and likely dispersal corridors. Breeding sites organized along a linear riparian corridor showed the highest levels of connectivity. In contrast, breeding sites separated even by short over-land distances showed high levels of genetic divergence and in some cases evidence of inbreeding and isolation.

Low elevation clusters or complexes of sites separated by short dispersal distances (< 2 km) showed the highest genetic diversity and the lowest levels of genetic differentiation. Sites at the top of headwater basins showed lower genetic diversity and higher genetic differentiation over the same distances. Although many high elevation sites undoubtedly have very small effective population sizes because breeding aggregations are composed of few individuals, even those with large breeding aggregations had low genetic diversity thus small effective population sizes.

1.3.5 How does the pattern of breeding sites within the watershed affect population structure?

Short beaver and long non-beaver watersheds showed significantly different average F_{ST} values for two distance classes (0-2.5, 2.5-7.5 km). The level of genetic differentiation exhibited over short and medium distances classes suggested population subdivision in long non-beaver watersheds but population connectivity in short beaver watersheds. Estimates of the population subdivision within these watersheds supported these conclusions. Other beaver and non-beaver watersheds examined in earlier studies have shown similar patterns (Funk et al. 2005). There are several possible explanations for these differences. In short beaver watersheds, the location of lentic habitat in riparian corridors may be important for maintaining connectivity between breeding sites, if dispersal success is higher along riparian corridors than over-land. Alternatively, larger breeding aggregations with a higher number of juvenile dispersers in short beaver watersheds would maintain lower genetic divergence. With only one exception, the numbers of frogs or egg masses observed at breeding sites within watersheds during sample collection suggest they represent breeding aggregations of typical (~50 individuals; Werner et al. 2004) or smaller sizes with no differences between watershed types.

1.3.6 Synthesis

As ecosystem engineers, beaver physically alter their environment changing the pattern of lentic habitat on the landscape (Power et al. 1996). Although many studies have examined how beaver have influenced the abundance, distribution, and diversity of biota (Naiman et al. 1986, Johnston and Naiman 1990, Snodgrass 1997, Stevens et al. 2007), none have linked these changes to population connectivity (Moore 2005). Because Columbia spotted frogs have limited vagility and stochastic recruitment (Funk et al. 2005a,b, Maxell unpub. data), dispersal is important for maintaining populations over time. By creating habitat, beaver increase the number of frog breeding sites and redistribute them across the landscape more evenly.

In general, breeding sites occurred in patches within the background of clustered lentic sites. The distribution of Columbia spotted frog breeding sites differed from the underlying pattern of lentic habitat, reflecting a combination of lentic site distribution patterns and dispersal ability. Median distances between breeding sites for all of the watersheds were within the range of estimated dispersal distances (1.6 km), and were in agreement with the most common dispersal distances from intensive mark-recapture studies of the species (< 2 km; Funk et al. 2005b). Breeding sites showed significant positive spatial autocorrelation over distances of approximately seven kilometers given the pattern of available lentic habitat.

The composition and configuration of breeding sites within watersheds in the landscape database was different between beaver and non-beaver watersheds. Beaver watersheds had four times the number of lentic and breeding sites than non-beaver drainages had. Beaver engineering altered the pattern of spotted frog breeding sites dispersing them across a wide range of elevations. In contrast, Columbia spotted frog breeding sites in non-beaver watersheds were strongly clustered, with most sites located in the upper portion of the drainage. Because most non-beaver watersheds had few lentic sites tightly clustered together – separated by distances less than five kilometers – pair-wise distances between breeding sites were short. The more dispersed patterns in beaver watersheds produced pair-wise distances between breeding sites at or above estimates of spotted frog dispersal distances. Beaver appear to alter the distribution of spotted frogs on the landscape by facilitating more movement amongst the available lentic habitat.

In the landscape analysis, the configuration of lentic habitat across all watersheds was similar: sites were clustered within the watershed, and this pattern held true for both beaver and non-beaver watersheds. However, the number and location of Columbia spotted frog breeding sites were very different between beaver and non-beaver watersheds. Because multiple breeding sites were necessary

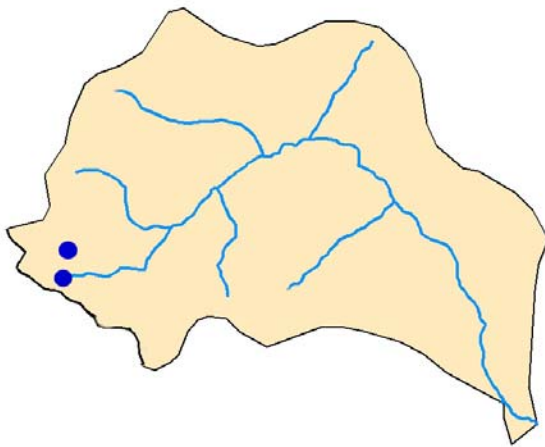
to address differences in population structure within watersheds, typical non-beaver watersheds couldn't be used. Instead, non-beaver watersheds with multiple breeding sites were selected (Fig. 1).

The fine scale population structure of spotted frogs in watersheds with contrasting habitat patterns was examined to investigate the effects of landscape configuration and beaver presence on population connectivity. Patterns of historic and contemporary gene flow were evident in the population structure. Watershed configuration affected the amount of genetic divergence between breeding sites and the fine scale population structure of Columbia spotted frogs. Specifically, variations in the landscape patterns of breeding sites altered the presence and strength of isolation by distance equilibriums and the amount of population subdivision within the watershed. Watersheds with beaver presence and an average distance of < 5 km between breeding sites showed higher levels of connectivity than did non-beaver watersheds with an average distance of > 5 km between them. More importantly, short beaver watersheds had lower levels of genetic divergence than long non-beaver watersheds for the same distance, even when the distance was within the commonly observed dispersal ability of the frogs.

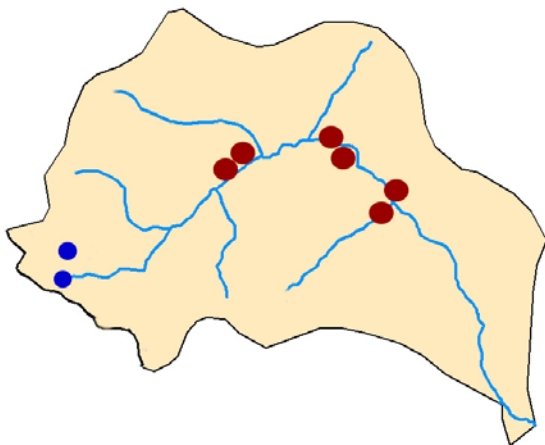
What do the observed differences in the Columbia spotted frog population structures tell us about the typical watersheds in southwestern Montana, based on their habitat configurations? First, historic patterns of migration and/or colonization may still be evident in fine-scale population structures. Large differences in the level of genetic differentiation between populations in short and long watersheds in the northern Bitterroots suggests historic patterns of dispersal or the geomorphology surrounding these watersheds are still strongly evident in population processes. The loss of beaver created spotted frog breeding sites detected during earlier amphibian surveys changed the average distance between breeding sites from short to long in Cache Creek. The effects of beaver alterations to landscape patterns may be temporary or transient and dependent upon current beaver occupancy. Within the Pintler and Pioneer ranges, beaver watersheds will have low levels of divergence between breeding sites separated by moderate distances (< 7.5 km) and will likely consist of a single population. This implies that even sites separated by long distances are not isolated from neighboring sites within the watershed. Third, non-beaver watersheds will have moderate to high levels of divergence between breeding sites separated by moderate distances. Since most non-beaver watersheds consist of a single breeding site, they represent isolated populations. In non-beaver watersheds where multiple breeding sites are found separated by moderate or longer distances, watersheds likely contain several isolated populations. Finally, in non-beaver watersheds even sites separated by short distances may have high levels of genetic divergence.

Although this study focused on one species, the Columbia spotted frog, the redistribution of lentic habitat may have similar effects on the population structure of other lentic breeding amphibians and suggests that subtle differences in landscape patterns may have far reaching population consequences. For beaver management, a more careful consideration of potential population effects for species using the lentic habitat they create is required. Limiting harvest of beaver in some areas may be important for maintaining existing populations of lentic breeding amphibians, or may improve connectivity among isolated populations. In some areas where limited habitat has led to the isolation of populations, beaver reintroductions may provide managers with the ability of connect low and high elevation populations, or to increase the number of breeding sites available within a watershed.

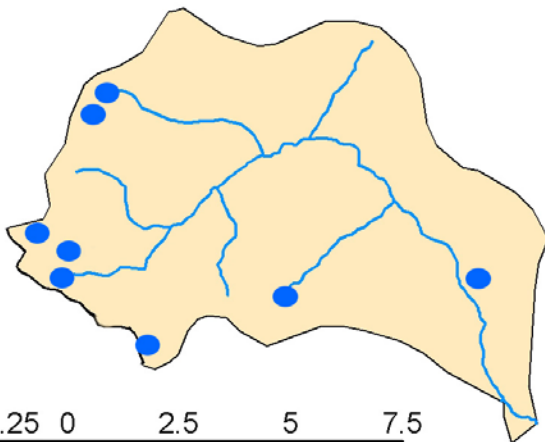
Figure 1. Diagram comparing Columbia spotted frog breeding site configurations for the three watersheds types described in this thesis. Blue dots represent breeding sites located in lentic habitat created through geomorphology while red dots are breeding sites located in lentic habitat created by beaver. Non-beaver landscape and beaver: typical non-beaver and beaver watersheds in the landscape database. Beavers alter the structure of the watershed by adding lentic sites used for spotted frog breeding along the riparian corridor. Beaver watersheds in the landscape and genetic analyses did not differ. Non-beaver genetic: the configuration of spotted frog breeding sites for watersheds used in the genetic analysis. These watersheds are atypical compared to most non-beaver watersheds in the landscape database because they have more sites which were widely dispersed within the watershed.



(a) Non-beaver landscape



(b) Beaver



(c) Non-beaver genetic



Chapter 2

Ecosystem engineering: beaver, landscape patterns of lentic habitat, and the distribution of Columbia spotted frogs in southwestern Montana

2.1 Abstract

Beavers (*Castor canadensis*) are considered ecosystem engineers, altering hydrologic regimes, ecosystem processes, and modifying community structure. The effect of beaver on the spatial pattern of lentic habitat and on populations using those habitats has not been examined. I used a database of over 100 watersheds in southwestern Montana to compare the scale and pattern of different lentic sites and their occupancy by Columbia spotted frogs (*Rana luteiventris*) between watersheds with and without signs of beaver activity. Univariate and point pattern statistics were used to analyze the observed patterns of lentic habitat and of spotted frog breeding habitat. Across all watersheds spotted frog breeding sites were more clustered than the underlying pattern of lentic habitat. Breeding sites were spatially autocorrelated up to distances of approximately seven kilometers. Clustering of breeding sites across all watersheds agreed with known dispersal distances for spotted frogs but may be limited by the configuration of lentic sites. More importantly, the composition and configuration of Columbia spotted frog breeding sites was different in beaver and non-beaver watersheds. Breeding sites were evenly distributed across the elevational gradient in beaver watersheds while they were centered above the watershed midpoint in non-beaver watersheds. In addition, beaver watersheds had four times as many lentic and breeding sites than non-beaver watersheds. Spotted frog breeding sites were more dispersed within beaver drainages, with positive spatial autocorrelation only up to distances of one kilometer. Non-beaver watersheds, in contrast, often had only one spotted frog breeding site and watersheds with two or more sites had a single

group of strongly clustered spotted frog breeding sites with positive spatial autocorrelation up to distances of five kilometers. Columbia spotted frog breeding sites were more dispersed within drainages with evidence of beaver presence than would be expected given the configuration of the underlying lentic habitat. In addition, beaver watersheds contained breeding sites where nearest neighbor distances exceeded estimated dispersal ability. Beaver altered the distribution of spotted frogs on the landscape by creating watersheds where spotted frog breeding sites were more widely dispersed. Isolated breeding sites have persisted despite being separated by distances larger than the frogs' dispersal ability.

2.2 Introduction

Ecosystem engineers physically change their environment, redistributing physical resources and altering landscape patterns (Jones et al. 1994, 1997, Moore 2005). Research on ecosystem engineers has primarily focused on small spatial and temporal scales (Moore 2005). The importance of ecosystem engineers on larger or longer scale processes, such as regional patterns of habitat or evolutionary processes have remained largely unexamined (Moore 2005). Although the ecological effects of keystone species and ecosystem engineers have been the focus of much study (see reviews Power et al. 1996, Jones et al. 1997, Moore 2005), mechanistic links between these species, landscape changes, and conservation consequences are not often demonstrated.

Beaver (*Castor canadensis*) are considered ecosystem engineers, changing hydrologic regimes and ecosystem processes, increasing species productivity and diversity within watersheds, improving riparian habitats, and modifying community structure (see reviews Naiman et al. 1986, Hammerson 1994, Collen and Gibson 2001). Previous research has quantified the rate and total area of lentic habitat created by beaver (Naiman et al. 1986, Johnston and Naiman 1990, Snodgrass 1997), but effects of beaver on the spatial pattern of lentic habitat and effects on populations using those habitats has not been examined.

The spatial pattern of lentic habitat (temporary and permanent water bodies) may be especially important for amphibians, because habitat loss and fragmentation are often cited as key factors behind population declines and decreasing overall diversity (e.g., Semlitsch 2002). For example, decreases in amphibian diversity in temperate regions of North America occurred from historic draining of wetlands and clearing of forests (Hecnar and M'Closkey 1996). In North America beaver have

historically created vast areas of lentic habitat, but dramatic decreases in beaver numbers due to the fur trade resulted in a considerable portion of the U.S wetlands converting to dry land (Naiman et al. 1986). In the upper Mississippi and Missouri river basins it was estimated only one percent of the historic area of beaver ponds remained in the mid-1990s (Hey and Phillippi 1995). This habitat loss is important for many species of lentic breeding amphibians (e.g., Russell 1999, e.g., Stevens et al. 2007). Beaver wetlands can serve as both over-wintering and breeding habitat, and may be especially important in arid regions with limited habitat. Disruption of the temporal and spatial patterns of these critical habitats may depress or fragment amphibian populations. Loss of beaver and the habitat they create may have played an important role in the decline of Columbia spotted frogs at the southern edge of its range (USFWS 2002). Although these effects have been suggested, the relationship between landscape patterns of beaver ponds and the distribution of amphibians has not yet been examined.

For many amphibian species in southwestern Montana, small population sizes and high variability in recruitment (Maxell 2000, Werner et al. 2004) may make the landscape pattern (configuration and composition) of habitat and the dispersal of individuals critical for long-term population persistence. Most water bodies in southwestern Montana are ephemeral (69%; Maxell, unpub. data), and rapid pond drying often results in the loss of a year class (pers. obs.) or in the decline and eventual extinction of a local amphibian breeding site (Semlitsch 2002). The extended hydroperiod of beaver sites may make them focal points for amphibian breeding, foraging, and over-wintering in arid regions. These more permanent sites potentially play an important role in maintaining and connecting lentic-breeding amphibian populations at a landscape scale. In addition, an increase in number of lentic sites within a watershed due to beaver activity might increase the number of breeding populations, insulating species against demographic stochasticity. Thus, in arid regions with limited lentic sites, species dependent on lentic habitat might be more widely distributed in beaver watersheds than in non-beaver watersheds.

The limited vagility, breeding site fidelity, and aquatic nature of Columbia spotted frogs make them a good candidate to examine how beaver alterations to the spatial pattern of lentic habitat across southwestern Montana influence another species. A common pond-breeding amphibian found in western North America, spotted frogs use lentic sites for both breeding and over wintering. Telemetry and capture-recapture studies for Columbia spotted frogs describe short distance (< 2 km) seasonal migrations and dispersals by adults (4% moved) and juveniles (25% moved) (Turner 1960, Pilliod et al. 2002, Funk et al. 2005b). Approximately 90% of movements occur over Euclidian

distances < 2 km (Funk et al. 2005b), with rare (2%) long distance dispersal by juveniles covering 5 to 7 km (Reaser 1996, Funk et al. 2005b). The population structure of spotted frogs in southwestern Montana show statistically and biologically significant genetic divergence between breeding sites beyond overland distances of 5 kilometers or riparian distances of 7.5 kilometers (Funk et al. 2005a, Amish Chap. 3).

I examined patterns of lentic habitat, beaver detection, and Columbia spotted frog detection in western Montana to investigate landscape-scale processes important to the distribution and population persistence of *R. luteiventris*. I addressed two main questions: 1) How do spotted frog detection patterns compare to the underlying lentic habitat distribution and their estimated dispersal distances? 2) How do lentic habitat and spotted frog detection patterns in beaver and non-beaver watersheds differ?

2.3 Materials and Methods

2.3.1 Database

I adapted an existing database developed for monitoring lentic amphibian presence in Montana to examine the spatial composition and configuration of lentic habitat within watersheds. The database consists of approximately 155 sixth hydrological unit code (HUC) watersheds that were randomly selected in southwestern Montana or chosen as focal watersheds for the collection of water quality, demographic, or genetic data. A 6th field HUC is a headwater watershed or subwatershed of 4,047 -16,188 hectares (federal standards for the delineation of hydrologic unit boundaries). The database was created by Bryce Maxell collaboratively with multiple state and federal agencies (Department of Environmental Quality, National Heritage Program, Montana Fish Wildlife and Parks, United States Forest Service) and is now overseen by the Montana Natural Heritage program. Most (92%) 6th field HUCs were selected using a stratified random cluster sampling design. Western Montana was stratified by level three ecoregions resulting in separate bioregions with similar abiotic conditions (Nesser 1997). Watersheds (6th field HUCs) within each ecoregion containing at least 25% federal or state land were randomly selected. The total area of the watersheds chosen within each ecoregion was proportional to the area of the ecoregion relative to the total area of all ecoregions (Maxell 2005).

Within each watershed, field crews surveyed all standing water bodies identified from topographic maps or aerial photos on public lands (and some private lands). Amphibians were counted using timed visual encounter and dip net sampling. In addition, habitat characteristics associated with site origin (glacial, beaver, river, human), site classification (pond, lake, wetland, oxbow), hydroperiod (permanent, temporary, dry) and spotted frog breeding (breeding observed, potential breeding) were recorded (for details on survey methods see Maxell 2004a,b). Site origin was determined by noting evidence of current or historic beaver activity, location of water body relative to current stream channel, or evidence that the site was modified or created by people. Hydroperiod of sites was estimated, based on water depth, the presence of inlets and outlets, and the type of emergent vegetation at the site. Water bodies with amplexed pairs, egg masses, or tadpoles were identified as spotted frog breeding sites. The physical characteristics required for a site to be classified as a potential breeding site for Columbia spotted frogs included the presence of the species, along with shallow water and emergent vegetation. A direct comparison of beaver created lentic habitat to sites of glacial, human, or riverine origin was not done because identifying historic beaver ponds can be difficult, beaver complexes are often multi-pooled sites covering large areas, and beaver residence times can vary greatly. Instead, beaver presence was denoted at the watershed level, when at least one survey site showed current or historic occupancy.

I projected survey data in ArcMap (version 9.1) and collected additional data on watershed geomorphology and composition to create a database of lentic habitat distribution and Columbia spotted frog detection for southwestern Montana. Digital USGS 7.5' maps of the study area and detailed Montana Fish Wildlife and Parks (MFWP) stream and lake layers were added to the database. I recorded elevations for different site types, as well as the intersection of the main drainage with the lower drainage boundary. In addition, I measured the shortest route between pairs of spotted frog breeding sites and pairs of potential breeding sites along riparian corridors. Creeks and rivers present on USGS maps or the MFWP stream layer were used to define riparian corridors. In areas where water was not indicated, I followed topographic relief indicative of potential riparian corridors. In areas of little or no topographic relief where maps did not indicate any riparian corridors, I measured the shortest straight-line path.

2.3.2 Data analyses

I identified variables from the database describing the composition and configuration of lentic habitat and spotted frog detection within watersheds. I ran a multivariate ordination on variables describing watershed characteristics, land ownership and survey characteristics, quantity of lentic habitat within a watershed, and the distribution of lentic habitat within a watershed (appendix A, table 1) and examined whether there were any biases in the data set that influenced my results. Specifically, I was interested in whether the proportion of sites surveyed and the proportion of sites on private land were negatively correlated with the total number of lentic sites detected or the number of sites where spotted frog breeding was detected. Binary variables such as beaver detection and ecoregion were examined within the ordination space to identify possible groupings or trends correlated with ordination axes.

I used PC-ORD (version 4) to run a non-metric multidimensional scaling ordination (NMS). I standardized all variables using z scores, and used Sorensen distances to place watersheds in the ordination space. Random starting coordinates were used and dimensionality was stepped down from six axes with a maximum of 200 iterations per cycle. I used a stability criterion of 0.0005 standard deviations over the last 10 iterations to determine the final stress of the solution.

I used SPSS version 11 for summary statistics, as well as univariate and non-parametric analyses to examine differences in the number of sites, the relative elevation of sites compared to the watershed's mid-elevation, and distances between sites in beaver and non-beaver watersheds and between ecoregions. The watershed's mid-elevation was estimated as the average elevation between the HUC's lower boundary and highest lentic site. The site's relative elevation was calculated as the difference between its elevation and the watershed's mid-elevation. Mann-Whitney U and Kolmogorov-Smirnov tests were run to determine whether beaver and ecoregion comparisons had significantly different medians or distributions for variables describing the composition or configuration of lentic habitat within watersheds and whether beaver watersheds had significantly different gradients or areas than non-beaver watersheds.

To investigate the configuration of lentic and spotted frog breeding sites and whether beaver altered these patterns I examined the pair correlation function in R using a combination of packages that allow mapped point pattern data to be projected and analyzed. Watershed boundaries were imported from shapefiles along with point data from the lentic habitat database using MAPTOOLS version 0.5-4. Pair correlation functions were run on point data using SPATSTAT version 1.8-5 (Baddeley & Turner 2005), SPSPATSTAT version 0.1-1, and SP version 0.8-9. Within watershed

patterns were aggregated across all watersheds after testing for regional differences. The pair-correlation function represents the cumulative frequency distribution of observations at a given point-to-point distance and captures the spatial structure of the variable. The pair correlation function of a stationary point process is

$$g(r) = K'(r) / (2 * \pi * r)$$

where $K'(r)$ is the derivative of $K(r)$, the reduced second moment function (aka "Ripley's K function") of the point process. For a stationary Poisson process the pair correlation function is equal to 1, with values $g(r) < 1$ suggesting inhibition between points (negative spatial correlation) and values greater than 1 suggesting clustering (positive spatial correlation). Default settings were applied (Epanechnikov smoothing kernel with a bandwidth = $h / \sqrt{5}$) following Stoyan and Stoyan (1994) with a translation correction for borders. Boundaries between adjacent watersheds were removed to reduce the effects of border correction. Patterns at scales greater than 20 km were not included because they reach beyond the within watershed scale and because Columbia spotted frog population structure suggests most watersheds represent multiple populations (Funk et al. 2005a, Amish Chap. 3). To determine whether breeding sites were themselves aggregated within the background of lentic sites a neutral landscape was created from the empirical data to use as a null model (Lancaster & Downes 2004, Lancaster 2006). A neutral landscape distribution was generated from the empirical distribution of lentic sites by permuting site type labels (i.e. potential breeding site, breeding site, unoccupied lentic site) among locations within watersheds 100 times to generate 100 simulated spotted frog breeding site patterns. I evaluated the intensity and pattern of the pair correlation functions to investigate differences among empirical breeding site configurations and neutral landscape patterns across all watersheds and between beaver and non-beaver drainages. Values greater than zero represent points which are more positively spatial correlated than expected by chance.

2.4 Results

2.4.1 Database biases

Of the 109 watersheds, 105 watersheds were used in the multivariate ordination to check for biases. The ordination converged on a solution with two axes having an R^2 of 0.875. The second axis explained most (74.2 %) of the variation in the watershed database. Variables with the highest correlation to the second axis described watershed composition, specifically the number of wet lentic sites, number of mid-elevation lentic sites, and number of dry lentic sites (appendix A, table 2). Watershed geomorphology variables strongly correlated with the second axis included the highest site elevation and watershed mid-elevation (appendix A, table 2). The first axis explained another 13.3% of the variation in the watershed database. Additional variables associated with watershed geomorphology had the highest correlation to the first axis and included the distance from the lowest site to the lower watershed boundary and the distance from the highest to the lowest lentic site (appendix A, table 2).

Variables associated with the proportion of sites surveyed and the proportion of sites on private land that might indicate sampling bias did not show strong correlation with either axis and were not significantly different between ecoregions or beaver watersheds (data not shown). Therefore, differences in the proportion of sites surveyed did not confound the composition and configuration of lentic sites within watersheds described in the database. In addition, variables that might suggest a correlation between lentic sites and local (i.e. watershed gradient) or regional (i.e. subbasin) topography were not strongly correlated with either axis (data not shown). For example, lower watershed gradient was not associated with a higher number of lentic sites. A correlation between local or regional topography with either axes would have confounded the interpretation of beaver effects on watershed patterns of lentic habitat.

There was no bias in the watershed database when patterns due to ecoregion, drainage, aspect, or beaver presence were examined. Ecoregions only varied significantly in detection rates for the number of watersheds with more than one Columbia spotted frog breeding site or the number of watersheds where beaver presence had been detected (Table 1). The southwestern ecoregion had more watersheds with greater than one frog breeding site and approximately twice as many beaver watersheds than the west-central ecoregion. Median number of all lentic habitat types and spotted frog detection reflected this regional pattern, with more lentic habitat in the southwestern ecoregion providing additional habitat for breeding (Table 1).

2.4.2 Lentic habitat patterns

Since no biases in the database were detected, survey results from both ecoregions were combined for further analyses. One hundred and fifty-five watersheds were surveyed and of those 109 watersheds contained wet lentic sites. In watersheds with wet lentic sites, beaver were detected in 44% and Columbia spotted frogs were found in 83%. Where *R. luteiventris* were observed, breeding was detected in 87%, while multiple breeding sites were detected in only 63% of the watersheds (Table 2).

2.4.3 Beaver and lentic habitat patterns

The number of lentic sites, and detection rates for both Columbia spotted frog presence and breeding were higher in beaver watersheds than non-beaver watersheds (Table 3). In beaver watersheds, spotted frogs were almost always detected breeding at multiple sites, while non-beaver watersheds had lower occupancy and breeding detection rates (Table 3). Beaver watersheds have four times the median number of lentic sites, potential spotted frog breeding sites, and detected spotted frog breeding sites than non-beaver watersheds (Table 3).

To investigate whether the larger number of breeding sites detected in beaver watersheds was a product of increased number of lentic sites, I examined the proportions of different *R. luteiventris* site types (potential breeding, breeding) versus total lentic sites in beaver versus non-beaver watersheds. Beaver watersheds had a higher proportion of lentic habitat important to the breeding and over-wintering of Columbia spotted frogs - permanent (0.423 vs. 0.284) and potential breeding (0.607 vs. 0.440) sites per wet lentic site, and a higher proportion of spotted frog detection - spotted frog detection per wet lentic (0.598 vs. 0.423). However, breeding occupancy rates - the proportion of spotted frog breeding sites per wet lentic site (0.318 vs. 0.265) and per potential breeding site (.523 vs. 0.603) were similar.

First, I compared watershed gradient and area across beaver and non-beaver watersheds to explore potential correlations in the data set. Gradient did not differ significantly between beaver and non-beaver watersheds, but beaver were detected in slightly lower gradient watersheds. Watershed area differed significantly, with the median area of beaver watersheds roughly 1000 hectares larger than non-beaver watersheds (Table 3).

Second, I examined whether beaver presence influenced distances between sites within watersheds with more than one breeding site. In general, riparian distances between sites were significantly longer in beaver than non-beaver watersheds. Specifically, the distance between the lowest and highest lentic sites, potential Columbia spotted frog breeding sites, and breeding sites were all approximately 1.5 times longer in beaver watersheds. Similarly, the longest nearest-neighbor distance between breeding sites was also significantly longer in beaver watersheds. The median distance among breeding sites was within the range of expected dispersal distances seen for spotted frogs (up to 5 – 7.5 km) and was not significantly different between beaver and non-beaver watersheds (Table 3). In beaver watersheds, longest nearest-neighbor distances were greater than 8 km, which were greater than observed dispersal distances. In non-beaver watersheds median pairwise distances for both potential and observed breeding sites were less than known spotted frog dispersal distances, including the median longest nearest-neighbor distance (Table 3).

Third, I investigated whether the relative elevation of sites differed between beaver and non-beaver watersheds. The median relative elevation and the distribution of relative elevations for spotted frog breeding sites are both significantly different ($p < 0.001$) between beaver and non-beaver watersheds. Columbia spotted frog breeding sites in non-beaver watersheds represent a similar range of elevations seen in beaver watersheds but the distribution was skewed ($g_1 = -0.563$) above the midpoint of the watershed. Breeding sites were distributed across the elevational gradient in beaver watersheds while they were centered above the watershed midpoint in non-beaver watersheds. Overall the median distance between breeding sites within watersheds was short, but beaver watersheds had more dispersed habitat and breeding activity (Table 3). Because there are more lentic sites in beaver watersheds, the larger area associated with beaver do not explain the longer distances or larger elevational gradient seen between different habitat types or between spotted frog breeding sites.

I also compared the distribution of observed Columbia spotted frog breeding sites to observed lentic sites across all watersheds. The pair correlation functions of all lentic sites and known breeding sites both showed sharp declines with distance, approaching no spatial correlation at distances of approximately 7 km. The spatial autocorrelation of sites was strongest over distances of < 2 km with weak clustering evident up to 7 km (Fig. 2a). Columbia spotted frog breeding sites showed stronger autocorrelation over distances from 0 - 5 km ($> g(r)$) than the underlying pattern of lentic habitat did (Fig. 2b). The neutral landscape of breeding sites generated from the permutation process showed spatial autocorrelation of the same intensity as observed lentic sites, with significant clustering at

distances up to 4 km (Fig. 2c). The difference in the observed breeding sites and the neutral landscape breeding sites revealed significant positive spatial correlation of spotted frog breeding sites up to distances of 7 km after accounting for the pattern and intensity of the available lentic sites on the landscape (Fig. 2d).

For beaver watersheds the pair correlation functions of observed lentic and breeding sites both showed a sharp decline with the spatial autocorrelation strongest over distances of < 3 km, a scale similar to what was seen across all watersheds (Fig. 3a,b). Columbia spotted frog breeding sites had a stronger autocorrelation over distances from 0 - 5 km than the underlying pattern of lentic habitat did, but of a weaker intensity than seen for breeding sites across all watersheds (Fig. 3b). The neutral landscape of spotted frog breeding sites for beaver watersheds showed a higher level of positive spatial autocorrelation than observed lentic sites, with significant clustering at distances up to 4 km (Fig. 3c). Despite the similarity in the lentic and breeding site distributions to those seen previously for all watersheds, the weaker intensity of the breeding site clustering and the higher intensity of the neutral landscape model of breeding sites resulted in positive spatial correlation only at very short distances for beaver watersheds (1 km; Fig. 3d).

For non-beaver watersheds the pair correlation functions of observed lentic and breeding sites also showed a sharp decline with the spatial autocorrelation strongest over distances of < 4 km (Fig. 4a,b). Non-beaver lentic sites had weak positive correlation up to distances of 9 km, while the pair correlation function for Columbia spotted frog breeding sites in these watersheds showed a scale of positive correlation more typical of the other distributions (4 km). The spotted frog breeding sites in non-beaver watersheds had stronger autocorrelation over distances from 0 - 5 km than the underlying pattern of lentic habitat did (Fig. 4b). The neutral landscape of breeding sites for non-beaver watersheds showed a weaker level of positive spatial autocorrelation than the observed lentic sites, with significant clustering at distances up to 3 km (Fig. 4c). The stronger breeding site clustering combined with the weaker pattern generated by the neutral landscape model resulted in strong positive spatial correlation at distances up to 5 km in non-beaver watersheds (Fig. 4d).

In general, spotted frog breeding sites were clustered at short distances across all watersheds (1 – 7 km). Beaver watersheds produced weaker clustering patterns than seen in non-beaver watersheds and had configurations reflecting no positive spatial correlation among spotted frog breeding sites. These results are supported by the univariate results examining distances between Columbia spotted breeding sites in beaver and non-beaver watersheds. The random distribution of sites within beaver watersheds resulted in more dispersed habitat and spotted frog breeding sites,

while the strong clustering in non-beaver watersheds was evident in the restricted distribution and availability of breeding and habitat (Table 3).

2.5 Discussion

The distribution of Columbia spotted frog breeding sites differed from the underlying pattern of lentic habitat, reflecting a combination of lentic site distribution patterns and dispersal ability. Median distances between breeding sites for all of the watersheds were within the range of estimated dispersal distances (1.6 km), and was in agreement with the most common dispersal distances from intensive mark-recapture studies of the species (< 2 km; Funk et al. 2005b). Breeding sites occurred in patches within the background of clustered lentic sites. Breeding sites showed significant positive spatial autocorrelation over distances of < 7 km given the pattern of available lentic habitat used to estimate the neutral landscape.

The composition and configuration of breeding sites was also different between beaver and non-beaver watersheds. Beaver watersheds had four times the number of lentic and breeding sites than there were in non-beaver drainages. Beaver activity also altered the pattern of spotted frog breeding sites, producing more dispersed distributions, with positive spatial correlation only up to distances of 1 km, and with sites distributed across a wider range of distances and elevations. In contrast, Columbia spotted frog breeding sites in non-beaver watersheds reflected the underlying distribution of lentic habitat. Spotted frog breeding sites were strongly clustered in non-beaver watersheds, with most sites located in the upper portion of the drainage with positive spatial correlation up to distances of 5 km. Finally, the longer distances between spotted frog breeding sites observed in beaver watersheds exceeded estimates of its dispersal ability based on mark-recapture studies. Beaver appear to alter the distribution of spotted frogs on the landscape by facilitating more movement amongst the available lentic habitat.

2.5.1 How do spotted frog detection patterns compare to the underlying lentic habitat distribution and their estimated dispersal distances?

The landscape structure of Columbia spotted frog breeding sites was more clustered than the underlying pattern of lentic habitat over distances up to 7 km. Because the configurations for lentic sites and potential lentic sites were similar, the availability of breeding habitat does not appear to limit

the distribution of frog breeding sites. At distances < 7 km spotted frog breeding sites were positively spatially correlated given the underlying pattern of lentic sites, suggesting the scale of their dispersal ability. The underlying lentic habitat displayed only weak positive correlation at larger distances, therefore the geomorphology associated with the distribution of lentic sites or the sampling of watersheds may have limited the ability to detect patterns at a larger scale. The pair correlation function is isotropic, so the direction of the structure function is undefined, thus observed spatial autocorrelation cannot be assumed to describe within watershed processes alone, especially at scales greater than the average shortest dimension of the drainages (~ 10 km). Because watershed shape was highly variable and a delineation of within and between watershed point patterns was not possible, both within watershed processes or regional processes such as geomorphology, may explain patterns at scales greater than 10 km. However, watersheds were aggregated where they shared boundaries (the window for the analysis was the perimeter around both watersheds), so the scale of the patterns described by the mapped lentic habitat data accurately reflects lentic site configurations over distances less than 20 km.

Possible mechanisms explaining the clustering of Columbia spotted frog breeding sites include limited dispersal and demographic stochasticity. Mark-recapture studies and landscape genetics work suggest dispersal of spotted frogs is common at distances less than 2 km and rare over distances of 5 - 7.5 km (Funk et al. 2005b, Amish Chap. 3). Columbia spotted frog dispersal could explain the higher clustering of breeding sites within the 2.5 - 6 km range than seen in the underlying pattern of lentic habitat. Dispersal appears to be more limited than might be predicted based on the availability of suitable habitat, but it is not possible to distinguish from the data whether spatial dependence (sites are too dispersed at longer distances) or an ecological spatial processes (physical limit to dispersal ability) has resulted in the observed pattern of spotted frog breeding sites.

2.5.2 How do lentic habitat and spotted frog detection patterns in beaver and non-beaver watersheds differ?

Although there were minor differences in the intensities of clustering seen for lentic habitat between beaver and non-beaver watersheds, a major difference between the pattern of breeding sites was seen. Beaver watersheds had much more dispersed Columbia spotted frog breeding sites than non-beaver watersheds. Distances between different habitat types (lentic sites, potential and breeding sites), as well as the longest nearest-neighbor distance were significantly longer in beaver watersheds

including many that are beyond the estimated dispersal distances for spotted frogs. In contrast, most non-beaver watersheds only had a single spotted frog breeding site, with shorter (2 to 4 km) median distances between all habitat types which did not exceed estimated dispersal distances.

The underlying pattern of lentic habitat in beaver watersheds was not more widely dispersed when compared to non-beaver watersheds, but spotted frog breeding sites were, suggesting that alternative hypotheses explaining the configuration need to be explored. The longest nearest-neighbor distances between breeding sites observed in beaver watersheds are slightly longer than current estimates of dispersal, and mechanisms explaining these distances need to be explored. First, beaver may indirectly increase successful dispersals because a higher number of lentic sites would produce larger population sizes, either locally (patches of breeding sites separated by short distances) or at the watershed scale. Either contemporary populations need to be large enough to produce this effect, or historic populations may have been large enough to establish outlying populations and they have been able to persist.

Second, temporal patterns of lentic habitat creation may have allowed Columbia spotted frogs to move throughout the watershed. This suggests that historic patterns of lentic habitat are reflected in the current distribution of breeding sites. As beaver moved up and down the watershed, they left a series of ponds available for breeding and overwintering. Spotted frogs may have colonized this new habitat and subsequently dispersed to new areas. Over time, some lentic sites may have been lost to spotted frogs through flooding or successional processes, leaving isolated populations on the landscape. Alternatively, since spotted frogs showed higher gene flow along riparian corridors (Amish Chap. 3), increased riparian area and improvements to the riparian corridors and creek flows may have made the intervening matrix between breeding sites more hospitable.

Finally, a combination of these alternatives may have produced the current pattern. Current populations may be large enough to maintain isolated populations established when historic population sizes were larger or when habitat was more continuously distributed in the watershed, while no longer being large enough to colonize new habitat. Genetic evidence and survey data suggest that historic processes may be the most likely source of the observed landscape pattern of breeding sites. Current estimates of gene flow in beaver watersheds revealed isolated populations at high elevations (Amish Chap. 3). Moreover, survey data collected during 2003-2004 was typical for the region (approx. 50 or fewer individuals per breeding site, Werner et al. 2005) and did not reflect higher population sizes in beaver watersheds.

As ecosystem engineers, beaver physically alter their environment changing the pattern of lentic habitat on the landscape (Power et al. 1996). Although many studies have examined how beaver have influenced the abundance, distribution, and diversity of biota (Naiman et al. 1986, Johnston and Naiman 1990, Snodgrass 1997, Stevens et al. 2007), none have linked these changes to population connectivity (Moore 2005). Because Columbia spotted frogs have limited vagility and stochastic recruitment (Funk et al. 2005a,b; Maxell unpub. data), connectivity is important for maintaining populations over time. By creating habitat, beaver redistribute frog breeding sites across the landscape more evenly, potentially altering their population structure (Amish Chap. 3). Larger populations and more connectivity between breeding sites on the landscape may reduce the threat of local extinction from demographic stochasticity and inbreeding.

Although this study focused on one species, the Columbia spotted frog, the redistribution of lentic habitat may have similar effects on the population structure of other lentic breeding amphibians and suggests that subtle differences in landscape patterns may have far reaching population consequences. For beaver management, a more careful consideration of potential population effects on species utilizing the lentic habitat they create is required. Limited harvest of beaver in some areas may be important for maintaining existing populations of lentic breeding amphibians, or may improve connectivity among isolated populations. In some areas where limited habitat has led to the isolation of populations, beaver reintroductions may provide managers with the ability of connect low and high elevation populations, or to increase the number of breeding sites available within a watershed.

Table 1. Summary of watershed detection rates (number of watersheds with activity detected) for beaver and Columbia spotted frogs (CSF) and median number of lentic habitat types observed or detected between two ecoregions and beaver and non-beaver watersheds. Variables include lentic sites holding water at time of survey (wet), permanent hydroperiod (perm), potential CSF breeding sites, and CSF breeding detected at one or more site.

	Watershed detection rates				Median number of sites within watersheds			
	Beaver	CSF presence	CSF breeding	>1 CSF breeding site	Wet lentic	Perm lentic	Potential CSF breeding	CSF breeding
West-central	27%	83%	74%	45%	6.5	2.5	3.0	1.5
Southwestern	53%	83%	71%	60%	11.0	3.0	5.0	2.0
Non-beaver	NA	70%	57%	65%	4	1	2	1
Beaver	NA	98%	92%	80%	16	6	8	4

Table 2. General detection patterns at the watershed scale for beaver presence, Columbia spotted frog (CSF) presence, and one or more CSF breeding sites.

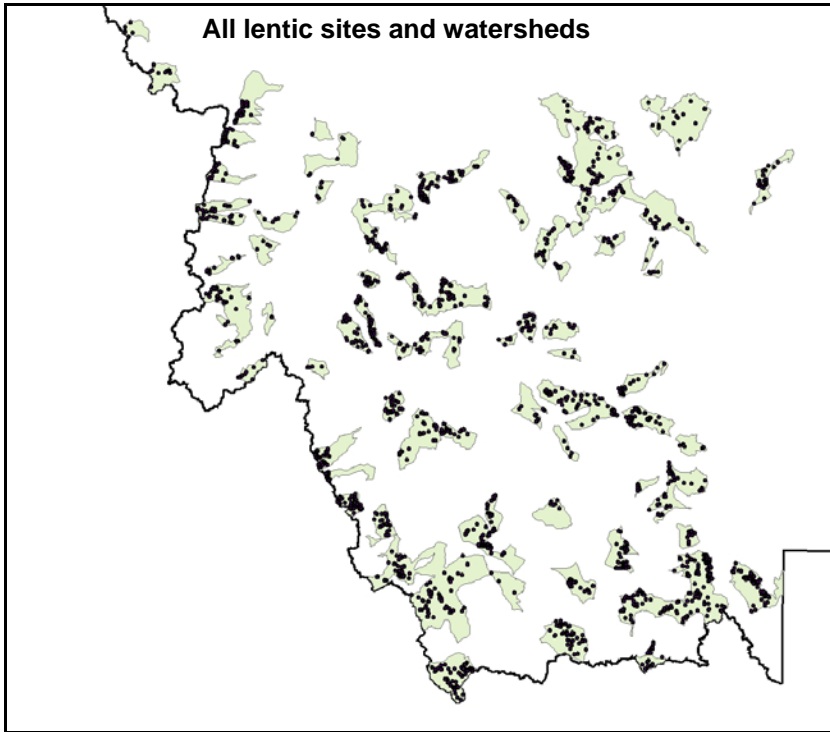
Survey Characteristic	Proportion of Watersheds	Percentage of Watersheds
Beaver	48 / 109	44%
CSF presence	90 / 109	83%
CSF breeding	78 / 109	72%
>1 CSF breeding site	57 / 109	52%

Table 3. A comparison of watershed characteristics and lentic habitat configurations for beaver and non-beaver watersheds. Gradient and median watershed area was investigated as possible sources of bias in the data set. Distances were measured along riparian corridors. Median values and Mann-Whitney U (M-W) and Kolmogorov-Smirnov (K-S) test p-values are reported.

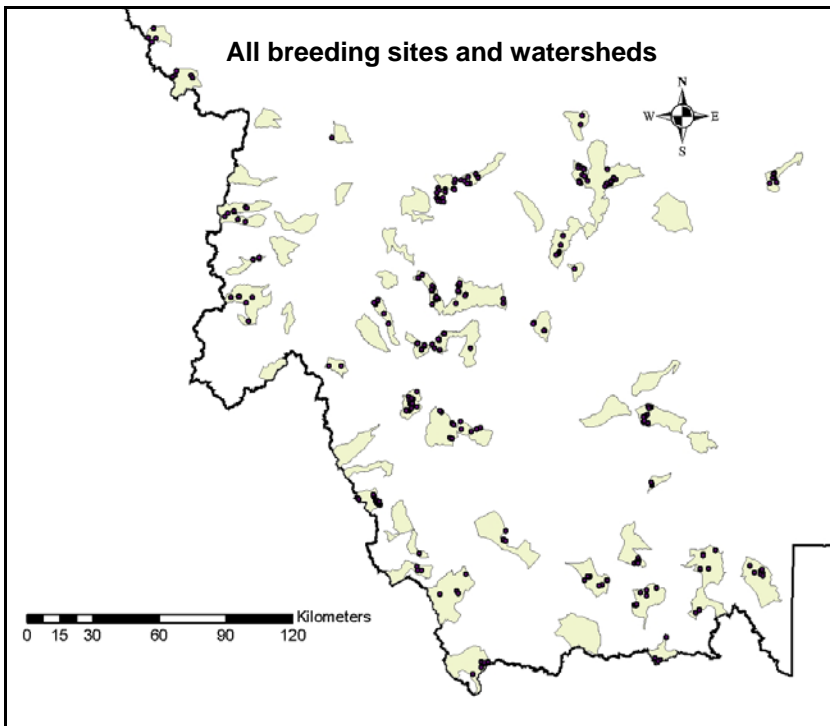
	All	Beaver	Non-beaver	p-value	
				M-W	K-S
Gradient (m/km)	46	41.3	49.6	0.158	0.142
Watershed area (hectares)	7111	8346	7067	0.040	0.212
Distance between lowest to highest lentic site (km)	12.1	15.1	9.8	0.005	0.008
Distance between lowest to highest potential CSF breeding sites (km)	7.0	9.2	5.1	0.032	0.041
Distance between lowest to highest CSF breeding sites (km)	7.7	8.6	5.1	0.002	0.013
Longest nearest-neighbor distance between breeding sites (km)	7.1	8.3	5.7	0.017	0.077
Distance between all CSF breeding sites (km)	1.6	1.9	1.2	0.586	0.978
Relative elevation lentic sites (m)	43	16	101	0.001	0.001
Relative elevation CSF breeding sites (m)	101	27	216	0.001	0.001

Figure 1. Map showing the distribution of lentic sites identified from maps and aerial photographs included in the southwestern Montana database. a) All lentic sites and the watershed boundaries created when adjacent drainages were aggregated. b) All spotted frog breeding sites and the corresponding watershed polygons. c) All spotted frog breeding sites in beaver watersheds. D) All spotted frog breeding sites in non-beaver watersheds. Each dot represents a single lentic site with watersheds as shaded polygons.

a)



b)



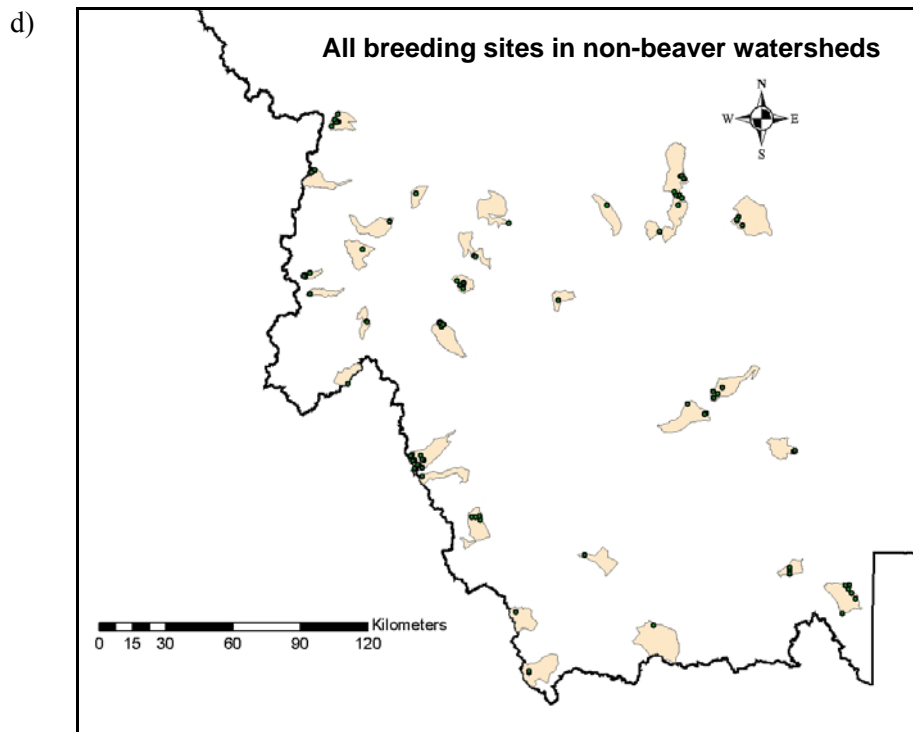
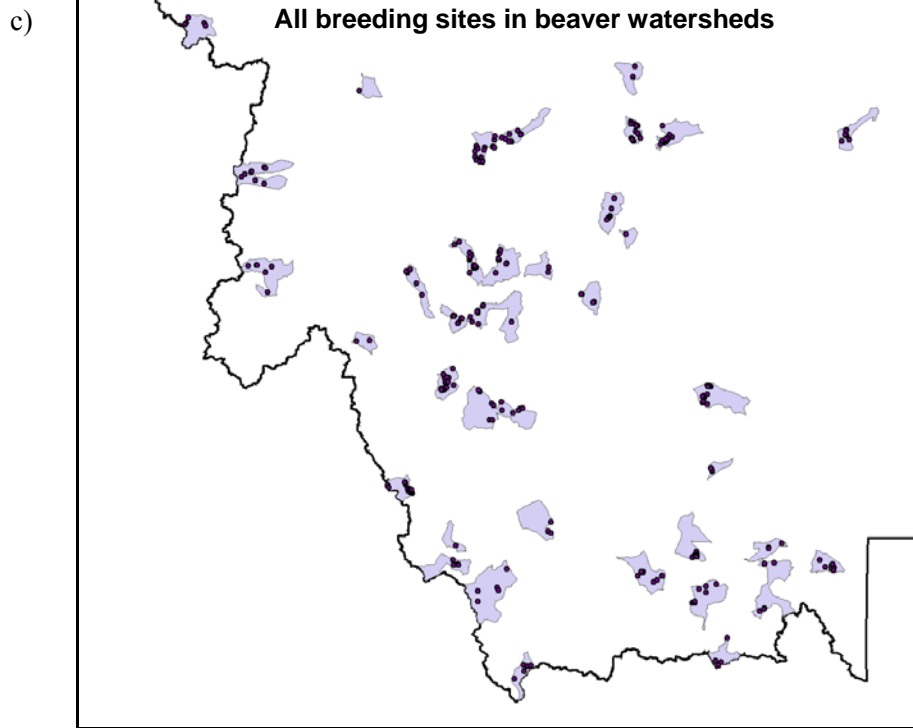
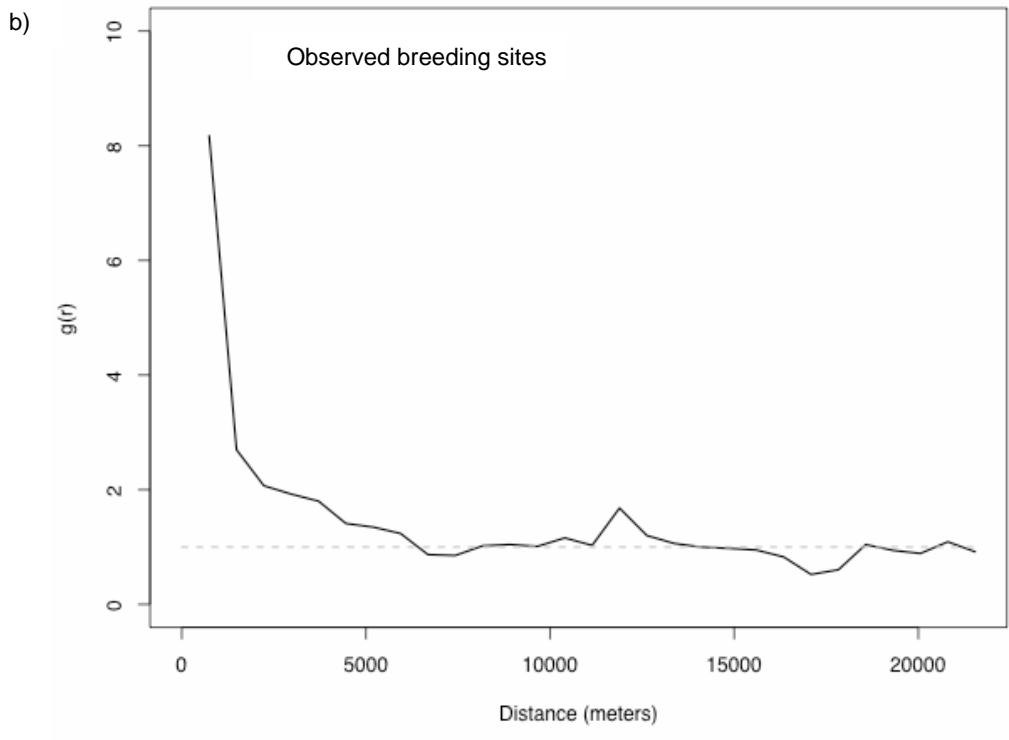
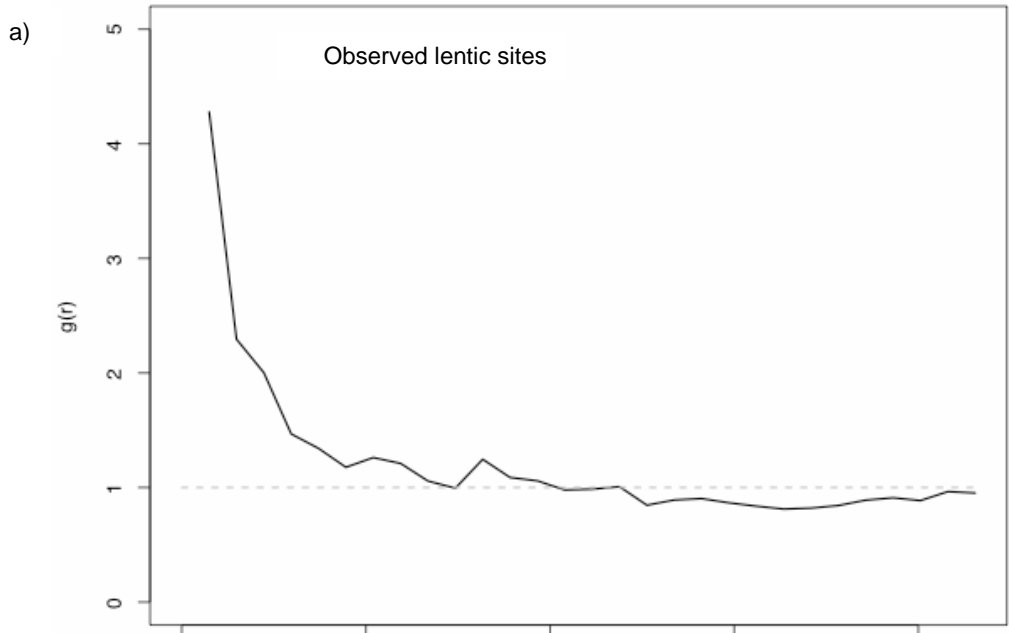


Figure 2. Spatial patterns of observed lentic and Columbia spotted frog breeding sites and for the neutral landscape breeding sites as given by the pair correlation function ($g(r)$). The x-axis represents the distance in meters between sites. The area above the grey dashed line at $g(r) = 1$ (for figures a - c) represents positive spatial correlation while below the line represents negative spatial correlation compared to a random distribution. In figure d, $g(r) = 0$ represents no spatial correlation after accounting for the correlation in the neutral landscape. The upper and lower bounds of the 95% confidence intervals are shown with red dotted lines. a) Observed lentic sites across all watersheds were positively spatially correlated at distances up to 10 km. b) Observed breeding sites across all watersheds were more strongly spatially correlated than lentic sites but only up to distances of approximately 7 km. c) Neutral landscape breeding sites were spatially autocorrelated at the same intensity as observed lentic sites, but only up to a distance of 5 km. d) Observed breeding sites were positively spatially autocorrelated after subtracting the neutral landscape at distances up to 7 km.



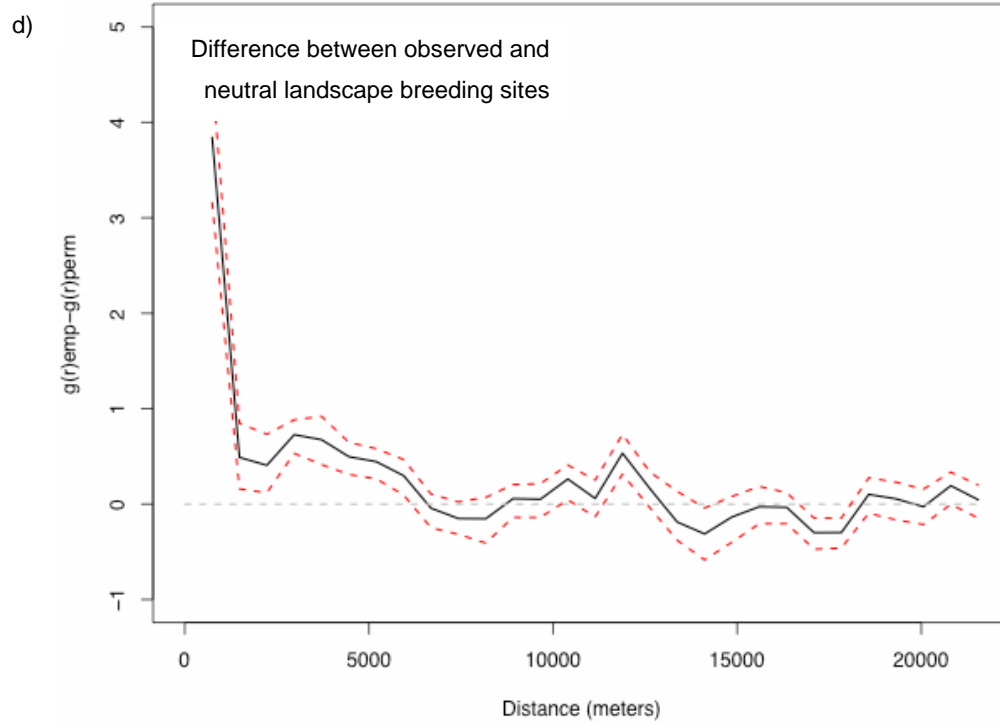
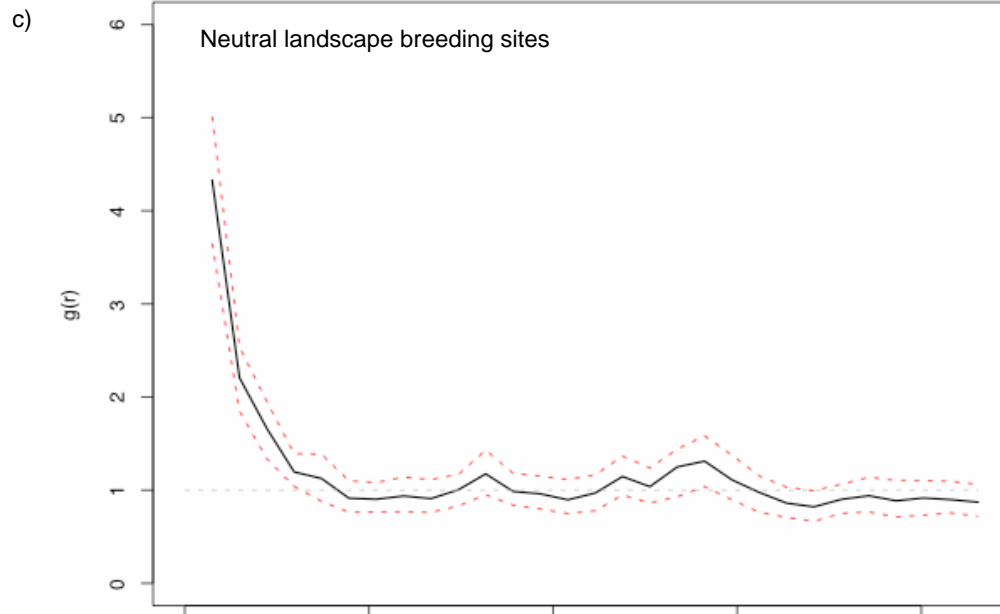
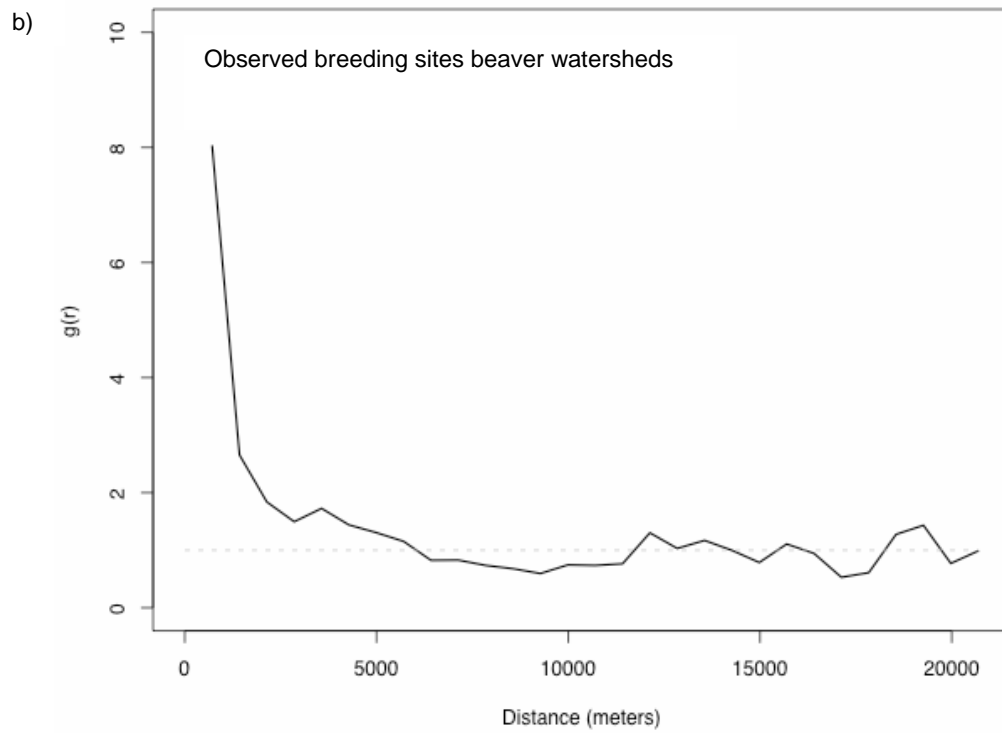
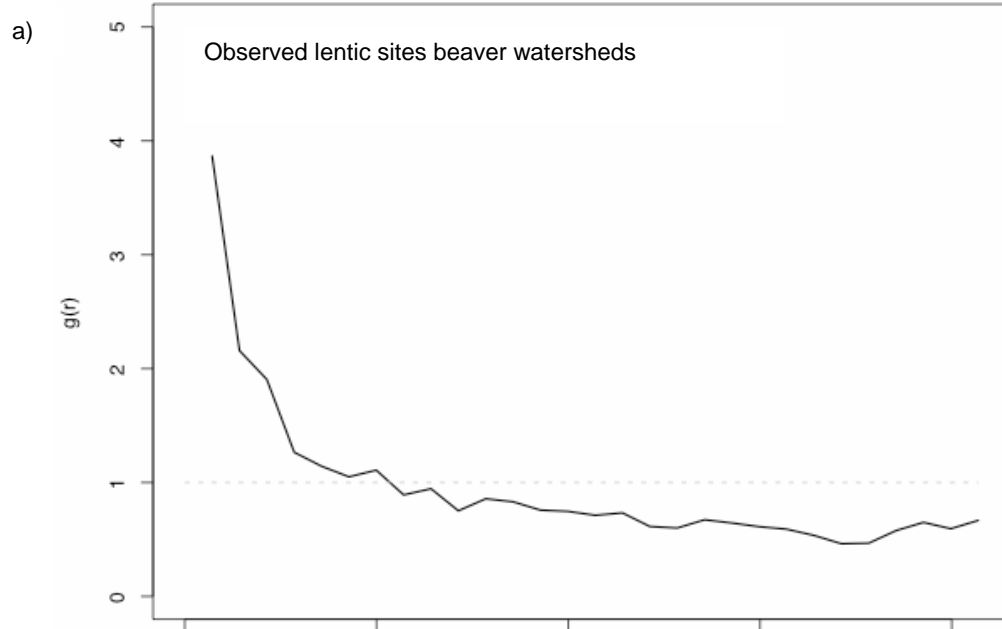


Figure 3. Spatial patterns of observed lentic and Columbia spotted frog breeding sites and for the neutral landscape breeding sites as given by the pair correlation function ($g(r)$) for beaver watersheds. The x-axis represents the distance in meters between sites. The area above the grey dashed line at $g(r) = 1$ (for figures a - c) represents positive spatial correlation while below the line represents negative spatial correlation compared to a random distribution. In figure d, $g(r) = 0$ represents no spatial correlation after accounting for the correlation in the neutral landscape. The upper and lower bounds of the 95% confidence intervals are shown with red dotted lines. a) Observed lentic sites across all beaver watersheds were positively spatially correlated at distances up to 5 km. b) Observed breeding sites across all beaver watersheds were more strongly spatially correlated than lentic sites over a slightly longer distance (approximately 7 km). c) Beaver neutral landscape breeding sites were spatially autocorrelated at the same intensity as observed lentic sites. d) Observed breeding sites in beaver watersheds were positively spatially autocorrelated after subtracting the neutral landscape only at very short distances (approximately 1 km).



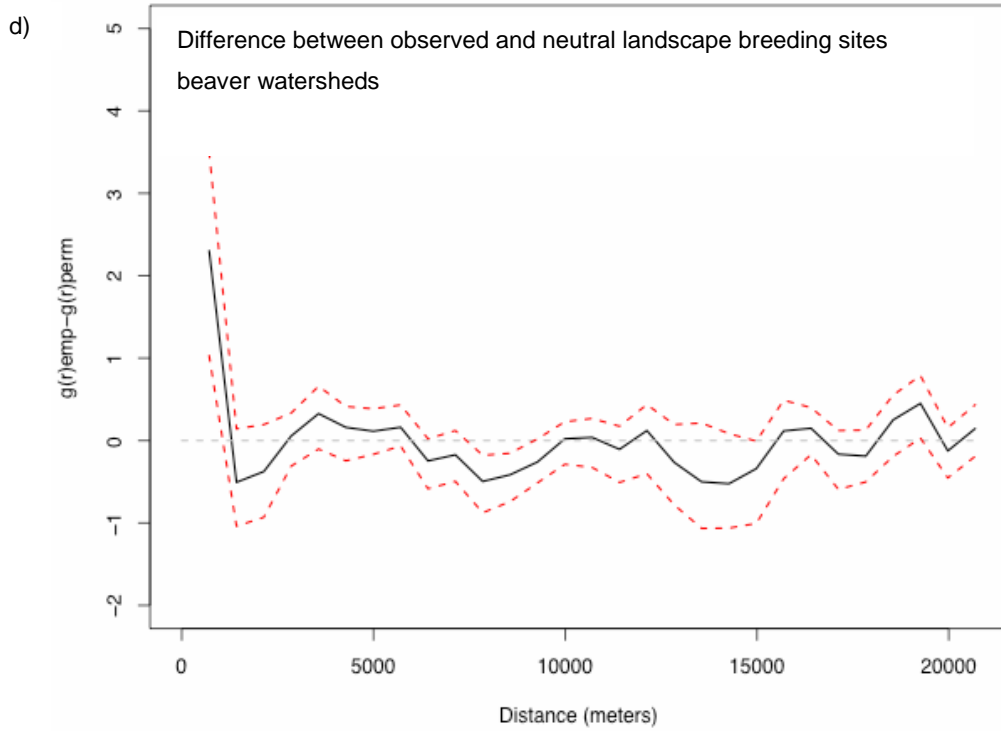
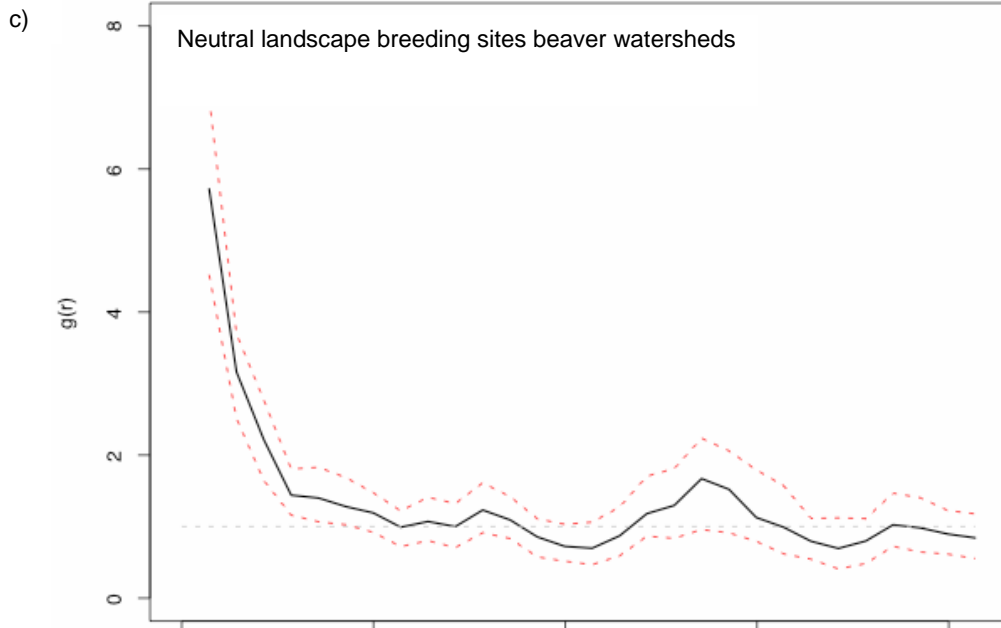
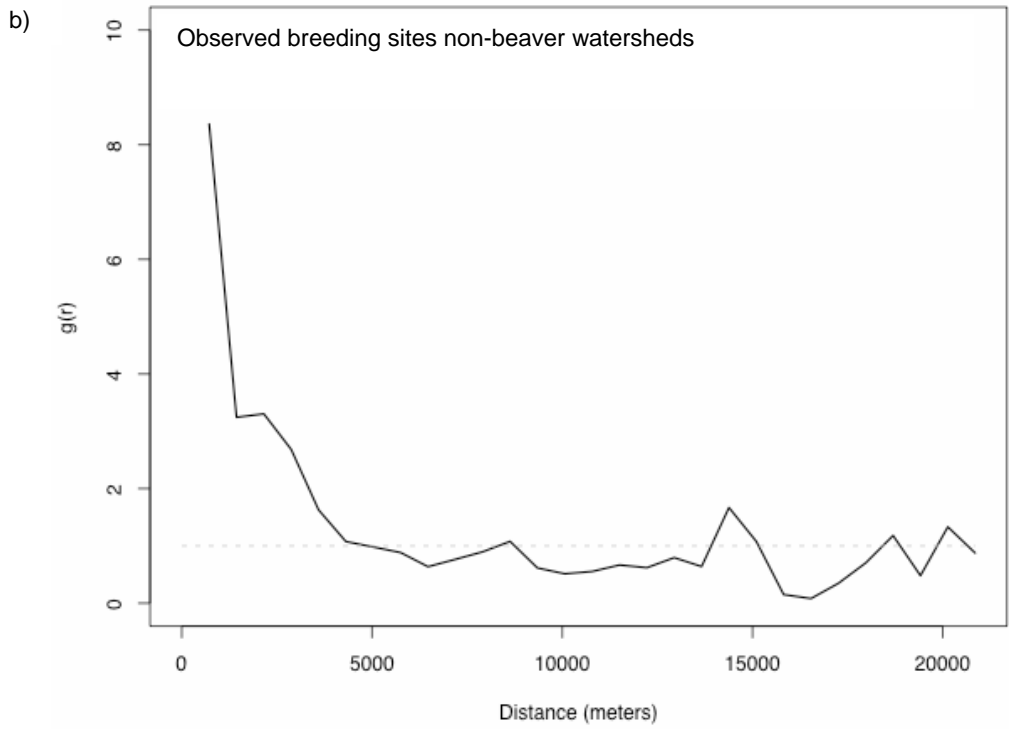
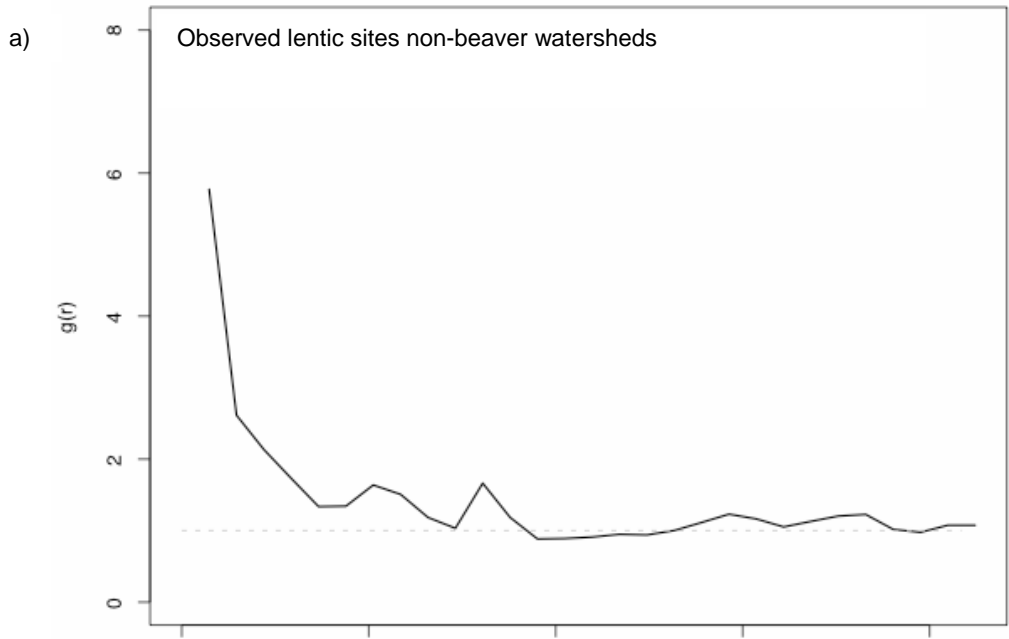
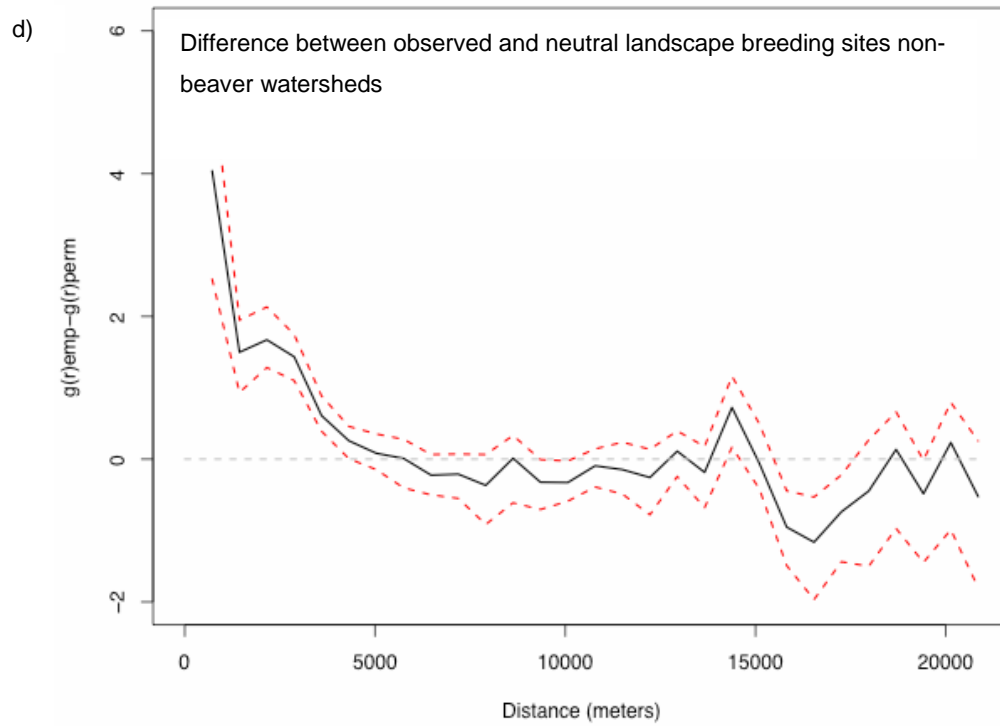
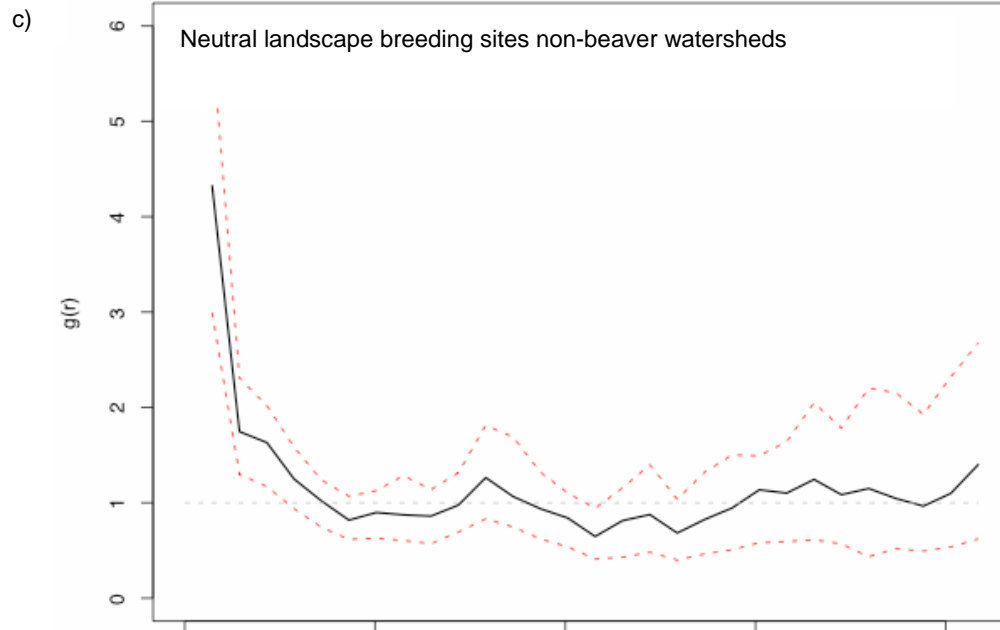


Figure 4. Spatial patterns of observed lentic and Columbia spotted frog breeding sites and for the neutral landscape breeding sites as given by the pair correlation function ($g(r)$) for non-beaver watersheds. The x-axis represents the distance in meters between sites. The area above the grey dashed line at $g(r) = 1$ (for figures a - c) represents positive spatial correlation while below the line represents negative spatial correlation compared to a random distribution. In figure d, $g(r) = 0$ represents no spatial correlation after accounting for the correlation in the neutral landscape. The upper and lower bounds of the 95% confidence intervals are shown with red dotted lines. a) Observed lentic sites across non-beaver watersheds were positively spatially correlated at distances up to 9 km. b) Observed breeding sites across non-beaver watersheds were more strongly spatially correlated than lentic sites but only up to distances of approximately 5 km. c) Non-beaver neutral landscape breeding sites were spatially autocorrelated at the same intensity as observed lentic sites, but only up to a distance of approximately 3 km. d) Observed breeding sites in non-beaver watersheds were positively spatially autocorrelated after subtracting the neutral landscape at distances up to 5 km.





Literature Cited

- Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Science* **38**:1507-1514.
- Berry, O., M. O. Tocher, D. M. Gleeson, and S. D. Sarre. 2005. Effect of vegetation matrix on animal dispersal: genetic evidence from a study on endangered skinks. *Conservation Biology* **19**:855-864.
- Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**:60-71.
- Bossart, J. L., and D. P. Prowell. 1998. Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution* **13**:202-206.
- Collen, P., and R. J. Gibson. 2001. The general ecology of beavers (*Castor spp.*), as related to their influence on stream ecosystems and riparian habitats, and the subsequent effects on fish - a review. *Reviews in Fish Biology and Fisheries* **10**:439-461.
- Double, M. C., R. Peakall, N. R. Beck, and A. Cockburn. 2005. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution* **59**:625-635.
- Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package). Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.
- Funk, W. C., M. S. Blouin, P. S. Corn, B. A. Maxell, D. S. Pilliod, S. Amish, and F. W. Allendorf. 2005a. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by landscape. *Molecular Ecology* **14**:483-496.
- Funk, W. C., A. E. Greene, P. S. Corn, and F. W. Allendorf. 2005b. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters* **1**:13-16.
- García-Paris, M., D. A. Good, G. Parra-Olea, and D. B. Wake. 2000. Biodiversity of Costa Rican salamanders: Implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proceedings of the National Academy of Sciences of the United States of America* **97**:1640-1647.
- Geffen, E., M. J. Anderson, and R. K. Wayne. 2004. Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology* **13**:2481-2490.

- Goudet, J. 1995. FSTAT version 1.2: a computer program to calculate F-statistics. *Journal of Heredity* **86**:485-486.
- Goudet, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics. *Molecular Ecology* **5**:184-186.
- Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* **48**:361-372.
- Hammerson, G. A. 1994. Beaver (*Castor canadensis*): ecosystem alterations, management, and monitoring. *Natural Areas Journal* **14**:44-57.
- Hansen, M. M., E. E. Nielsen, and K.-L. D. Mensberg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology* **6**:469-474.
- Hecnar, S. J., and R. T. M'Closkey. 1996. Regional dynamics and the status of amphibians. *Ecology* **77**:2091 - 2097.
- Hey, D. L., and N. S. Phillippi. 1995. Flood reduction through wetland restoration: The upper Mississippi River Basin as a case study. *Restoration Ecology* **3**:4-17.
- Hitchings, S. B., and T. J. C. 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.
- Hoffman, E. A., F. W. Schueler, and M. S. Blouin. 2004. Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. *Evolution* **58**:2536-2545.
- Hutchinson, D. W., and A. R. Templeton. 1999. Correlation of the pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**:1898-1914.
- Johansson, M., C. R. Primmer, and J. Merilä. 2006. History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology* **15**:975-983.
- Johnston, C. A., and R. J. Naiman. 1990. The use of a geographic information system to analyze long-term landscape alteration by beaver. *Ecology* **71**:5-19.
- Jones, C. G., J. H. Lawton, and M. Shachak. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* **78**:1946-1957.

- Jones, C. G., J. H. Lawton, and M. Shackck. 1994. Organisms as ecosystem engineers. *Oikos* **69**:373-386.
- Koenig, W. D., D. V. Vuren, and P. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution* **11**:514-517.
- Lougheed, S. C., C. Gascon, D. A. Jones, J. P. Bogart, and P. T. Boag. 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a poison-dart frog (*Epipedobates femoralis*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **266**:1829-1835.
- Lowe, W. H., G. E. Likens, M. A. McPeck, and D. C. Buso. 2006. Linking direct and indirect data on dispersal: isolation by slope in a headwater stream salamander. *Ecology* **87**:334-339.
- Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics* **152**:1753-1766.
- Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* **18**:189-197.
- Marsh, D. M., and P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**:40-49.
- Maxell, B. A. 2000. Management of Montana's amphibians: a review of factors that may present a risk to population viability and accounts on the identification, distribution, taxonomy, habitat use, natural history, and the status and conservation of individual species. Report to USFS Region 1. University of Montana, Missoula, MT.
- McGarigal, K., and S. A. Cushman. 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. *Ecological Applications* **12**:335-345.
- Mills, L. S., and P. E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations. *The American Naturalist* **144**:412-431.
- Monsen, K. J., and M. S. Blouin. 2003. Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology* **12**:3275-3286.
- Monsen, K. J., and M. S. Blouin. 2004. Extreme isolation by distance in a montane from *Rana cascadae*. *Conservation Genetics* **5**:827-835.
- Moore, J. W. 2005. Animal Ecosystem Engineers in Streams. *BioScience* **56**:237-246.
- Naiman, R. J., J. M. Melillo, and J. Hobbie. 1986. Ecosystem alteration of boreal forest streams by beaver. *Ecology* **67**:1254-1269.

- NatureServe. 2006. NatureServe Explorer: An online encyclopedia of life [web application]. NatureServe, Arlington, Virginia.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583-590.
- Peakall, R., M. Ruibal, and D. B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution* **57**:1182-1195.
- Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology* **6**:288-295.
- Pilliod, D. S., C. R. Peterson, and P. I. Ritson. 2002. Seasonal migration of Columbia spotted frogs (*Rana luteiventris*) among complementary resources in a high mountain basin. *Canadian Journal of Zoology* **80**:1849-1862.
- Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. *BioScience* **46**:609-620.
- Raybould, A. F., R. T. Clarke, J. M. Bond, R. E. Welters, and C. J. Gliddon. 2002. Inferring patterns of dispersal from allele frequency data. Pages 89-110 in J. M. Bullock, R. E. Kenward, and R. S. Hails, editors. *Dispersal Ecology: the 42nd symposium of the British Ecological Society held at the University of Reading 2-5 April 2001*. Blackwell Science Ltd, Malden, MA.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity* **83**:248-249.
- Reaser, J. K. 1996. *Rana pretiosa* (spotted frog) vagility. *Herpetological Review* **27**:196-197.
- Rieman, B. E., and J. B. Dunham. 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish* **9**:51-64.
- Russell, K. R. 1999. Amphibian and reptile communities associated with beaver (*Castor canadensis*) ponds and unimpounded streams in the Piedmont of South Carolina. *Journal of Freshwater Ecology* **14**:149-158.
- Scribner, K. T., J. W. Arntzen, N. Cruddace, R. S. Oldham, and T. Burke. 2001. Environmental correlates of toad abundance and population genetic diversity. *Biological Conservation* **98**:201-210.
- Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. *Conservation Biology* **16**:619-629.

- Shaffer, H. B., G. M. Fellers, A. Magee, and S. R. Voss. 2000. The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation polymorphism analysis (SSCP) and mitochondrial DNA sequence data. *Molecular Ecology* **9**:135-147.
- Skelly, D. K., E. E. Werner, and S. A. Cortwright. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* **80**:2326-2337.
- Smith, M. A., and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**:110-128.
- Snodgrass, J. W. 1997. Temporal and spatial dynamics of beaver-created patches as influenced by management practices in a south-eastern North American landscape. *The Journal of Applied Ecology* **34**:1043-1056.
- Stevens, C. E., C. A. Paszkowski, and A. L. Foote. 2007. Beaver (*Castor canadensis*) as a surrogate species for conserving anuran amphibians on boreal streams in Alberta, Canada. *Biological Conservation*:1-13.
- Tallmon, D. A., H. M. Draheim, L. S. Mills, and F. W. Allendorf. 2002. Insights into recently fragmented vole populations from combined genetic and demographic data. *Molecular Ecology* **11**:699-709.
- Tallmon, D. A., W. C. Funk, W. W. Dunlap, and F. W. Allendorf. 2000. Genetic differentiation among long-toed salamander (*Ambystoma macrodactylum*) populations. *Copeia* **2000**:27-35.
- Team, R. D. C. 2005. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Turner, F. B. 1960. Population structure and dynamics of the Western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. *Ecological Monographs* **30**:251-278.
- USFWS, U. S. F. a. W. S. 2002. 12-month finding for a petition to list the Wasatch Front Columbia spotted frog as threatened throughout its range. Pages 55758-55767 in U. S. D. o. Interior, editor. Federal Register.
- Vos, C. C., A. G. A.-d. Jong, P. W. Goedhart, and M. J. M. Smulders. 2001. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **86**:598-608.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**:1358-1370.

- Werner, J. K., B. A. Maxell, P. Hendricks, and D. L. Flath 2004. Amphibians and reptiles of Montana. Mountain Press Publishing Company, Missoula, Montana.
- Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity* **82**:117 - 125.
- Wiens, J. A. 1997. Metapopulation dynamics and landscape ecology. Academic Press, San Diego.

Chapter 3

Landscape genetics of *Rana luteiventris*: landscape patterns and fine scale population structure

3.1 Abstract

Examining dispersal patterns across different landscapes is essential for understanding population connectivity as well as how humans are altering it. How the frequency and importance of these dispersal events vary with changes in habitat configuration is largely unknown. This uncertainty makes possible sources of habitat fragmentation difficult to identify and the importance of dispersal to specific populations hard to estimate. The fine scale population structure of Columbia spotted frogs (*Rana luteiventris*) in watersheds with contrasting habitat patterns was examined to investigate the effects of landscape configuration and beaver presence. I estimated genetic connectivity for spotted frogs from eight microsatellite markers using tissue from tadpoles. The observed heterozygosity and number of alleles were similar to levels detected in previous studies using tissue from adults. Hierarchical analysis confirmed that watersheds within regions and breeding sites within watersheds were both statistically significant groupings of genetic variation. Different patterns of historic and contemporary gene flow were evident in the fine scale population structure of spotted frog breeding sites between ecoregions. The genetic divergence seen within watersheds revealed that landscape configuration affected the fine scale population structure of Columbia spotted frogs. Landscape patterns of breeding sites were reflected in the presence and strength of isolation by distance equilibriums and the overall level of population subdivision within watersheds. Watersheds with beaver presence and an average distance of less than five kilometers between breeding sites showed higher levels of connectivity than did non-beaver watersheds with an average distance of more than five kilometers between breeding sites. More importantly, short beaver watersheds had lower levels of genetic divergence between breeding sites than those in long non-beaver watersheds separated by the same distance, even when distances were within the commonly observed dispersal ability of the frogs.

3.2 Introduction

Metapopulation theory is often invoked in discussions about the conservation or management of species at the landscape and regional scale because of its ability to tie population and landscape processes together (McCullough 1996). A key result of the theory is that the size, number, and distribution of habitat patches affects the dynamics and long-term persistence of populations (Rieman and Dunham 2000). Despite current concerns about habitat fragmentation few studies have compared movement patterns for landscapes differing in the amount and configuration of critical habitat for a species (Wiens 1997, Smith and Green 2005). Consequently, links between habitat patterns and population dynamics and their potential short and long term ecological consequences are poorly understood (McGarigal and Cushman 2002, Smith and Green 2005).

Several characteristics of pond breeding amphibian populations suggest conservation plans may need to account for the number and distribution of habitat patches: they vary widely in abundance, have occasional and irregular recruitment, experience local extinctions, have limited dispersal ability, and demonstrate high natal site fidelity (Skelly et al. 1999, Semlitsch 2002). Some general landscape characteristics and anthropogenic activities have already been demonstrated to affect dispersal and the subsequent population structure of amphibians (reviewed in Marsh and Trenham 2001). Mountain ridges limit gene flow between populations in the frog species *Epipedobates femoralis* (Lougheed et al. 1999), *Rana luteiventris*, (Funk et al. 2005a) and likely several other amphibians (García-Paris et al. 2000, Shaffer et al. 2000, Tallmon et al. 2000). In addition interpopulation distance, land use, and roads have all shown negative correlations to demographic and genetic parameters for several European amphibians (Hitchings and Beebee 1997, Scribner et al. 2001, Vos et al. 2001). In undeveloped and rural landscapes possible sources of habitat fragmentation may be less obvious. Changing land use and beaver extirpation have been implicated in the declines of amphibians in North America (Hecnar and M'Closkey 1996), including Columbia spotted frog populations at the southern periphery of the species' range (USFWS 2002).

Historically much of the lentic and riparian habitat in North America was created through beaver activity. The dramatic decrease in beaver numbers due to overexploitation during the fur trade resulted in a large change in the landscape, converting a considerable portion of the U.S wetlands to dry land (Naiman et al. 1986). Current and historic harvest pressures as well as the loss of riparian vegetation due to ungulate and livestock over-grazing have likely resulted in population numbers far

below historic levels in many areas (Jonas 1955). In the intermountain west, reductions in beaver numbers has led to alterations in the hydrology and nutrient flow of subalpine and midelevation valleys and subsequently the characteristics of these aquatic and riparian systems (Neff 1957, Dahm and Sedell 1986, Maret and Fanin 1987).

Although community and ecosystem effects from beaver have been demonstrated, population effects for species directly affected by their ecosystem engineering have not been examined (Moore 2005). For many species of lentic breeding amphibians in arid landscapes, beaver wetlands provide over-wintering and breeding habitat and may be vital for establishing connections between widely dispersed permanent water bodies. Disruption of the temporal and spatial distribution of these critical habitats may fragment amphibian populations that evolved in a landscape shaped by beaver activity. These landscape changes have the potential to strongly influence Columbia spotted frog populations because the species is highly aquatic and has limited dispersal abilities.

Current patterns of lentic habitat in watersheds with beaver presence show very different configurations of Columbia spotted frog breeding than watersheds where they were not detected. In western Montana, beaver watersheds have more spotted frog breeding sites distributed across a broader elevational range than non-beaver watersheds. Non-beaver watersheds typically had a single or a small group spotted frog breeding sites clustered in the upper portion of the watershed. Beaver altered the distribution of spotted frogs on the landscape by producing watersheds where breeding sites were more widely dispersed and have persisted despite being separated by distances larger than its dispersal ability (Amish Chap.2).

These differences in the presence of Columbia spotted frogs between beaver and non-beaver watersheds suggest different population processes may be operating within watersheds with different breeding site configurations. Although range-wide differentiation (Green et al. 1996) and possible patterns of regional isolation have been described (Funk et al. 2005a), the level and importance of current gene flow for local population persistence is still unknown for spotted frogs (USFWS 2002) and other threatened *Rana* species in the West. I investigated both among and within watershed patterns of Columbia spotted frog genetic variation to address the following questions: (1) how are Columbia spotted frog populations structured? And (2) how does the configuration of breeding sites within watersheds affect population structure?

3.3 Materials and Methods

3.3.1 Columbia spotted frog natural history

Columbia spotted frogs are common in Pacific Northwest and the Rocky Mountains where they are continuously distributed from eastern Washington, to western Montana and northward to southeast Alaska. Disjunct populations occur on isolated mountains and in arid-land springs in eastern Oregon, northern Nevada and Utah, and southern Idaho. Isolated southern populations in the Great Basin (Idaho, Nevada) are declining due to habitat loss and degradation from dewatering and exotic species (NatureServe 2006).

Spotted frogs are usually not found far from the edge of lentic or riparian areas used for foraging. Adults generally over winter in large permanent water bodies or in springs (Turner 1960, Pilliod et al. 2002) while breeding typically occurs after snowmelt or pond ice-out in shallow water among emergent vegetation. In Montana most breeding sites consist of fewer than 50 individuals and can contain anywhere from two to several hundred egg masses and demonstrate high annual variation in recruitment (Werner et al. 2004, B. Maxell pers. comm.).

Capture-recapture and telemetry studies for Columbia spotted frogs describe adult seasonal migrations and common short distance (< 2 km) dispersals by adults and juveniles (Turner 1960, Pilliod et al. 2002, Funk et al. 2005b). Approximately 90% of movements occur over Euclidian distances of less than 2 kilometers (Funk et al. 2005b), with rare long distance dispersals covering 4 to 7 kilometers (Reaser 1996, Funk et al. 2005b). Juveniles are the primary dispersers with annual rates up to 68% recorded (Funk et al. 2005b). Dispersers display high breeding site fidelity with 95% of all movements permanent (Funk et al. 2005b). In two watersheds with large Columbia spotted frog populations, estimates of migration based on pair-wise genetic divergence matched dispersal frequencies seen in capture-recapture data (Funk et al. 2005b), but we do not know how well this applies to populations of a more typical size or the importance of landscape patterns at the local scale for shaping population connectivity.

3.3.2 Study design and sample collection

I selected one pair of headwater watersheds (6th code HUCs) from three mountain ranges in two ecoregions of western Montana: the northern Bitterroots, the Pioneers, and the Pintlers (Fig. 1). I

assumed shorter distances between breeding sites would be the largest effect of beaver presence on the fine scale population structure of spotted frogs. Paired watersheds were less than 30 km apart, similar in geomorphology, climate and size and were paired based on differences in average distance between breeding sites (short < 5 km, long > 5 km) and beaver presence (Table 1, Fig. 2). Within these six watersheds I sampled all potential spotted frog breeding sites identified from topographic maps, aerial photos, and previous amphibian surveys.

Whenever tadpole numbers permitted thirty samples were collected from each breeding site by removing 1 cm of tissue from the tip of its tail. Overall 1267 tissue samples from 48 breeding sites in western Montana were analyzed. Tadpole tail-clips were used for tissue samples instead of adult toes to facilitate obtaining samples across a large area (see Appendix A). Collecting tadpoles may lead to a sample representing the reproduction of only a few adults (e.g., Allendorf and Phelps 1981, Hansen et al. 1997). To avoid this problem, I collected tadpoles from throughout the entire breeding site. General survey information including number of egg masses, tadpoles, juveniles and adults was repeatedly gathered during the field season to establish relative population sizes.

3.3.3 Microsatellites

Eight microsatellite loci originally developed for use with Oregon spotted frog (*Rp 3*, *Rp 15*, *Rp 17*, *Rp 23*, *Rp 193*) and Columbia spotted frog (*SFC 128*, *SFC 134*, *SFC 139*) were amplified. Loci specific annealing temperatures and repeat sizes for *Rp 17*, *Rp 193*, *SFC 128*, *SFC 134*, and *SFC 139* can be found in Monsen & Blouin (2003) while *Rp 3*, *Rp 15*, and *Rp 23* can be found in Funk et al. (2005a). I amplified loci using fluorescently labeled primers in two multiplex polymerase chain reactions (PCR) following K. Goldberg (pers. comm.) and the Multiplex PCR Kit following the manufacturer's instructions (Qiagen). I conducted PCR in a PTC-100 thermocycler (MJ Research) with a total reaction volume of 10 μ l. Capillary electrophoresis of microsatellite PCR product was done on an ABI 3130 sequencer. Allele sizes were scored using the program Genemapper version 3.7.

3.3.4 Data analyses

I set a minimum sample size of 10 individuals, and aggregated breeding sites less than 100 meters apart. Allele frequencies, observed and expected heterozygosities, average number of alleles,

F_{IS} and mean within breeding site relatedness (Lynch and Ritland 1999) were calculated using GENALEX version 6 (Peakall and Smouse 2006). I estimated exact probabilities for Hardy-Weinberg proportions (Guo and Thompson 1992), exact probabilities for genotypic disequilibrium, and pair-wise F_{ST} (Weir and Cockerham 1984) using Genepop version 3.4 (Raymond and Rousset 1995). All watersheds had sites separated by a range of Euclidian and riparian distances from 0.5 kilometer to 22 kilometers. I calculated Euclidian distances between sites from UTM coordinates and measured riparian distances in ARCMAP version 9 using a GIS database and digital USGS 7.5 minute maps.

To investigate large-scale patterns which might be present in the genetic variation of watersheds, the hierarchical structure of genetic variation in the data was investigated and isolation by distance plots from different mountain ranges and ecoregions were compared. Nested hierarchical levels of genetic variation based on ecoregion, mountain range, watershed, breeding site and individual were computed and tested for significance using the package HIERFSTAT version 0.04-2 (Goudet 2005) in the program R version 1.13 (R Development Core Team 2005). Tests for statistically significant differences in the genetic variation between ecoregions and among mountain ranges, watersheds, and breeding sites were computed based on a generalized likelihood ratio using 1000 iterations. I plotted genetic distance between sites ($F_{ST} / 1 - F_{ST}$) against geographic distance to check for patterns of isolation by distance (IBD). Plots examining the correlation of pair-wise genetic and geographic distance measures assume a stepping stone model of dispersal and compare the relative effects of random genetic drift and gene flow between pairs of sampling points (Hutchinson and Templeton 1999). If sampling points in the study area are in migration-drift equilibrium a linear relationship between genetic and geographic distance is expected. I used FSTAT version 2.9.3 (Goudet 1995) for Mantel's tests of global correlation between genetic and geographic distance matrices with significance based on 2000 randomizations.

I also investigated whether watershed characteristics describing the pattern of sites (e.g. average distance between breeding sites) was evident in the population structure of Columbia spotted frogs. Short (average distances between breeding sites < 5km) and long (distances > 5km) watersheds were selected to obtain pairs with contrasting configurations of known breeding sites at the watershed scale. I compared average F_{ST} between short and long watershed types (Table 1) for three distance classes and used Mantel's tests and IBD plots to compare levels of genetic divergence between watershed pairs.

Finally, I used the Bayesian clustering algorithm in the program STRUCTURE version 2.1 (Pritchard et al. 2000) to estimate the number of populations (K) breeding sites within each watershed

represented. I used the admixture and correlated allele frequencies models which assume gene flow among populations and that historic allele frequencies should be similar based on shared ancestry or migration. Each breeding site was assigned to the population in which it had the highest proportion of membership. For each watershed, I ran two sets of simulations estimating the number of populations from one to the total number of breeding sites sampled. The most parsimonious model with the largest natural log of the probability of the data given the number of populations ($\ln P(X/K)$) was taken as the best estimate of the number of populations in each watershed (Pritchard et al. 2000). If more than one model converged on a similar value of $\ln P(X/K)$, the one with the smallest number of populations where breeding site membership was not distributed symmetrically among clusters was used (Pritchard et al. 2000).

3.4 Results

3.4.1 Sampling and locus variation

From the 48 breeding sites, 1267 samples were successfully run at all eight microsatellite profiles. Between five (*SFC128*) and 18 (*SFC139*) alleles were observed at each locus, with an average of 9 ± 2.85 (95% CI), with per locus expected heterozygosities ranging from 0.292 (*Rp17*) to 0.723 (*SFC139*). The allele frequency distributions tended to be multimodal, with the exceptions of *SFC128* and *Rp23*, which were generally bimodal.

3.4.2 Tests of disequilibrium and intrapopulation structure

Genotypic frequencies generally conformed to Hardy Weinberg proportions (HWE). If tadpole samples represent the reproductive output of a few adults, the sampling scheme may generate significant heterogeneity among and within sampling sites (Allendorf and Phelps 1981) and may be more sensitive to tests of HWE and linkage disequilibrium. Specifically, heterozygote excess at loci or higher levels of linkage disequilibrium may result from sampling tadpoles instead of adults at breeding sites. Fifty-seven of 472 tests departed from HWE instead of the 24 expected by chance ($p < 0.05$). Of these statistically significant departures, 17 were by locus *SFC139*, while all other loci had at least three and no more than nine. When grouping by locus and using sequential Bonferroni correction for multiple tests, five tests at three loci remained significant ($p < 0.05$). Loci *SFC139* and

Rp3 both deviated from HWE twice and had an excess of heterozygotes in all four occurrences. Locus *Rp193* was out of HWE once, and showed a deficit of heterozygotes (App. B, Table 1). Previous work using *Rp193* reported no evidence of a null allele (Monsen and Blouin 2003, 2004), and locus amplification at the site in question was consistent. When grouping by site and using sequential Bonferroni correction for multiple tests, 16 tests representing samples taken from 14 different breeding sites showed significant departures from HWE proportions ($p < 0.05$) (App. B, Table 2). Of the sixteen sites not conforming to HWE, only sites A2 and C2 had more than one significant result after sequential Bonferroni correction. At site A2, both loci indicate heterozygote excess, with *SFC139* having an $F_{IS} = -0.046$ and *Rp23* having an $F_{IS} = -0.512$, suggesting either recent admixture or non-representative sampling. At site C2 the two loci are split, with *Rp3* showing a slight heterozygote deficit with an $F_{IS} = 0.092$ while *Rp17* has a heterozygote excess with an $F_{IS} = -0.613$.

Of 1652 possible comparisons, 50 exhibited significant linkage disequilibrium after sequential Bonferroni correction for loci pairs ($p < 0.05$) (App. B, Table 3). Linkage was detected in 20 different loci pairs in 27 different breeding sites across the study area. One loci pair, *SFC139* and *Rp3* accounted for 15 of the significant results while another loci pair, *Rp23* and *Rp193* had five (App. B, Table 3). Weak linkage between *SFC139* and *Rp3* was suggested previously (Funk et al. 2005a). Fourteen loci pairs with one to three significant linkage disequilibrium tests, showed no linkage during earlier testing (Monsen and Blouin 2003, 2004, Funk et al. 2005a). Five loci pairs with significant tests represent previously untested combinations: *Rp23* and *Rp193*, *Rp3* and *Rp193*, *SFC128* and *Rp15*, *SFC128* and *Rp23*, and *Rp193* and *Rp15*. Multiple significant tests, listed here in parentheses following the site number, at S13 (14), A2 (7), and S12 (4) suggest some degree of population subdivision within these breeding sites (App. B, Table 4). None of the previously untested loci pairs had more than one significant test result after accounting for subdivided sites with multiple significant tests.

3.4.3 Tadpole sampling

Overall levels of genetic variation were in agreement with earlier work done on Columbia spotted frog adults. At the watershed scale, there were no statistically significant differences between the average numbers of alleles, mean expected heterozygosity, and pair-wise F_{ST} values observed for samples collected from tadpoles instead of adult frogs (Table 2). Across the study area and within watersheds the genetic characteristics of sites spanned a wide range. The total number of alleles per

site varied from 14 (site A6) to 44 (site C1). For breeding sites the average expected heterozygosity ranged from 0.259 (site A6) to 0.645 (site NF4), while the average observed heterozygosity varied from 0.281 (site C4) to 0.695 (site C1). Among watersheds, average expected heterozygosities and average number of alleles were similar, with the highest values being observed in the North Fork of Fish Creek ($H_e = 0.588$, $N_a = 4.125$) and the lowest values being observed in Alder Creek ($H_e = 0.442$, $N_a = 3.357$)(Table 1).

3.4.4 Hierarchical structure of genetic variation

Fine scale groupings of samples explained the largest portion of the genetic variation in the data set. F-statistics were computed for a nested hierarchy with five levels: ecoregion, mountain range, watershed, breeding site, and individual. Grouping data by breeding site explained 23.9%, by watershed 18.1%, by mountain range 13.6%, and by ecoregion 14.9% of the total variation in the data. Differences in patterns of genetic divergence were apparent between ecoregions ($p = 0.068$, $nperm = 1000$) and among mountain ranges ($p = 0.17$, $nperm = 1000$) but were not statistically significant. Fine scale patterns of genetic divergence were evident as tests among watersheds within ecoregions and among sites within watersheds were both significant ($p = 0.001$, $nperm = 1000$).

In agreement with the hierarchical analysis, the significance and level of correlation observed for Mantel's tests of genetic and geographic distance measures increased as the scale of the ecological groupings decreased. When points from all six watersheds were aggregated Mantel's tests detected a significant linear correlation for both Euclidian (SL) and riparian distance (RP) measures with genetic distance (SL $R^2 = 0.0725$, $p = 0.0015$; RP $R^2 = 0.1474$, $p = 0.0005$). When points were grouped by ecoregion, riparian distance explained four times as much of the genetic variation in the west-central ecoregion than in the southwestern ecoregion (Table 3). Within the southwestern ecoregion, the Pintler range watersheds showed a significant correlation between Euclidian and genetic distances while the Pioneer range watersheds had no significant pattern (Pintlers $R^2 = 0.17$, Table 3). Genetic and geographic distance were most strongly correlated in the northern Bitterroot watersheds, where the highest levels of genetic differentiation were observed for distances greater than ~3 km Euclidian or ~9 km riparian (Fig. 3).

Within watersheds, two drainages contained small isolated populations demonstrating high genetic divergence despite the presence of neighboring sites at relatively short distances. In Alder Creek, an inbred (mean $r = 0.359$), genetically isolated (mean pair-wise $F_{ST} = 0.206$) breeding site

only 2 kilometers Euclidian distance from the nearest neighboring breeding site was excluded. In Pintler Creek, a similar outlier (mean $r = 0.252$, mean pair-wise $F_{ST} = 0.165$) just outside of the watershed boundary but only 1 - 2 kilometers from several breeding sites was also excluded. An IBD pattern was not seen in Alder Creek before removing the outlier, but was seen using riparian distance once the outlier was excluded. Excluding the outlier did not change the IBD pattern in Pintler Creek (Fig. 3).

3.4.5 Within watershed population structure

Different equilibrium and non-equilibrium conditions were detected within watersheds pairs despite being separated by less than 30 km and having similar levels of genetic variation (N_a , H_e ; Table 1). Mantel's tests were significant for Seymour, Alder, and Cache Creeks between genetic and riparian distance, and between genetic and Euclidian distance for Seymour Creek. Neither Pintler Creek nor Squaw Creek showed any correlation between genetic and geographic distance measures while a weak non-significant pattern was evident in the North Fork of Fish Creek when using Euclidian distance (Table 3, Fig. 3). Low pair-wise F_{ST} values between breeding sites in Squaw creek even at long distances suggest high levels of gene flow caused non-equilibrium conditions, while the high pair-wise F_{ST} values in Pintler Creek, Cache Creek and the North Fork of Fish Creek even at short distances suggest they are dominated by genetic drift (Fig. 3).

Bayesian analysis of the breeding site allele frequencies subdivided most watersheds into three or more populations (Fig. 2). Watersheds averaged 2.8 populations; the northern Bitterroot drainages averaged four while basins in the Pintler and Pioneer ranges averaged 2.3. General patterns of watershed subdivision fit well with drainage topography and likely dispersal corridors. Selection of the most parsimonious number of populations in each watershed was straightforward except for Seymour Creek. The two largest values for the natural log of the probability of the data given the number of populations ($\ln P(X/K)$) were -7263.0 for $K=10$ and -7265.0 for $K=1$ which suggested that 10 populations was the highest probability solution. However, the run data for $K=10$ revealed that each breeding site had a symmetrically distributed proportion of membership (0.1 for each population) and was an over-estimate of the true number of populations (Pritchard et al. 2000).

3.4.6 Watershed characteristics and population subdivision

Watersheds were paired based on the average distance between breeding sites and beaver presence to examine how the pattern of breeding sites affected the spotted frog population structure. Average distance was used to select drainages instead of beaver presence and absence so a comparison between beaver and non-beaver watersheds could be made while limiting possible effects on spotted frog population structure due differences in the number and configuration of breeding sites within each watershed type. The absence of breeding activity at several low elevation sites in the northern Bitterroot watersheds during sampling in 2003 and 2004 reversed the beaver and average distance relationship for this pair. The Cache Creek watershed had breeding sites separated by longer distances than any other drainage without the riparian breeding sites detected earlier (Fig. 3). The N. Fork of Fish Creek became a short watershed when no breeding was detected at two sites separated from the others by long distances. Although levels of genetic differentiation and population subdivision were much higher in the northern Bitterroot watersheds, differences observed between short and long watersheds were in agreement with those seen in the Pintler and Pioneer watersheds. With the loss of beaver created sites the configuration of spotted frog breeding in Cache Creek resembles the long non-beaver watersheds in the Pintlers and Pioneers (Fig. 2). Similarly, although distances in the N. Fork of Fish Creek are relatively short, the watershed differs from short beaver drainages in the relative location of breeding sites. The most direct route between breeding sites involves overland travel, without a direct connection along downstream riparian corridors (Fig. 2).

When points from short and long watersheds in the Pintlers and Pioneers were plotted using genetic and Euclidian distance, an IBD pattern was seen but long watersheds had no correlation with distance (Fig. 4). A Mantel's test of the correlation between these two distance measures for short watersheds was significant ($R^2 = 0.2345$, $p = 0.0005$). Long watersheds had a non-equilibrium pattern with high pair-wise F_{ST} values at all distances suggesting they were dominated by genetic drift (Fig. 4). Over distances up to 7.5 km, average pair-wise F_{ST} values were significantly lower in short than in long watersheds (Fig. 5). Patterns of population subdivision for short and long watersheds reflected the patterns of genetic divergence already described using IBD and pair-wise F_{ST} data. As suggested by the low levels of genetic differentiation, the two short watersheds (Squaw and Seymour Creek) each consisted of a single population. Similarly, the level of genetic divergence in the two long watersheds (Pintler and Alder Creek) was consistent with population subdivision. Pintler Creek consisted of 3 populations while Alder Creek was subdivided into 4 populations (Fig. 2).

3.5 Discussion

At the watershed scale, Columbia spotted frog breeding sites displayed migration – genetic drift equilibrium suggesting fine scale patterns of population structure. Differences in the IBD patterns between regions and overall levels of genetic differentiation suggest ecoregions have experienced different colonization or dispersal histories. The average distance between breeding sites within a watershed was reflected in current gene flow patterns and the level of population subdivision. Short beaver watersheds were characterized by a single population with very low levels of genetic differentiation between breeding sites while long non-beaver watersheds were subdivided into multiple populations and had higher levels of genetic differentiation over the same distance.

3.5.1 Genetic variation

Population structure for Columbia spotted frogs in six western Montana watersheds varied widely from a single population to five populations for each of the five breeding sites sampled (Fig. 2). In general watersheds were characterized by low genetic connectivity between breeding sites with moderate levels of within population genetic diversity. The level of genetic structure seen ($F_{ST} = 0.01 - 0.232$) in this study across scales of 1 to 25 km is similar to recent work done on *R. luteiventris* (Funk et al. 2005a) and *R. cascadae* (Monsen and Blouin 2004). Lower values for the same scale ($F_{ST} = 0.04 - 0.09$) are seen for *R. temporaria* (Johansson et al. 2006) across a landscape with less physical relief and a more hospitable matrix. Estimated levels of expected heterozygosity were within the range seen in other anuran studies (reviewed Hoffman et al. 2004, Monsen and Blouin 2004).

Across the study area, watershed structure determines the distribution of spotted frog populations. Similar to results in Funk et al. (2005a), basin or watershed groupings of breeding sites explained the highest portion of loci variation (18.1%) after breeding sites (23.9%). Landscape structures associated with watersheds boundaries (like ridges) have been seen to be important for structuring populations of Columbia spotted frogs (Funk et al. 2005a) and are well supported for other amphibians (García-Paris et al. 2000, Shaffer et al. 2000, Tallmon et al. 2000, Monsen and Blouin 2004). The strong genetic subdivisions seen in two montane frog species (Monsen and Blouin 2004,

Funk et al. 2005a, this study) and known effects from ridges suggest headwater watersheds are well suited for use as conservation and management units.

Regional patterns in genetic variation and divergence evident in previous work (Funk et al. 2005) and this study suggest that watersheds separated by distances of 100-200 km may have experienced very different colonization or dispersal histories. Within this study IBD patterns and levels of population subdivision within watersheds were different between ecoregions and are reflected in Columbia spotted frog population structure even though the hierarchical analysis found differences between ecoregion's genetic variation to be non-significant. Differences in effective population size do not appear to be responsible, as expected heterozygosity and the average number of alleles were similar across ecoregions. Geomorphology or patterns of human settlement may have influenced colonization and dispersal histories between regions. Landscape analyses of the configuration of lentic habitat in the west-central and southwestern ecoregions of Montana suggested similar geomorphology for most watersheds. However, the valley at the bottom of these headwater watersheds may important for colonization and dispersal dynamics (Funk et al. 2005a). Source populations may be limited for watersheds without stable low elevation breeding sites, changing the frequency of dispersal into headwater areas (Funk et al. 2005a). In addition, human settlement may have altered dispersal patterns through the draining of wetlands and the removal of beaver.

3.5.2 Population structure within watersheds

Within watersheds, both landscape patterns of sites and a sites relative location within a watershed had effects on site levels of genetic subdivision. Bayesian analysis of breeding site allele frequencies subdivided most watersheds into three or more populations (Fig. 2). The range of population subdivision seen agreed with earlier work (Funk et al. 2005) and suggests fine-scale population structure for spotted frogs varies widely. General patterns of watershed subdivision fit well with drainage topography and likely dispersal corridors. Breeding sites organized along a linear riparian corridor showed the highest levels of connectivity (Fig. 2). In contrast, even breeding sites separated by short over-land distances showed high levels of genetic divergence and in some cases evidence of inbreeding and isolation.

Clusters or complexes of sites separated by short dispersal distances (< 2 km) showed higher genetic diversity and low genetic differentiation, especially at lower elevations. At these distances enough migration between breeding sites may exist to maintain a higher level of genetic diversity

than would otherwise be possible. Sites at the top of headwater basins showed lower genetic diversity, and higher genetic differentiation over the same distances. Although many high elevation sites undoubtedly have a very small effective population sizes because breeding aggregations are composed of only a couple of individuals, even those with large breeding aggregations had low genetic diversity and small effective population sizes (e.g. Table 1, A3 & S14, but not P6). Variation associated with anuran demography increases at higher elevations (shorter growing season, variation in snow pack) and implies that complexes or clusters of sites may be vital for maintaining population processes in headwater basins and for the long-term persistence of isolated populations.

3.5.3 How does the pattern of breeding sites within the watershed affect population structure?

Short beaver and long non-beaver watersheds showed significantly different average F_{ST} values for the two shortest distance classes (0-2.5, 2.5-7.5 km) (Fig. 5). The level of genetic differentiation exhibited over short and medium distances classes suggested population subdivision in long non-beaver watersheds but population connectivity in short beaver watersheds. Estimates of the population subdivision within these watersheds supported these conclusions. Other beaver and non-beaver watersheds have examined in earlier studies have shown similar patterns (Funk et al. 2005). There are several possible explanations for these differences. For short beaver watersheds, the location of lentic habitat in riparian corridors may be important for maintaining connectivity between breeding sites if dispersal success is higher along riparian corridors than over-land. Alternatively, larger breeding aggregations with a higher number of juvenile dispersers in short beaver watersheds would maintain lower genetic divergence. With one exception (S14), the numbers of frogs or egg masses observed at breeding sites within watersheds during sample collection suggest they represent breeding aggregations of typical (~50 individuals; Werner et al. 2004) or smaller sizes with no differences between watershed types.

What do the observed differences in the Columbia spotted frog population structures tell us about the typical watersheds in southwestern Montana, based on their habitat configurations? First, historic patterns of migration and/or colonization may still be evident in fine-scale population structures. Large differences in the level of genetic differentiation between populations in short and long watersheds in the northern Bitterroots suggests historic patterns of dispersal or the geomorphology surrounding these watersheds are still strongly evident in population processes. In

addition, the loss of beaver created spotted frog breeding sites detected during earlier amphibian surveys changed the watershed characteristics of Cache Creek. The effects of beaver alterations to landscape patterns may be temporary or transient and dependent upon current beaver occupancy. Within the Pintler and Pioneer ranges, beaver watersheds will have low levels of divergence between breeding sites separated by moderate distances (< 7.5 km) and will likely consist of a single population. This implies that even sites separated by long distances are not isolated from neighboring sites within the watershed. Third, non-beaver watersheds will have moderate to high levels of divergence between breeding sites separated by moderate distances. Since most non-beaver watersheds consist of a single breeding site, they represent isolated populations. In non-beaver watersheds where multiple breeding sites are found separated by moderate or longer distances, watersheds likely contain several isolated populations. Finally, in non-beaver watersheds even sites separated by short distances may have high levels of genetic divergence.

Although this study focused on one species, the Columbia spotted frog, the redistribution of lentic habitat may have similar effects on the population structure of other lentic breeding amphibians and suggests that subtle differences in landscape patterns may have far reaching population consequences. For beaver management, a more careful consideration of potential population effects on species utilizing the lentic habitat they create is required. Limiting harvest of beaver in some areas may be important for maintaining existing populations of lentic breeding amphibians, or may improve connectivity among isolated populations. In some areas where limited habitat has led to the isolation of populations, beaver reintroductions may provide managers with the ability of connect low and high elevation populations, or to increase the number of breeding sites available within a watershed.

Table 1. Sampled breeding sites organized by ecoregion, mountain range, and watershed: Site number; watershed type based on average distance between breeding sites (S = short or < 5 km , L = long or > 5 km) and beaver presence; number of complete genotypes (N); average number of alleles (N_a); expected heterozygosity (H_e); number of egg masses detected; site elevation (meters); Universal Transverse Mercator coordinates (UTME & UTMN).

Location & Site Number	Watershed Type		N	N_a	H_e	Egg Masses	Elevation	UTM		
	Distance	Beaver						Zone	UTME	UTMN
<i>West-central Montana Ecoregion</i>										
<i>Northern Bitterroot Mountains</i>										
Cache Creek	L	Y								
C1			25	5.500	0.628	-	1195	11	677396	5184482
C2			25	3.625	0.531	-	1280	11	678066	5183416
C3			32	4.000	0.524	-	1899	11	670567	5186025
C4			32	2.000	0.286	-	1921	11	669314	5183148
C5			32	2.875	0.424	-	1927	11	670643	5178585
Watershed average				3.600	0.479		1645			
North Fork Fish Creek	S	N								
NF1			33	3.750	0.519	-	1899	11	658511	5203903
NF2			32	4.250	0.616	-	1829	11	659861	5199601
NF3			16	3.500	0.522	-	1909	11	656897	5199001
NF4			31	4.375	0.645	-	1976	11	658304	5197711
NF5			34	4.875	0.634	-	1757	11	656302	5197843
NF6			20	4.000	0.594	-	1915	11	655038	5200819
Watershed average				4.125	0.588		1881			
<i>Southwestern Monatan Ecoregion</i>										
<i>Pioneer Range</i>										
Alder Creek	L	N								
A1			18	4.125	0.543	-	2184	12	336002	5074716
A2			31	3.625	0.532	-	2626	12	333746	5072359
A3			29	3.875	0.438	16	2621	12	333581	5072493
A4			25	3.375	0.418	-	2631	12	333950	5071851
A5			13	2.625	0.465	2	2808	12	333333	5025469
A6			10	1.750	0.259	1	2863	12	331887	5068054
A7			25	4.125	0.436	4	2760	12	334274	5067413
Watershed average				3.357	0.442		2642			
Squaw Creek	S	Y								
SQ1			10	3.125	0.507	-	2161	12	323919	5070170
SQ2			15	3.875	0.468	-	2174	12	324156	5070337
SQ3			24	3.625	0.510	-	2471	12	325837	5067442
SQ4			23	3.875	0.476	-	2403	12	326050	5067875
SQ5			14	3.750	0.452	-	2386	12	327346	5069299
Watershed average				3.650	0.483		2319			
<i>Pintler Range</i>										
Pintler Creek	L	N								
P1			39	4.625	0.508	-	2147	12	309924	5076789
P2			30	4.000	0.526	-	2156	12	311048	5078736
P3			31	4.125	0.456	-	2147	12	310324	5080659
P4			31	4.375	0.396	9	2198	12	308580	5083413
P5			16	2.625	0.351	-	2829	12	304679	5087382
P6			32	4.125	0.496	-	2737	12	303806	5086491
P7			15	2.750	0.368	-	2856	12	304612	5087454
P8			29	3.500	0.474	-	2917	12	304239	5088054
P9			32	3.875	0.505	-	2733	12	305631	5089061
PX			15	2.125	0.370	-	2706	12	303523	5085827
Watershed average				3.613	0.445		2543			
Seymour Creek	S	Y								
S1			32	3.875	0.502	-	2042	12	330416	5088038
S2			34	4.125	0.515	5	2181	12	332365	5090859
S3			18	4.125	0.497	-	2174	12	331977	5091255
S4			32	4.000	0.529	-	2236	12	332929	5092162
S5			33	4.375	0.572	-	2413	12	330247	5093579
S6			36	4.500	0.547	-	2454	12	330224	5094284
S7			31	4.375	0.580	-	2467	12	330129	5094520
S8			30	4.000	0.520	-	2311	12	330781	5094870
S9			30	4.375	0.546	-	2324	12	330883	5095427
S10			28	4.000	0.546	-	2372	12	330883	5095724
S11			30	4.125	0.566	20	2348	12	330799	5095724
S12			28	3.625	0.484	-	2377	12	330130	5096333
S13			32	3.375	0.511	-	2617	12	325691	5100797
S14			31	3.000	0.471	111	2863	12	323931	5099542
Watershed average				3.991	0.528		2370			

Table 2. Comparison of genetic variation and relatedness when sampling Columbia spotted frog tadpoles and adults: regional comparison of tadpole and adult Columbia spotted frog study areas listed by mountain range, average number of alleles (N_a), mean expected heterozygosity (H_e), and watershed and study wide means of F_{ST} and relatedness (r).

	Avg N_a *	Mean H_e	Watershed Average	
			F_{ST} **	r
C. Bitterroot Range (Amish)	3.886	0.539	0.097	0.1343
Anaconda Range (Amish)	3.833	0.493	0.039	0.0471
Pioneer Mountains (Amish)	3.538	0.452	0.046	0.0563
Tadpole Study Mean (Amish)	3.766	0.492	0.066	0.0682
95% CI (+)	3.978	0.541	0.102	0.122
95% CI (-)	3.554	0.444	0.030	0.014
Cabinets & Cour D'Alene Range (Funk)	5.083	0.601	0.067	
S. Bitterroot Range (Funk)	3.000	0.405	0.093	
Bighorn Crag Mountains (Funk)	2.800	0.322	0.149	
Adult Study Mean (Funk)	3.628	0.443	0.067	
95% CI (+)	4.892	0.586	0.109	
95% CI (-)	2.197	0.281	0.019	

* Average number of alleles computed based on 6 microsatellite markers used by Funk plus an additional 2 markers for Amish.

** Average based on pair-wise F_{ST} values within watersheds.

Table 3. Summary of Mantel's tests of the correlation between genetic and geographic distance across a spatial hierarchy: Geographic and genetic distance *correlation*, *beta* for the geographic distance measure with its *p-value*, and the R^2 for the model are shown. Significant values are in bold.

Ecoregion	Range	Watershed	Correlation	Straight-line			Riparian			
				Beta	P(Beta)	R^2	Correlation	Beta	P(Beta)	R^2
<i>West central</i>			0.5343	0.000023	0.003	0.286	0.7312	0.000016	0.0015	0.535
	N. Bitterroots		0.5343	0.000023	0.003	0.286	0.7312	0.000016	0.0015	0.535
	Cache		0.1493	0.000006	0.6705	0.0223	0.6364	0.000013	0.0425	0.405
	N. Fork Fish		0.4058	0.000009	0.1290	0.1647	-0.0271	0.000000	0.9210	0.0007
<i>Southwestern</i>			0.3449	0.000003	0.0005	0.119	0.3430	0.000002	0.0005	0.118
	Pioneers		0.1334	0.000003	0.5185	0.0179	0.3034	0.000003	0.1280	0.0922
	Alder		0.0494	0.000001	0.8410	0.0024	0.5465	0.000007	0.0265	0.299
	Squaw		0.1607	0.000004	0.6615	0.0258	-0.3161	-0.000003	0.3760	0.0999
	Pintlers		0.4150	0.000004	0.0005	0.172	0.3794	0.000002	0.0005	0.144
	Pintler		-0.1744	-0.000001	0.3285	0.0304	-0.2265	-0.000001	0.2015	0.0513
	Seymour		0.6423	0.000006	0.0005	0.413	0.6012	0.000004	0.0005	0.361

Figure 1. Map of study area in southwestern Montana: Focal watershed pairs are shown in beige; northern Bitterroots pair due west of Missoula, Pintler range pair shown on the north side of the Big Hole River, with the Pioneer range pair to the south.

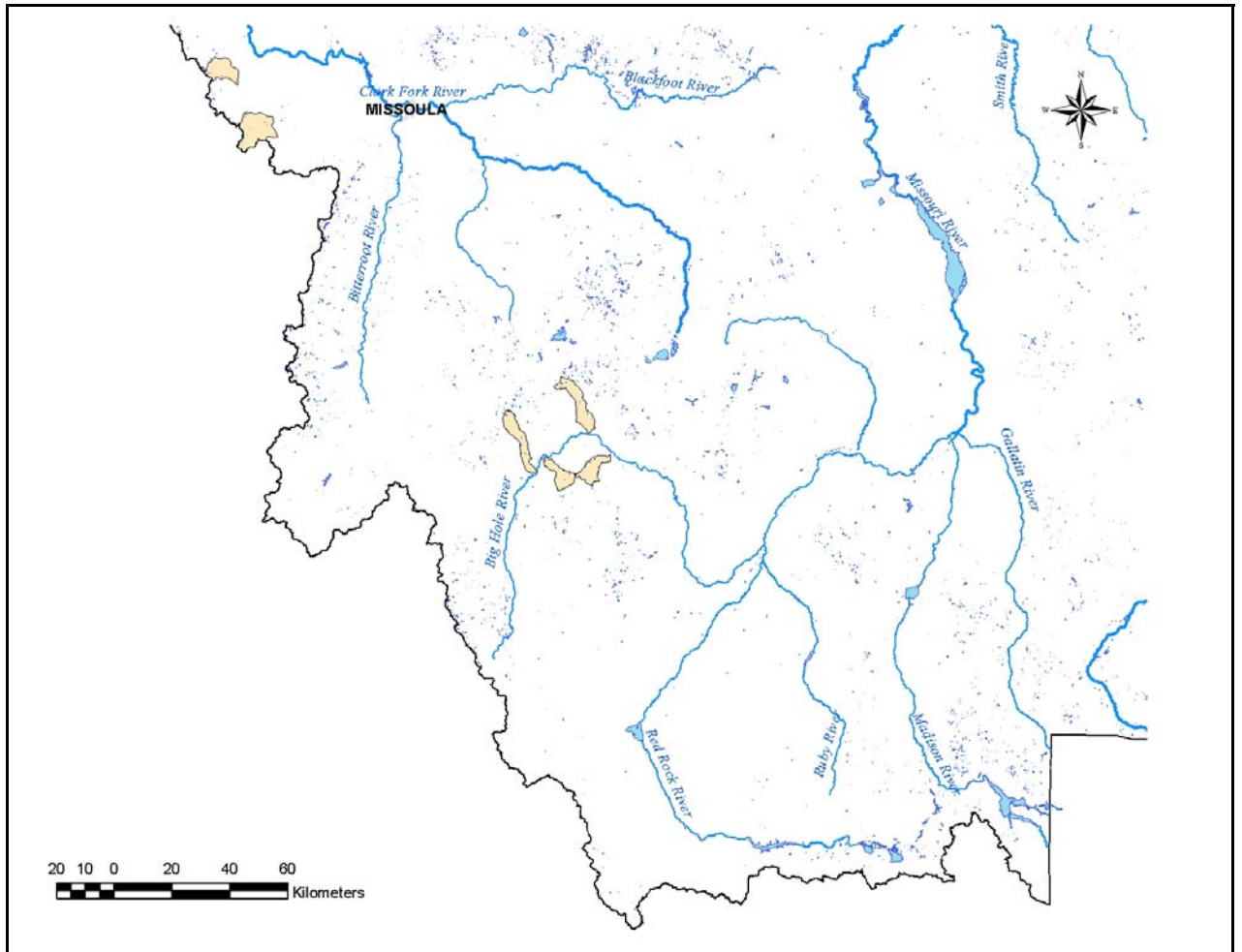
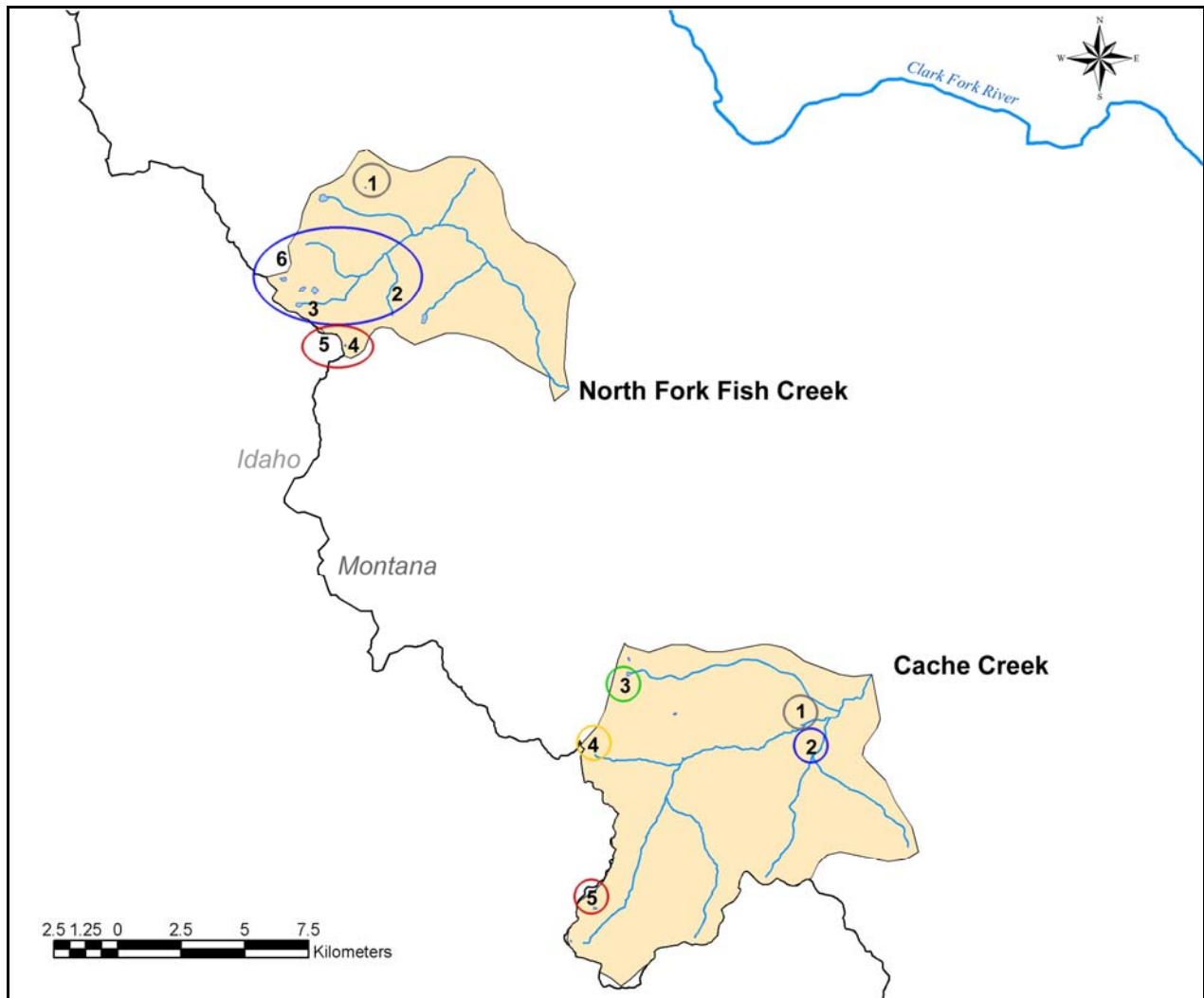


Figure 2. Detail of watershed pairs: northern Bitterroot range watersheds are the North Fork Fish Creek and Cache Creek in the Lolo National Forest; Pintler range watersheds are Pintler Creek and Seymour Creek; Pioneer range are Alder Creek and Squaw Creek; breeding sites are numbered from the bottom to the top of the watershed; colored circles denote groupings from the most parsimonious STRUCTURE model with sites being assigned to the cluster from which individuals had the highest proportion of membership.



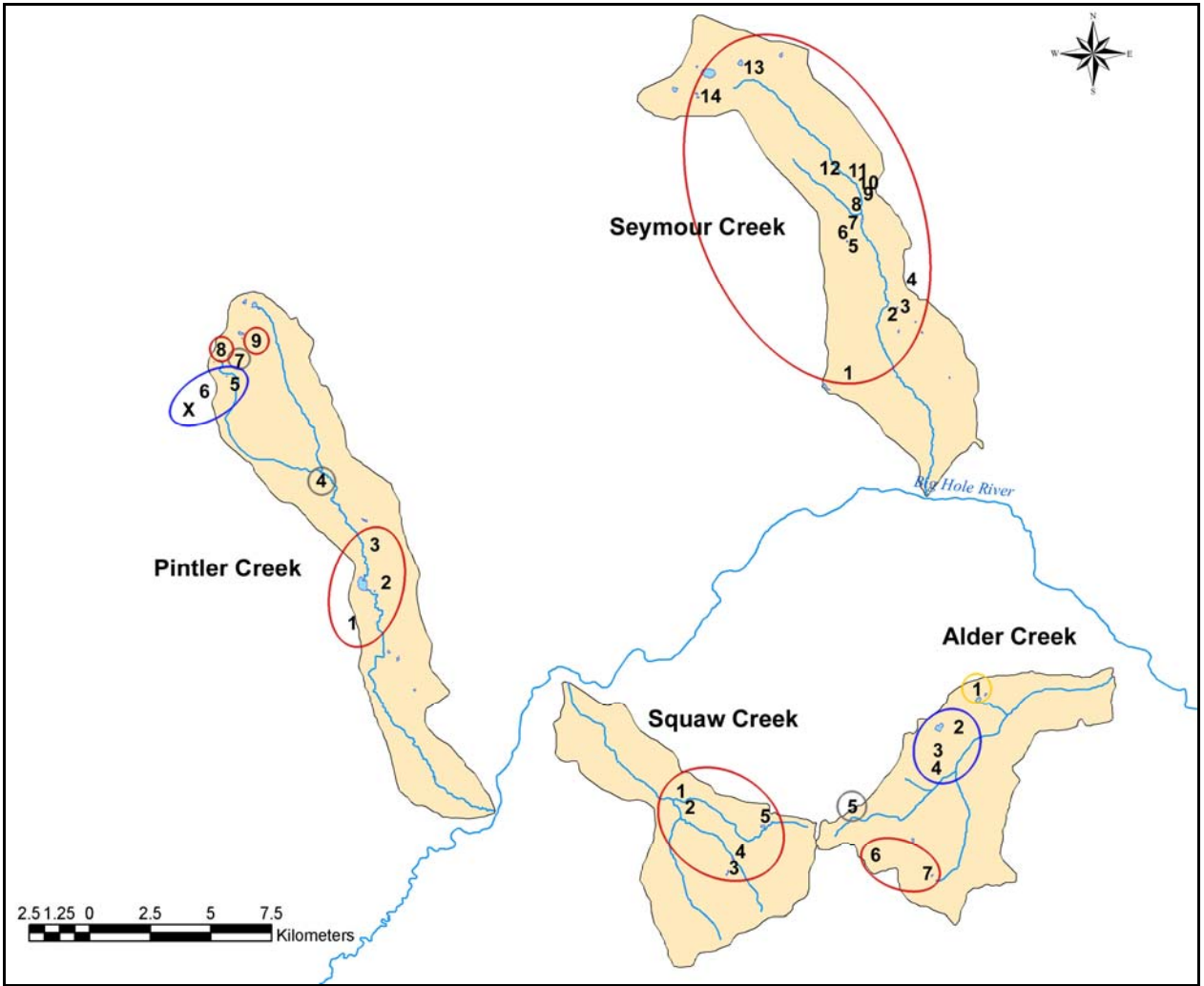
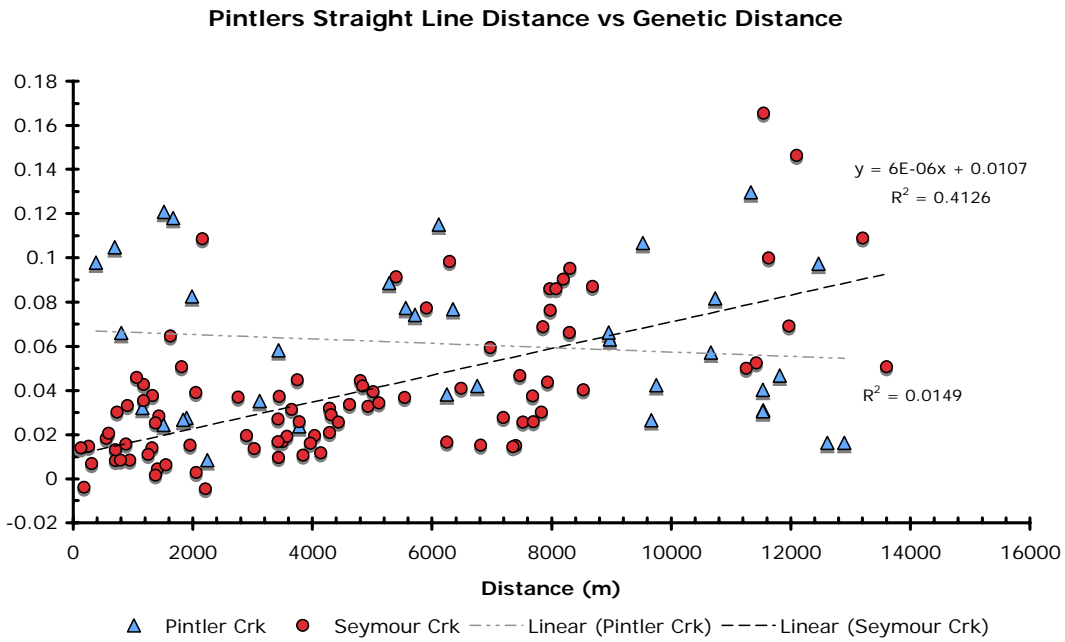
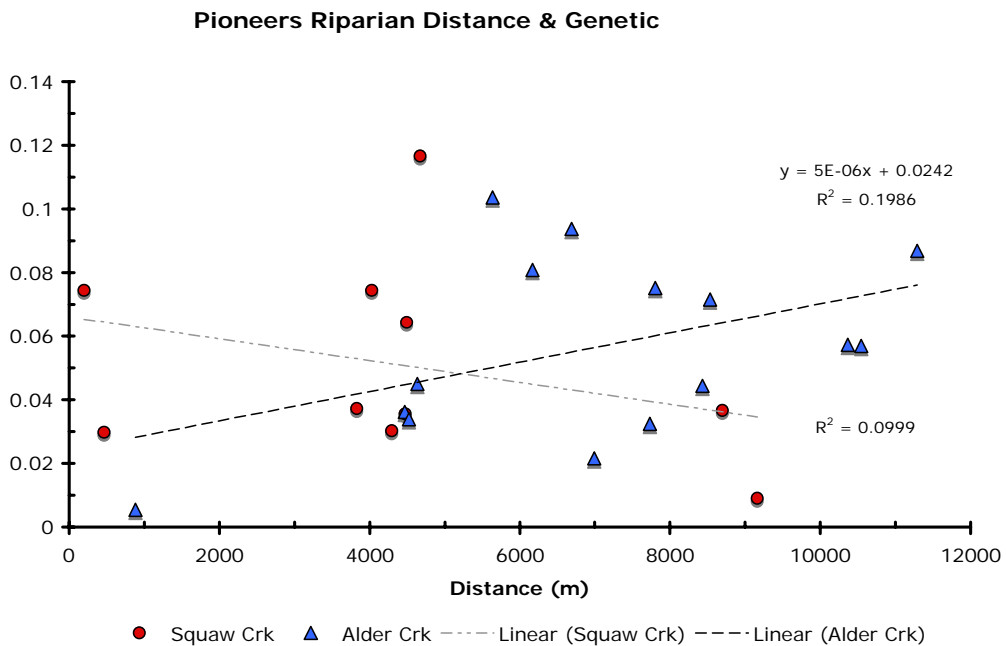


Figure 3. Isolation by distance graphs A - F: Graphs are identified by the mountain range where the watersheds are located, with watersheds labeled by creek. Distance in meters is shown on the X-axis, while genetic distance ($F_{ST} / 1 - F_{ST}$) is shown on the Y-axis.

A)

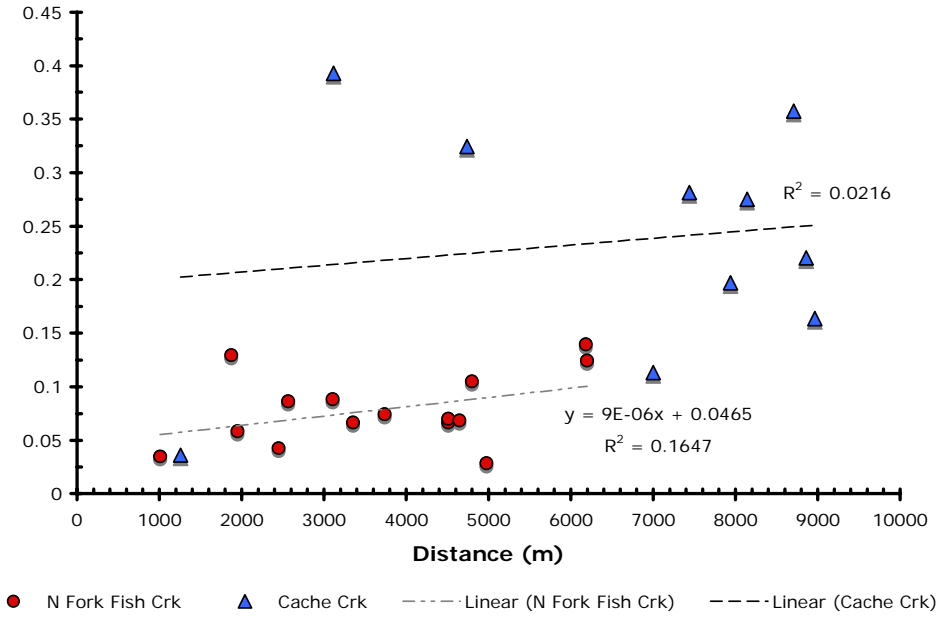


B)



C)

N Bitterroot Straight Line Distance vs Genetic Distance



D)

N Bitterroot Riparian Distance & Genetic Distance

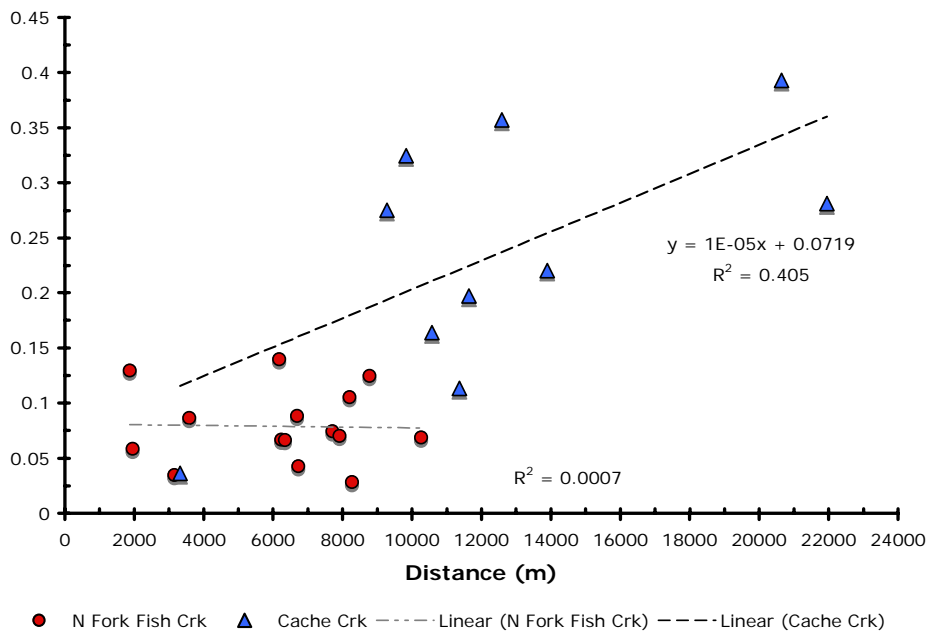


Figure 4. Isolation by distance graph for short and long watersheds: Watersheds are identified by the average distance between breeding sites (short and long), with Euclidian distance plotted against genetic distance. Short watersheds show a significant IBD pattern while long watersheds show a drift dominated pattern with no significant correlation between genetic and geographic distance.

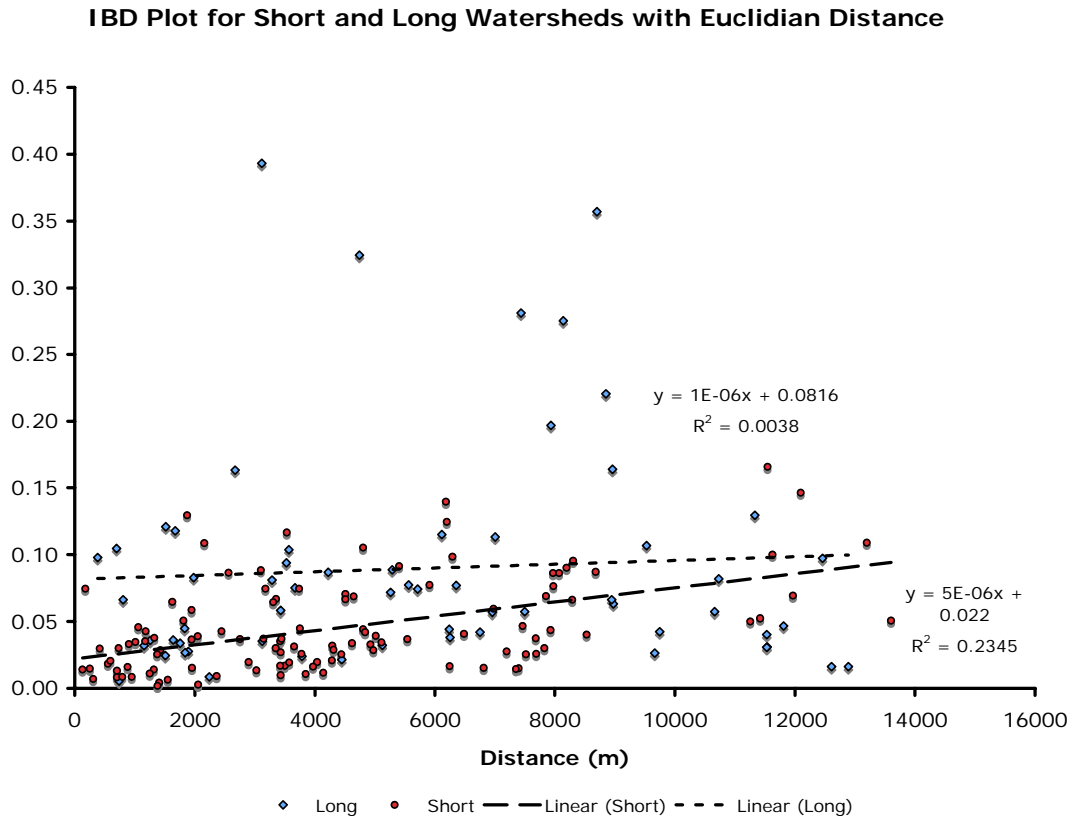
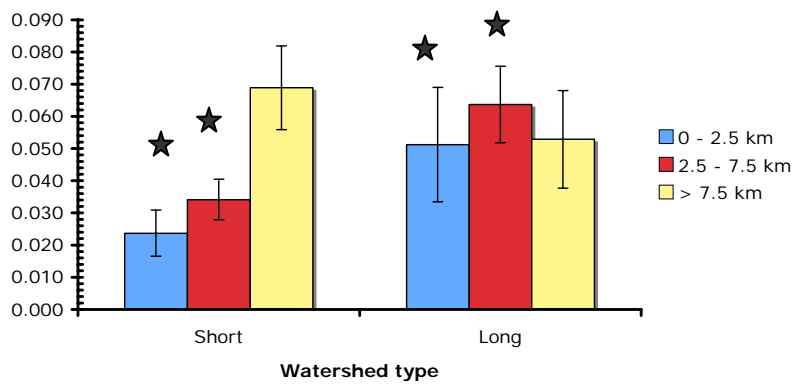


Figure 5. Pair-wise F_{ST} over three geographic distance classes when watersheds are classified by average distance between breeding sites: Two short beaver watersheds and two long non-beaver watersheds were used to investigate the effects of the distribution of lentic sites on the relationship between genetic divergence and the Euclidian distance between the sites. Distance categories with significantly different average pair-wise F_{ST} values (non-overlapping 95% CI) between watershed types are starred.



Literature Cited

- Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Science* **38**:1507-1514.
- Collen, P., and R. J. Gibson. 2001. The general ecology of beavers (*Castor spp.*), as related to their influence on stream ecosystems and riparian habitats, and the subsequent effects on fish - a review. *Reviews in Fish Biology and Fisheries* **10**:439-461.
- Dahm, C. N., and J. R. Sedell. 1986. The role of beaver on nutrient cycling in streams. *Journal of the Colorado-Wyoming Academy of Science* **18**:32.
- Funk, W. C., M. S. Blouin, P. S. Corn, B. A. Maxell, D. S. Pilliod, S. Amish, and F. W. Allendorf. 2005a. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by landscape. *Molecular Ecology* **14**:483-496.
- Funk, W. C., A. E. Greene, P. S. Corn, and F. W. Allendorf. 2005b. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters* **1**:13-16.
- García-Paris, M., D. A. Good, G. Parra-Olea, and D. B. Wake. 2000. Biodiversity of Costa Rican salamanders: Implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proceedings of the National Academy of Sciences of the United States of America* **97**:1640-1647.
- Goudet, J. 1995. FSTAT version 1.2: a computer program to calculate F-statistics. *Journal of Heredity* **86**:485-486.
- Goudet, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics. *Molecular Ecology* **5**:184-186.
- Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* **48**:361-372.
- Hammerson, G. A. 1994. Beaver (*Castor canadensis*): ecosystem alterations, management, and monitoring. *Natural Areas Journal* **14**:44-57.
- Hansen, M. M., E. E. Nielsen, and K.-L. D. Mensberg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology* **6**:469-474.
- Hecnar, S. J., and R. T. M'Closkey. 1996. Regional dynamics and the status of amphibians. *Ecology* **77**:2091 - 2097.
- Hey, D. L., and N. S. Phillippi. 1995. Flood reduction through wetland restoration: The upper Mississippi River Basin as a case study. *Restoration Ecology* **3**:4-17.
- Hitchings, S. B., and T. J. C. 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.
- Hoffman, E. A., F. W. Schueler, and M. S. Blouin. 2004. Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. *Evolution* **58**:2536-2545.
- Hutchinson, D. W., and A. R. Templeton. 1999. Correlation of the pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**:1898-1914.
- Johansson, M., C. R. Primmer, and J. Merilä. 2006. History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology* **15**:975-983.

- Johnston, C. A., and R. J. Naiman. 1990. The use of a geographic information system to analyze long-term landscape alteration by beaver. *Ecology* **71**:5-19.
- Jones, C. G., J. H. Lawton, and M. Shachak. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* **78**:1946-1957.
- Jones, C. G., J. H. Lawton, and M. Shackck. 1994. Organisms as ecosystem engineers. *Oikos* **69**:373-386.
- Lougheed, S. C., C. Gascon, D. A. Jones, J. P. Bogart, and P. T. Boag. 1999. Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a poison-dart frog (*Epipedobates femoralis*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **266**:1829-1835.
- Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics* **152**:1753-1766.
- Maret, T. J., and T. E. Fanin. 1987. The effect of beaver ponds on the nonpoint source water quality of a stream in southwestern Wyoming. *Water Research* **21**:263.
- Marsh, D. M., and P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**:40-49.
- Maxell, B. A. 2000. Management of Montana's amphibians: a review of factors that may present a risk to population viability and accounts on the identification, distribution, taxonomy, habitat use, natural history, and the status and conservation of individual species. 43-0343-0-0224, University of Montana, Missoula, MT.
- McGarigal, K., and S. A. Cushman. 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. *Ecological Applications* **12**:335-345.
- Monsen, K. J., and M. S. Blouin. 2003. Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology* **12**:3275-3286.
- Monsen, K. J., and M. S. Blouin. 2004. Extreme isolation by distance in a montane from *Rana cascadae*. *Conservation Genetics* **5**:827-835.
- Moore, J. W. 2005. Animal Ecosystem Engineers in Streams. *BioScience* **56**:237-246.
- Naiman, R. J., J. M. Melillo, and J. Hobbie. 1986. Ecosystem alteration of boreal forest streams by beaver. *Ecology* **67**:1254-1269.
- NatureServe. 2006. NatureServe Explorer: An online encyclopedia of life [web application]. *in.* NatureServe, Arlington, Virginia.
- Neff, D. J. 1957. Ecological effects of beaver habitat abandonment in the Colorado Rockies. *Journal of Wildlife Management* **21**:80.
- Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology* **6**:288-295.
- Pilliod, D. S., C. R. Peterson, and P. I. Ritson. 2002. Seasonal migration of Columbia spotted frogs (*Rana luteiventris*) among complementary resources in a high mountain basin. *Canadian Journal of Zoology* **80**:1849-1862.
- Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. *BioScience* **46**:609-620.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity* **83**:248-249.
- Reaser, J. K. 1996. *Rana pretiosa* (spotted frog) vagility. *Herpetological Review* **27**:196-197.
- Rieman, B. E., and J. B. Dunham. 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish* **9**:51-64.

- Russell, K. R. 1999. Amphibian and reptile communities associated with beaver (*Castor canadensis*) ponds and unimpounded streams in the Piedmont of South Carolina. *Journal of Freshwater Ecology* **14**:149-158.
- Scribner, K. T., J. W. Arntzen, N. Cruddace, R. S. Oldham, and T. Burke. 2001. Environmental correlates of toad abundance and population genetic diversity. *Biological Conservation* **98**:201-210.
- Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. *Conservation Biology* **16**:619-629.
- Shaffer, H. B., G. M. Fellers, A. Magee, and S. R. Voss. 2000. The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation polymorphism analysis (SSCP) and mitochondrial DNA sequence data. *Molecular Ecology* **9**:135-147.
- Skelly, D. K., E. E. Werner, and S. A. Cortwright. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* **80**:2326-2337.
- Smith, M. A., and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**:110-128.
- Snodgrass, J. W. 1997. Temporal and spatial dynamics of beaver-created patches as influenced by management practices in a south-eastern North American landscape. *The Journal of Applied Ecology* **34**:1043-1056.
- Stevens, C. E., C. A. Paszkowski, and A. L. Foote. 2007. Beaver (*Castor canadensis*) as a surrogate species for conserving anuran amphibians on boreal streams in Alberta, Canada. *Biological Conservation*:1-13.
- Tallmon, D. A., W. C. Funk, W. W. Dunlap, and F. W. Allendorf. 2000. Genetic differentiation among long-toed salamander (*Ambystoma macrodactylum*) populations. *Copeia* **2000**:27-35.
- Team, R. D. C. 2005. R: a language and environment for statistical computing. *in*. R Foundation for Statistical Computing, Vienna, Austria.
- Turner, F. B. 1960. Population structure and dynamics of the Western spotted frog, *Rana p. pretiosa* Baird & Girard, in Yellowstone Park, Wyoming. *Ecological Monographs* **30**:251-278.
- USFWS, U. S. F. a. W. S. 2002. 12-month finding for a petition to list the Wasatch Front Columbia spotted frog as threatened throughout its range. Pages 55758-55767 *in* U. S. D. o. Interior, editor. Federal Register.
- Vos, C. C., A. G. A.-d. Jong, P. W. Goedhart, and M. J. M. Smulders. 2001. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **86**:598-608.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**:1358-1370.
- Werner, J. K., B. A. Maxell, P. Hendricks, and D. L. Flath. 2004. Amphibians and reptiles of Montana, 1st edition. Mountain Press Publishing Company, Missoula, Montana.
- Wiens, J. A. 1997. Metapopulation dynamics and landscape ecology. Academic Press, San Diego.

Appendix A

Watershed database

Appendix A, table 1. Database variables related to watershed geomorphology and lentic habitat composition used in multivariate ordination.

Variable	Explanation
<i>Land Ownership Characteristics</i>	
Proportion of sites on private land	Proportion of all potential lentic sites on private land
<i>Survey Characteristics</i>	
Proportion of sites surveyed	Proportion of all potential lentic sites successfully surveyed
<i>Watershed Characteristics</i>	
Ecoregion	Level three ecoregion
Orientation	General aspect / orientation of the watershed
Subbasin	Level four hydrologic unit code (HUC) that contains the sixth code HUC
Beaver	Beaver created lentic sites detected within the watershed
Lower boundary elevation	Lowest point along the watershed boundary
Watershed mid-elevation	Mid-point between lower boundary elevation and highest lentic site
Change in elevation	Change in elevation between lower boundary and highest lentic site
<i>Quantity of Lentic Sites within Watersheds</i>	
Wet lentic	Lentic sites with water during survey
Dry lentic	Lentic sites without water during survey
Permanent lentic site	Lentic sites holding water year-round
Riparian lentic	Lentic sites created originating from beaver activity or river activity
Lentic with CSF	Lentic sites where CSF were also detected
Lentic with CSF breeding	Lentic sites where CSF breeding was also detected
Lentic with potential CSF breeding	Lentic sites where CSF were detected and breeding habitat was present
Lentic sites at midelevation	Lentic sites within the mid-elevation zone (1/4 change in elevation above and below watershed mid-elevation)
Lentic sites with CSF breeding at midelevation	Lentic sites where CSF breeding was detected within mid-elevation zone
<i>Distribution of Lentic Sites within Watersheds</i>	
Lowest site elevation	Lowest potential lentic site within watershed
Distance lowest site to watershed boundary	Distance from lowest potential lentic site to lowest boundary elevation
Highest site elevation	Highest lentic site
Distance highest site to watershed boundary	Distance from highest site to nearest watershed boundary
Distance highest to lowest sites	Distance from lowest site to highest site

Appendix A, table 2. Variables correlated with the two axes of the NMS ordination related to watershed composition and geomorphology, with rankings based on average of r^2 (Pearson's correlation coefficient) and τ (Kendall correlation coefficient).

Ranking Watershed Composition		Axis	r^2	τ
1	# Wet lentic sites	2	0.643	-0.669
2	# Mid-elevation lentic sites	2	0.598	-0.658
3	# Dry lentic sites	2	0.459	-0.618
4	# Riparian lentic sites	2	0.325	-0.391

Ranking Watershed Geomorphology		Axis	r^2	τ
1	Highest site elevation	2	0.461	-0.462
2	Watershed mid-elevation	2	0.432	-0.466
3	Distance lowest site to boundary	1	0.723	-0.671
4	Distance lowest to highest site	1	0.371	0.451

Appendix B

Tadpole sampling

Introduction

Tadpole tail-clips were used for tissue samples instead of adult toes to facilitate obtaining samples across a large area. There are several reasons why sampling tadpoles instead of adults may be preferable for a landscape genetics study: females often migrate to nearby foraging areas immediately after reproduction (Pilliod et al. 2002), different post-breeding migration patterns have been observed by age and sex (Turner 1960, Pilliod et al. 2002), provides a longer sampling window, effort per site is lower so more sites can be sampled, and high elevation sites may be difficult to access until after breeding has occurred. However, collecting tadpoles may lead to a sample representing the reproduction of only a few adults (e.g., Allendorf and Phelps 1981, Hansen et al. 1997). To avoid this problem, I collected tadpoles from the entire breeding site and gathered general survey information including number of egg masses, tadpoles, juveniles and adults repeatedly during the field season.

Methods

Samples

Tissue samples were collected at a subset of sites over two years to estimate annual variation in genetic data. At higher elevations females are believed to breed every 2-3 years (Turner 1960) and have variable recruitment (Turner 1960, Funk et al. 2005b) potentially leading to temporal differences in the genetic signature of a breeding site. Two watersheds in the Pioneers were sampled in 2003 and 2004, while watersheds in the northern Bitterroots and the Pintlers were sampled in 2004. Only samples from one site in Alder Creek were successfully run for two years.

Data analyses

Allele frequencies, observed and expected heterozygosities, average number of alleles, F_{IS} and mean within breeding site relatedness (Lynch and Ritland 1999) were calculated using GenAEx

version 6 (Peakall and Smouse 2006). I estimated exact probabilities for Hardy-Weinberg proportions (Guo and Thompson 1992), exact probabilities for genotypic disequilibrium, and pair-wise F_{ST} (Weir and Cockerham 1984) using Genepop version 3.4 (Raymond and Rousset 1995).

To evaluate whether tadpole sampling produced a representative sample of the breeding population, I used expected heterozygosities, average number of alleles, F_{IS} , and relatedness. Data were checked for patterns indicative of sampling a limited number of breeding pairs. If tadpole sampling produced a sampling bias, I would expect low allelic richness, high relatedness, and an excess of heterozygotes across most populations. In addition, I used number of egg masses, adults, and tadpoles, as well as breeding site size and location in conjunction with genetic data to examine whether sampling reflected general patterns of population size observed within watersheds. I compared samples from one breeding site collected in both 2003 and 2004 to estimate annual variation in allele frequencies. Expected heterozygosities, average number of alleles, pair-wise F_{ST} , and relatedness were used to estimate the magnitude of yearly variation.

Results

Annual variation

I examined temporal variation at one breeding site in the Alder Creek watershed (A5), with 23 individuals collected in 2003 and 13 collected in 2004 where complete genotypes across all eight loci amplified successfully. All alleles in the 2004 sample except two were found in the 2003 sample, while eight alleles found in the 2003 sample were not found in 2004. In 2003, the average number of alleles (N_a) was 4.25 with an average expected heterozygosity (H_e) of 0.373 (95% CI = 0.274 to 0.464) compared to 2004 when the N_a was 2.63 with a H_e of 0.465 (95% CI = 0.342 to 0.589). Differences in the average number of alleles may be due to sample size alone.

Relatedness values for the two samples suggest individuals in the 2003 sample are more highly related (0.137) than individuals in the 2004 sample (0.086). Breeding surveys during 2003 found three egg masses while two were found during 2004. Average relatedness between individuals for these two years at this site represents the second and third highest values seen in the watershed, in general agreement with the higher number of egg masses found at other breeding sites in the watershed.

The pair-wise F_{ST} between the two years (0.066) was approximately three times higher than the lowest pair-wise F_{ST} values seen between other sites sampled during 2004. UPGMA clustering based on Nei's genetic distance grouped the two A5 samples together as an out-group to the rest of the Alder Creek samples. Average pair-wise F_{ST} values for this site in 2003 and 2004 with other breeding sites sampled in the watershed during 2004 reflect higher allele frequency similarities between samples taken during the same year (2004 A5 – Alder Creek sites avg. F_{ST} = 0.071, 2003 A5 – Alder Creek sites avg. F_{ST} = 0.142). Unfortunately, poor success at amplifying samples collected during the 2003 field season has so far precluded evaluation at more than one site.

Tadpole sampling

If tadpole samples represent the reproductive output of a few adults, the sampling scheme may generate significant heterogeneity among sampling sites (Allendorf and Phelps 1981) and may be more sensitive to tests of HWE and linkage disequilibrium. Specifically, heterozygote excess across loci may be the result of sampling tadpoles instead of adults at breeding sites. Both regional and study means of the average number of alleles and mean H_e conducted on tadpoles and adult Columbia spotted frogs overlap (Table 3, Figure 3). Expected heterozygosities for the two studies were nearly identical, with H_e varying from 0.259 to 0.645 in this study, and from 0.23 to 0.70 when sampling adult Columbia spotted frogs (Table 3, Figure 3). When examining tadpole samples across all breeding sites, F_{IS} varied from -0.387 to 0.161, and over all sites was significantly less than zero ($mean = -0.05$, 99% $CI = -0.076$ to -0.023 , Table 3), reflecting a slight excess of heterozygotes. Similarly, mean relatedness per breeding site appears low (average $r = 0.0665$, 95% $CI = 0.037$ to 0.096 , Table 3) for a species with limited dispersal abilities and strong breeding site fidelity. High variation in mean breeding site relatedness both within watersheds and across all sites, suggested that representative samples were successfully collected at most sites ($r = -0.032$ to 0.359). Mean relatedness values mirrored the survey data collected at sites (number of egg masses, tadpoles, juveniles, or adults detected) and apparent site isolation.

Discussion

Overall levels of genetic variation are in accordance with earlier work done on Columbia spotted frogs and other species in the family *Ranidae* (Monsen and Blouin 2003). The low level of mean relatedness and the high variation in relatedness within and across all watersheds suggests tadpole sampling reflects the variation in population sizes within and across watersheds. Tadpole sampling provides several advantages for projects surveying landscape genetics of amphibian species when care is taken to collect a representative sample. Large areas can be surveyed efficiently while avoiding possible biases associated with sex and age biased migration patterns.

However, temporal variation seen in Alder Creek (A5) reflects substantial genetic differentiation between sampling years 2003 and 2004. It is possible that this level of differentiation is amplified due to the relatively small N_e of the site. In very small populations, demographic stochasticity associated with marginal habitats and extreme environmental fluctuations along with genetic drift can produce discontinuities in the distribution of allele frequencies. The sharp contrast in differentiation between 2003 and 2004 samples at this site with the rest of the breeding sites in Alder Creek may be the result of demographic synchrony at either the site or watershed level. Site level annual variation may be developed by small populations, high site fidelity (isolation), an inhospitable matrix, and alternate year breeding of both sexes. Explosive breeders might display allele frequency synchrony across sites at the watershed level. If sites are not normally connected by dispersal, but N_e fluctuates with environmental variables across the watershed then explosive breeding may lead to occasional synchronous episodes of migration when population sizes are large enough. Alternatively, even if demographic synchrony is not present, occasional population explosions at a limited number of sites may be enough to increase migration between sites across the watershed and result in high annual variation.

Comparisons of annual variation between low and high elevation populations would clarify whether females at low and high elevation populations exhibit different lags between breedings. More samples collected at sites over the same two years need to be analyzed to whether synchrony is evident across the watershed, and how strongly annual variation is affected by population size. If similar levels of genetic subdivision are seen multiple sites and synchrony is not evident at the watershed scale, several years of data need to be collected if tadpole samples are going to be used to estimate genetic distances. However, sampling of adults is not easily applied to landscape genetic

questions for amphibians, and may mask synchrony if differences in allele frequencies are generated by sampling breeding and non-breeding individuals.

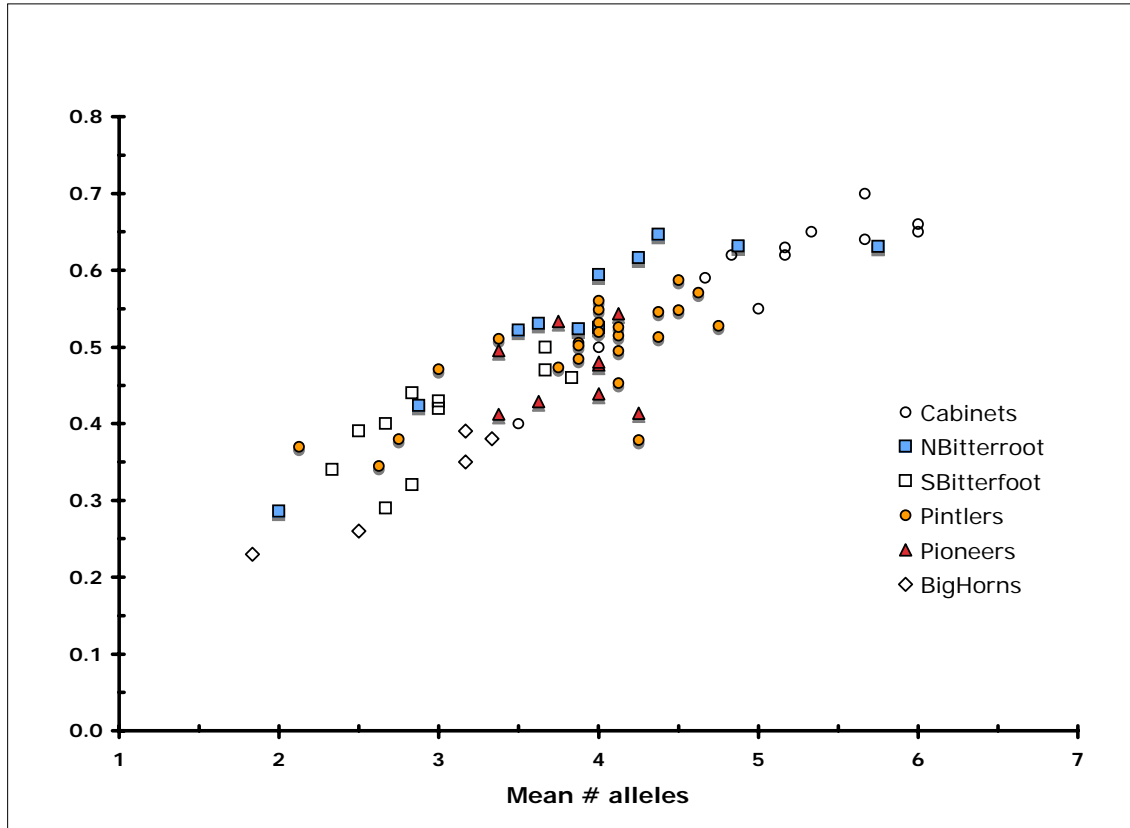
Appendix B, Table 1. Comparison of genetic variation and relatedness when sampling Columbia spotted frog tadpoles and adults: regional comparison of tadpole and adult Columbia spotted frog (CSF) study areas listed by mountain range, average number of alleles (N_a), mean expected heterozygosity (H_e), and watershed and study wide means of F_{ST} and relatedness (r).

	Avg N_a *	Mean H_e	Watershed Average	
			F_{ST} **	r
C. Bitterroot Range (Amish)	3.886	0.539	0.097	0.1343
Anaconda Range (Amish)	3.833	0.493	0.039	0.0471
Pioneer Mountains (Amish)	3.538	0.452	0.046	0.0563
Tadpole Study Mean (Amish)	3.766	0.492	0.066	0.0682
95% CI (+)	3.978	0.541	0.102	0.122
95% CI (-)	3.554	0.444	0.030	0.014
Cabinets & Cour D'Alene Range (Funk)	5.083	0.601	0.067	
S. Bitterroot Range (Funk)	3.000	0.405	0.093	
Bighorn Crag Mountains (Funk)	2.800	0.322	0.149	
Adult Study Mean (Funk)	3.628	0.443	0.067	
95% CI (+)	4.892	0.586	0.109	
95% CI (-)	2.197	0.281	0.019	

* Average number of alleles computed based on 6 microsatellite markers used by Funk plus an additional 2 markers for Amish.

** Average based on pair-wise F_{ST} values within watersheds.

Appendix B, Figure 1. CSF Regional Genetic Variation: mean number of alleles is given on the x-axis, while expected heterozygosity is given on the y-axis. Breeding sites sampled by collecting tissue from adult CSF from Funk et al. 2005 are shown using hollow symbols. Breeding sites sampled by collecting tissue from tadpoles are shown using solid symbols. Legend lists watersheds in order from North to South.



Appendix C

Tests of disequilibrium and intrapopulation structure

Appendix C, Table 1. Summary of per-locus tests and information: the number of significant tests using a $p < 0.05$ without and with sequential Bonferroni correction (SBC), whether the F_{IS} indicated a deficit (-) or excess (+) of heterozygotes, the number of alleles, and the number of populations where a locus was monomorphic.

HWE Summary by Locus with All Sites

Locus	# Signif Heterozygosity			# Monomorphic	# Alleles	# Signif Heterozygosity		
	P<.05	-	+			SBC	-	+
SFC139	17	4	12	0	18	2	0	2
SFC134	5	2	3	1	6	0	0	0
SFC128	7	5	2	2	5	0	0	0
RP3	8	1	7	0	10	2	0	2
RP23	4	2	2	2	6	0	0	0
RP193	9	6	3	1	10	1	1	0
RP17	4	3	1	4	8	0	0	0
RP15	3	1	2	1	9	0	0	0
Total	57					5		

Appendix C, Table 2. Summary of per-site tests and information: the number of significant tests using a $p < 0.05$ without and with sequential Bonferroni correction (SBC), whether the F_{IS} indicated a deficit (-) or excess (+) of heterozygotes, the average F_{IS} across these loci, the sample size (N), and the number of loci indicating an excess or deficit of heterozygotes after SBC.

HWE Summary by Population with All Sites

Pop	# Loci	# Hets .05 sig		Avg F_{IS}	N	# Hets SBC	
		-	+			-	+
11_30	4	2	2	0.122	32	0	0
11_10	3	2	1	0.167	32	1	0
19_03	3	2	1	0.155	31	1	0
24_05B	3	3	0	0.077	23	1	0
24_70	3	0	3	0.557	31	0	2
35_26	3	3	0	-0.279	39	1	0
57_04	3	0	2	-0.113	23	0	1
995_37	3	1	2	-0.054	32	0	1
11_02	2	0	2	0.111	25	0	0
11_12	2	1	1	0.098	25	1	1
35_41	2	1	1	-0.061	29	0	1
35_49	2	0	2	0.398	15	0	0
995_26	2	0	2	-0.052	33	0	1
995_35	2	1	1	-0.147	28	1	0
11_20	1	1	0	0.512	32	1	0
57_03B	1	1	0	0.116	24	1	0
995_80	1	1	0	0.431	32	1	0
Total	40					9	

Appendix C, Table 3. Summary of linkage disequilibrium by loci pair: the two loci in the pair, and the number of significant tests after correcting for multiple tests using sequential Bonferroni correction ($p < 0.05$).

Linkage Disequilibrium Detail by Loci Pair

Locus#1	Locus#2	SBC
SFC139	RP3	15
RP23	RP193	5
SFC134	RP193	3
RP3	RP15	3
RP3	RP193	3
SFC139	RP15	2
RP3	RP23	2
SFC134	RP23	2
SFC128	RP193	2
RP3	RP17	2
SFC139	RP193	2
SFC139	RP23	1
RP23	RP17	1
SFC134	SFC128	1
SFC128	RP15	1
SFC134	RP3	1
SFC139	SFC134	1
RP17	RP15	1
SFC128	RP23	1
RP193	RP15	1
total		50

Appendix C, Table 4. Summary of linkage disequilibrium by population: the number of significant tests without correcting for multiple tests, after correcting for multiple tests using Bonferroni correction, and sequential Bonferroni correction, all using $P < 0.05$.)

Linkage Disequilibrium Detail by Population
Loci Pairs

Pop	P = 0.05	BC	SBC
995_37	20	12	14
24_70	14	7	7
995_35	10	4	4
11_12	8	3	3
11_20	6	3	3
19_03	8	3	3
995_19	5	3	3
11_30	3	2	2
24_05	5	2	2
35_26	4	2	2
35_41	7	2	2
57_04	6	2	2
995_24	6	2	2
995_34	5	2	2
11_02	4	1	1
19_104	3	1	1
19_11	3	1	1
24_05B	2	1	1
24_06	6	1	1
35_34	2	1	1
35_45	3	1	1
35_49	2	1	1
995_28	2	1	1
995_29	6	1	1
995_32	3	1	1
995_33	1	1	1
995_42	6	1	1
11_10	2	0	0
19_07	4	0	0
19_103	2	0	0
19_12	7	0	0
24_03	2	0	0
24_08	3	0	0
24_12	4	0	0
35_31	3	0	0
35_39	2	0	0
35_40	5	0	0
35_51	2	0	0
35_53	3	0	0
57_03B	6	0	0
57_09B	5	0	0
995_11	3	0	0
995_22	2	0	0
995_31	3	0	0
995_80	6	0	0
Total	214	60	64

