DISSERTATION

DISPERSAL LIMITATION AND THE SPATIAL ORGANIZATION OF COMMUNITIES AND POPULATIONS IN ALPINE HEADWATER STREAMS.

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DEBRA SUZANNE FINN ENTITLED DISPERSAL LIMITATION AND THE SPATIAL ORGANIZATION OF COMMUNITIES AND POPULATIONS IN ALPINE HEADWATER STREAMS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF **DISSERTATION**

DISPERSAL LIMITATION AND THE SPATIAL ORGANIZATION OF COMMUNITIES AND POPULATIONS IN ALPINE HEADWATER STREAMS.

The alpine zone encompasses all areas higher in elevation than the permanent tree line and is characterized by harsh climatic conditions and extreme topographic relief. In the Rocky Mountains, permanent alpine streams represent patches of stable aquatic habitat embedded in this relatively harsh matrix. Alpine stream insect communities are isolated from one another by the landscape and by physiological constraints confining them to the upper extent of the river continuum. The objective of my dissertation research was to use these spatially-structured systems to assess the effects of dispersal limitation on different biological levels across a relatively fine spatial scale in and around Rocky Mountain National Park, Colorado. On a more general level, comprehensive studies of alpine streams are lacking, and understanding these systems will be important to help assess the potential impact of climate change and other anthropogenic disturbance.

Using a rapidly-evolving mitochondrial marker, I found evidence of significant geographic population genetic structure in two strictly-alpine black fly (Simuliidae) species: *Prosimulium neomacropyga* and *Metacnephia coloradensis*. The latter is rare, extremely habitat-specific (at productive lake outlets), and has several life history traits related to limited adult flight dispersal. Surprisingly, population genetic subdivision was significantly greater in *P. neomacropyga*, possibly because local populations of *M. coloradensis* are extraordinarily dense, allowing for a greater number of dispersal propagules. Across the study region, grouping the more highly structured *P.*

neomacropyga populations according to "island" of contiguous alpine zone explained a significant proportion of total genetic variation, despite streams on such islands occupying different major watersheds. This structure is probably a signature of historic allopatric fragmentation driven by post-glacial climatic warming. Within alpine islands, I found strong isolation by distance, and geographic distance was highly correlated with more biologically realistic measures of isolation (such as steep ridgelines).

I compared spatial population genetic structure of the more common *P. neomacropyga* to spatial structure of an entire benthic community under the framework of neutral theory, which yields equivalent spatial predictions at the population [genetic] and the community levels, assuming dispersal limitation. A pattern analogous to genetic isolation by distance known as "distance decay" is expected at the community level such that more isolated communities are more different than close ones. I found no evidence for this pattern, despite spatially implicit evidence that communities are dispersal-limited. Ultimately, I reasoned that neutral community theory is a useful heuristic tool, but most real communities probably do not meet all of the assumptions required for a practical application of the theory.

I also made direct observations of the flight activity of the benthic insect community emerging from an alpine headwater reach of the S. Fork Cache la Poudre River in northern Rocky Mountain National Park. Using perpendicular transects of Malaise traps set to a distance of 60 meters in 2002 and 2003, I found that most flight activity was concentrated near the stream and that most flying insects were generalists, as opposed to alpine zone specialists, which may be poorly adapted for dispersal because their habitat is relatively rare and stable. Sex ratios of most taxa were strongly skewed towards the sex that is traditionally more active in flight, suggesting that the more extreme climatic conditions of the alpine zone may intensify these differences. The prevalent westerly winds, however, did not affect the direction of flight activity, possibly because insects do not initiate flight when it is windy. Between years, emergence time varied drastically for most common taxa, probably due to different winter precipitation amounts. 2003 was a very snowy year compared to 2002, and a dense snowpack covered the stream until a much later date, apparently causing much later emergence. Because the emergence season is already short in the alpine zone, these differences have strong implications given continued climate change, under which winter precipitation is expected to increase.

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"Acknowledgments." Don't you think that's a bit of an understatement? To "acknowledge" is the absolute minimal form of recognition. If you are not ignoring someone, then I guess you must be acknowledging their presence. The notion may be that this is a section where, as my dad has profoundly suggested, "you have to thank a bunch of assholes that didn't really do that much for you." I hope I am not the only one around who disagrees. There are so many people that I feel I am indebted to beyond words. Furthermore, anyone who thinks they can get away with writing a dissertation and claiming the right to sole authorship is kidding themselves. The massive work before you was authored by Finn... *et al.* And I propose to unofficially change the name of this section to "Extensions of my deepest and most humble appreciation."

I'll start with my oft-neglected family. We Finns have always been considered a bit "whacked out" (words of a dear ex-roommate). For some reason, we seem to just do what we wanna do, and we don't care much about social norms. My parents are to thank for that, I suppose, and it is with deep gratitude that I do thank them, particularly for never once in my or my two awesome sisters' lives intimating that success be defined in terms of dollars. The best and most fun things that you can do in the world have zero correlation with making a lot of money. "Follow your folly," as they say at a well-known local brewery.

Next on the list is LeRoy Poff, my approachable yet seemingly superhuman adviser through both my MS and PhD adventures. I began as a converted ski bum, and with a little bit of luck and some good grades going in, LeRoy took me under his wing as his first grad student. He gave me the intellectual freedom, with just enough guidance (primarily by way of immense scribbling all over every paper I ever wrote), to do what I wanted to do. He gave me optimism that I could do things on my own, like design research, find money, give talks at meetings, and publish. And for a man who can apparently do it all, he always listened without scorn during the doubtful times. He also introduced me to his figurative extended family – the North American Benthological Society – and I thank NABS also for taking me in and showing me the satisfaction of science as a group endeavor.

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Boris, it's not just me - I hope you understand how much so many people appreciate you. Peter H. Adler, Rich Durfee, and Dave Ruiter were other key taxonomists that revealed unanticipated joy in vials full of dead insects.

Dave Theobald has also been a key influence later in my grad student career. He provided enthusiastic help with GIS, is a co-author on the Chapter 3 manuscript, and is responsible for Figure 3.2.

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There is fun in the interstices of hard work. Thank you Poff lab members, for keeping light-hearted and fun-loving. Jeremy Monroe, Dave Pepin, Julian Olden, Aaron Hoffman, Christine Albano, and Angie Moline: we were multiple-year colleagues and I hope that going separate ways doesn't mean that we're not still in this together. Heidi

Luter was a wonderful and loyal (3 years!!) work/study in the lab, and I thank her, Nora Machuca, and Sudah Balakhani for dedicated bug-picking. Big thanks also to Aaron Flohrs and Adam Valenti, who were undergrad summer field assistants and braved the extremes of the alpine zone for weeks at a time to help keep my main field studies up and running.

To all the rest of my friends, and to the CSU Challenge Course, thank you for keeping me sane and maintaining balance in my life. I hope that will continue. See you in Jackson Hole next year.

I feel like I've been extremely lucky, but maybe my luck was simply in stumbling across the right people in my life. Good teachers, for example, know what they are up to; it is not a matter of luck once you are in their hands. I took a freshman botany class from Dave Steingraeber and was fully converted to Biology then and there. In high school, Bob Reed taught me that writing can be an extension of your very essence, and he helped me realize - in Texas of all places - my true calling to the wild. Even earlier teachers (e.g. June Burt, Gloria Daniels) had profound impacts on my confidence and character. We're talking about public school here. Let us try to treat these people with appreciation and respect.

At the end, as I always did in the field, I thank the streams. Thank you Roaring Fork and Ship's Prow Creek and North Big Thompson and all the rest. Thank you SFP and the "Shangri-La." You've given me what I've asked for. I will do the best I can to repay you.

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CHAPTER 1

INTRODUCTORY COMMENTS

> "With increasing elevation, mountain 'islands' become progressively insular as area decreases and isolation increases. Alpine tundra streams are indeed tiny isolated habitats; although contiguous with downstream reaches, physical and ecological constraints limit colonization by species and populations occurring at lower elevations." J.V. Ward, *Ecology of alpine streams* (1994)

Alpine tundra streams are broadly defined as headwater streams occurring at elevations above the permanent tree line (>ca. 3300 m in northern Colorado). I became interested in studying these habitats for my dissertation research for a variety of reasons that I will attempt to condense here.

Before graduate school, most of the experience I had with alpine streams came from my free time spent recreating in the mountains. A pervasive feature that I encountered above tree line was that even in the summer, ambient conditions were cold and windy; furthermore, a thunderstorm hit nearly every afternoon. Part of what kept me coming back to these conditions was that they seemed to be correlated with an absence of annoying and biting insects (although alpine areas are awe-inspiring in other ways as well). Edward Abbey (1984) noted this condition in the Arctic eloquently: "The breeze continues to blow from downriver, a chill wind off the ice pack ninety miles north, but welcome to us here and now because it keeps the mosquitoes busy. They cling to the brush and weeds when the wind blows, come forth for a blood transfusion only when it stops…"

Later, a big part of my MS research compared longitudinal gradients of invertebrate community structure among four physical similar Rocky Mountain streams. One of the most interesting results revealed a strong pattern of decreasing community similarity among streams with increasing elevation (Finn & Poff 2005). The highest-elevation sample sites were in the alpine zone. This result recalled memories of the lack of annoying insects above tree line. After all, how *do* insects that incorporate a flying adult stage as an essential part of their life history deal with the generally harsh terrestrial conditions of the alpine zone? For my dissertation research, I hypothesized that the combined harsh terrestrial conditions and insularity (*cf.* Ward 1994) of alpine areas leads to a decreased amount of insect flight activity and dispersal compared to lower elevations. Streams, particularly, would be isolated because they are distinct patches of aquatic habitat embedded in a harsh terrestrial matrix. As such, decreased between-stream dispersal may have contributed to the decreased community similarity that I observed among alpine streams compared to those at lower elevations.

To test this hypothesis, I used a variety of approaches ranging from theoretical to empirical and looking at independent as well as potentially linked responses at two levels of biological organization: the population and the community levels. Prior to my research, little was known about dispersal in the alpine zone, although much could be speculated (e.g. Downes 1965, Ward 1994). A few population genetics studies had been done in mountainous regions and suggested that high-elevation drainage divides might cause limited dispersal (e.g. Hughes *et al.* 1999, Monaghan *et al.* 2002), although none focused on species confined to the alpine zone. Furthermore, no one had done direct studies of flight activity and lateral dispersal for alpine stream insects, although a few such lateral dispersal studies had been done in mainly lower-elevation, forested streams (e.g. Kuusela & Huusko 1996, Petersen *et al.* 1999, Briers *et al.* 2002).

Chapters 3 and 4 of my doctoral dissertation report results concerning the population genetic structure of two stream insect species that are alpine specialists. In

Chapter 5, I report on a detailed study on the lateral flight dispersal of the insect community emerging from an alpine stream reach. Chapter 2 compares spatial patterns at the community and the population genetic levels under the theoretical assumption that strong dispersal limitation has similar effects at both levels of organization (based on Hubbell 2001).

Each of the following four chapters have been written as independent entities formatted for journal submission. The order of the chapters reflects the order in which I wrote them; therefore, later chapters may refer to findings from earlier ones. They are otherwise independent and need not be read in the order that they are presented. Because much of the work was collaborative, there are additional coauthors on each; however, while I recognize and greatly appreciate their myriad contributions, I stress that this dissertation is the product of my own creative efforts.

Chapter 2: Finn, D.S. and Poff, N.L. Comparing community and population genetic structure to test spatial predictions of neutral theory in a dispersal-limited alpine stream metacommunity.

Chapter 3: Finn, D.S., Theobald, D.M., Poff, N.L. and Black, W.C. Spatial population genetic structure and limited dispersal in a Rocky Mountain alpine stream insect.

Chapter 4: Finn, D.S. and Adler, P.H. Population genetic structure of a rare high-elevation black fly, *Metacnephia coloradensis*, occupying Colorado lake outlet streams.

Chapter 5: Finn, D.S. and Poff, N.L. Lateral flight activity of insects from a Rocky Mountain alpine stream.

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CHAPTER 2

COMPARING COMMUNITY AND POPULATION GENETIC STRUCTURE TO TEST SPATIAL PREDICTIONS OF NEUTRAL THEORY IN A DISPERSAL-LIMITED ALPINE STREAM METACOMMUNITY

SUMMARY

1. Empirical tests of neutral community theory have been problematic due to the difficulty in distinguishing neutral vs. niche-based explanations for observed patterns. Here I present a novel approach that takes advantage of the mathematical unity between neutral theories at the community and population genetic levels, which propose analogous processes occurring at these two levels of organization. A key expectation is that under neutrality and dispersal limitation, the spatial distribution of species relative frequencies will parallel the distribution of neutrally-evolving allele frequencies from single-species populations occupying shared localities.
2. To test this expectation, I sampled dispersal-limited benthic insect communities of five environmentally similar alpine stream sites within a small region of contiguous alpine zone in Rocky Mountain National Park, Colorado, USA. Neutral population genetic structure was analyzed using a portion of the mitochondrial COI gene in the blackfly species *Prosimulium neomacropyga* (Peterson). Community structure was assessed using species relative frequencies of ecologically similar species in the family Chironomidae collected from the same localities.
3. Species richness was positively correlated with genetic diversity at the locality scale ($r = 0.37$), and analysis of molecular variance (AMOVA) revealed significant spatial structure at both the population genetic ($F_{ST} = 0.23$) and the community ($F_{ST} = 0.16$) levels. A more spatially-explicit comparison of pairwise

community dissimilarity and genetic distance, however, revealed no evidence of correlation (Mantel test $p = 0.54$; $r = -0.18$).

4. Results suggest that both dispersal limitation and unaccounted variance in local environment probably contributed to structuring this metacommunity. I conclude with suggestions for how this newly-tested empirical approach may be applied in future studies.

INTRODUCTION

The unified neutral theory of biodiversity and biogeography (Hubbell 2001) challenges traditional views of niche theory and has generated a great deal of discussion in the literature (e.g. McGill 2003, Nee & Stone 2003, Volkov *et al.* 2003, Harte 2003, Maurer & McGill 2004). The heuristic value of the theory (cf. Chave 2004) has thus become apparent, irrespective of whether or not it universally represents the "real" world. "Neutrality" assumes that individuals of all species in a metacommunity are, on average, competitively equivalent, they have equal realized dispersal abilities, and relative abundances of species fluctuate randomly in local communities having constant total numbers of individuals. "Ecological drift" is the term used for these random fluctuations and is analogous to genetic drift at neutral molecular loci within local populations. The ecological equivalence assumption is critical to the idea of drift and implies negligible effects of biotic interaction and niche partitioning. This assumption is realized by defining a community as only including those species using the same food resource and by inferring that if species co-occur, then they are, on average, selectively equivalent (Hubbell 2001, 2005). Random ecological drift combined with dispersal limitation leads to the further expectation of "distance decay," which refers to an increasing dissimilarity of local communities with greater geographical distance (isolation) separating them.

To date, most approaches to "testing" Hubbell's neutral theory have focused on one of two primary predictions. One key theoretical and empirical focus has been on the predicted species-abundance distribution (e.g. McGill 2003, Volkov *et al.* 2003, Poulin 2004), a lognormal-like distribution with a negative skew driven by more rare and fewer abundant species. By contrast, some studies have focused on the distance decay prediction (e.g. Condit *et al.* 2002, Chave & Leigh 2002). Unfortunately, both approaches have practical problems associated with empirical hypothesis testing. Specifically, many non-neutral community theories predict very similar species-abundance distributions that are unlikely to be differentiated statistically using observational data (Harte 2003, Nee & Stone 2003, Chave 2004, Maurer & McGill 2004). Distance decay can also typically be explained under non-neutral, niche-based concepts: more distant local sites also tend to be more environmentally different (Nekola & White 1999, Condit *et al.* 2002, Chave 2004; but see Gilbert & Lechowicz 2004). In this paper, I present a novel approach to testing spatial predictions of neutral community theory that avoids these common problems.

Linking population and community patterns under neutral theory

It is widely recognized that Hubbell's (2001) neutral theory of biodiversity has the same mathematical basis as the neutral theory of molecular evolution (Kimura 1968, 1983). There are thus several analogies between the population and community levels of biological organization under neutrality (Chave & Leigh 2002, Etienne & Olff 2004, see also Nee 2005). Different alleles of a gene within a population are analogous to different species of individuals within a community; mutation is analogous to speciation in creating new forms of these entities; and random genetic drift is analogous to ecological drift. Spatially, a metapopulation can be a single-species subset of a metacommunity occupying the same local sites across a region. It therefore follows that the more spatially-explicit expectations of genetic isolation by distance (Wright 1943) and community-level distance decay are also analogous between levels.

In this paper, I follow recent metacommunity terminology (Leibold *et al.* 2004) and define a "locality" as a habitat patch that is capable of holding a local community and/or population, "dispersal" as the movement of alleles and/or individuals between localities, a "metapopulation" as a set of local populations (single species) that are linked by dispersal, a "metacommunity" as a set of local communities (multiple co-occurring species) that are linked by dispersal, and a "region" as a larger area containing multiple localities and capable of holding a metacommunity/metapopulation.

Given the mathematical unity of neutral theories at the community and population levels, the spatial pattern of species distribution across a neutrally-assembled metacommunity is expected to parallel the spatial pattern of neutrally-evolving alleles across a metapopulation occupying the same localities, given that dispersal is limited to the same extent at both hierarchical levels. Indeed, it has been proposed that genetically diverse populations should occur in the same localities as very speciose communities and vice-versa (Vellend 2003, 2004, Vellend & Geber 2005, Etienne & Olff 2004) and that a demonstration of a positive correlation between genetic and species diversity would provide evidence in support of Hubbell's (2001) theory (Etienne & Olff 2004). On a more spatially-explicit level, pairwise patterns of neutral community dissimilarity between localities ought to parallel patterns of neutral genetic distance. Moritz (2002), for example, proposed that major regions of biogeographic vicariance may be revealed for conservation purposes by concordant patterns at the community and population genetic levels across the landscape. Effectively, pairs of sites that have been more isolated from one another should show both greater distance decay (community-level) and stronger isolation by distance (population level).

Some of the aforementioned studies (Vellend 2003, 2004, Moritz 2002) are among only a handful thus far that have addressed the question of concordant spatial patterns between the community and the population genetic levels, and they have produced conflicting results. Importantly though, none of these studies was designed explicitly as a test of neutral theory predictions, and communities were not chosen as per Hubbell (2001) to be comprised only of ecologically similar species sharing the same trophic resource. Additionally, environmental conditions among localities were variable, thereby influencing local community structure. A more explicit test of neutral spatial concordance between the two levels of biological organization would include a stricter community definition, and localities would have more similar environments. Under these stricter conditions, a positive correlation between both genetic and species diversity among localities and between community-level distance decay and population-level isolation by distance would provide some support for neutral community processes in the metacommunity under study. According to neutral theory, these spatial patterns would be driven primarily by dispersal limitation.

Dispersal limitation and neutral theory

Dispersal is important in all types of metacommunity theory, both neutral and non-neutral, due to the spatial structure of metacommunities (reviewed in Leibold *et al.* 2004). The patch dynamics framework, for example, is a metacommunity concept in which between-locality dispersal ability counteracts local competitive exclusion. Similarly, under neutral theory, in the absence of dispersal into local communities (or local speciation across very long time periods), ecological drift would eventually lead to random “fixation” of a single species. As such, there are only two major influences (outside of locality size) on local diversity under neutral theory: metacommunity diversity (i.e., regional species pool) and species dispersal rate (Hubbell 2001, Maurer & McGill 2004, Etienne 2005).

Dispersal is arguably the most important aspect of Hubbell's theory; indeed, it is often termed a "dispersal-assembly" (as opposed to "niche-assembly") theory. Increased dispersal increases spatial autocorrelation (Bell 2001) and increases the proportion of the regional species pool that is present in each locality. Hence, high beta and low alpha diversities are expected in low-dispersal metacommunities and vice-versa. Dispersal limitation therefore drives the distance decay effect (analogously to dispersal limitation driving genetic isolation by distance at the population genetic level, Wright 1943). A low-dispersal metacommunity is therefore an ideal one look for the regional-scale patterns predicted by neutral theory. Here, stronger patterns of distance decay will be detectable at a finer spatial scale of resolution, and differences in community structure due to long environmental gradients will be minimized.

Study system

I used alpine stream insect communities in northern Rocky Mountain National Park (RMNP), Colorado, USA to test spatial predictions of neutral theory at the community and population levels. Occurring above the permanent treeline, alpine streams are typically small headwaters and island-like with respect to their primary inhabitants, insects (Ward 1994). Typically, even neighboring streams occur in different major drainage basins, and because the range of many resident species is limited to the cold, higher-elevation reaches, between-stream dispersal occurs only by flight of the adult stage that emerges during the brief summer growing season.

I hypothesized that these communities are dispersal-limited for three main reasons. First, aquatic insects in general have been shown to concentrate most activity in the vicinity of their emergence location (e.g. Griffith, Barrows & Perry 1998, Petersen 1999, Briers, Cariss & Gee 2002). Second, the alpine terrestrial environment is characterized by many features that negatively affect insect flight, including low air temperatures and high winds (Choe *et al.* 1984, Roberts & Irving-Bell 1996, Briers, Cariss & Gee 2003, D.S. Finn unpublished data), as well as significant topographical relief (Hughes *et al.* 1999, Wishart & Hughes 2003). Finally, the alpine stream metacommunity in the study area has relatively low alpha and high beta diversity (Finn & Poff 2005), which is suggestive of dispersal limitation under neutral theory (Hubbell 2001).

METHODS

Benthic insect communities of five first-order streams occurring within a 30 km^2 region of contiguous alpine zone were quantitatively sampled by taking three replicate Surber samples (900 cm^2 of stream bed) per locality once each August in 2000-2003. Sample sites occurred within an altitudinal range of 3355-3470 m a.s.l., and although they were minimally geographically distant, in most cases streams were separated by steep drainage divides extending up to 600 vertical meters above the streams (Fig. 2.1). At each site, samples were taken only in areas of loosely-organized rocky substrate and low local channel slope, thereby minimizing environmental heterogeneity among sample sites. Samples were then preserved in 70% ethanol and separated and identified in the lab.

Due to the high functional diversity among benthic species in this area (Finn & Poff 2005) and to taxonomic uncertainty for some insect groups, I used only members of the family Chironomidae (dipteran "midges") in community-level analyses. Alpine stream Chironomidae are predominantly classified as "collector/gatherers" (*sensu* Merritt & Cummins 1996), a term reserved for generalist feeders that consume a variety of small particulate resources (primarily detritus) found on the streambed and in the water column. Although some chironomids can be classified into different trophic categories (e.g. Subfamily Tanypodinae are predators), these were found in minimal abundance in the current collections and were left out of the analysis. The community was thus ecologically similar, as per Hubbell (2001), who has also suggested that generalist feeders may be the most appropriate type of community in which to look for evidence of neutral assembly. The Chironomidae were also a taxonomically diverse group at my sites, and all individuals were identifiable to the species level.

The representative species used for population genetics was another dipteran, the blackfly (Simuliidae) *Prosimulium neomacropyga* (Peterson). Although this species occupies a different family than the representative community, there are several reasons to use it for this analysis. First, it is one of very few species that was found across the five localities. Second, *P. neomacropyga* is unusual among the blackflies in that the females are obligately autogenous (mouthparts incapable of taking a blood meal), an important quality that makes this species more similar in dispersal behavior and potential to the Chironomidae assemblage than to other species in its own family, which may disperse long distances in search of a blood meal. Flight dispersal barriers can reasonably be assumed to be the same for *P. neomacropyga* as for members of the

Chironomidae; therefore, spatial patterns driven by dispersal limitation are also expected to be similar among these taxa. Third, a simuliid species is a good option for molecular work because methodology is well-documented for this family, which has been the subject of extensive research due to the human impact of some species as disease vectors and pests.

In August 2003, 50 individuals of *P. neomacropyga* were collected from each of the five sample streams and preserved in 70% ethanol for DNA isolation, which was accomplished by a basic salt extraction method (Black & DuTeau 1997). Polymerase chain reaction (PCR) was used to amplify a 307-bp section of the 3' end of the mitochondrial COI gene using primers UEA9 and UEA10 listed by Lunt *et al.* (1996). This region of the genome was chosen for its simple (nonrecombining) haploid inheritance and rapid rate of neutral evolution, which were necessary due to the close geographical proximity of the study populations. Haplotypes (haploid alleles) were assigned to all sampled individuals according to sequence variation.

I tested for positive correlation between local species and genetic diversity (cf. Vellend 2003) by comparing average chironomid species richness (across sample years) to haplotype richness at the *P. neomacropyga* mitochondrial marker. Because there were only five local sample sites in the region used for the current study, two additional sites were included in the correlation analysis to increase statistical power. Both added localities occupy an alpine region ca. 20 km south of the sites described and were sampled using the same protocols and taxa as described above.

To test for concordance between patterns of distance decay (at the community level) and isolation by distance (at the population level), I first used analysis of molecular variance (AMOVA) in the Arlequin software package (Schneider, Roessli & Excoffier 2000) to estimate F_{ST}, a measure of total partitioning of variance among vs. within the five localities in the region. The inputs to AMOVA were haplotype frequencies for the population-level and relative species frequencies (averaged across the four sample years) for the community-level analyses. These overall F_{ST} values measure the degree and significance of spatial structuring across the region (beta diversity). Arlequin was also used to calculate Slatkin's linearized F_{ST} (Slatkin 1995) for all pairs of sites, which effectively measured genetic distance at the population level and dissimilarity at the community level between each pair of localities. Finally, a Mantel test was used to measure the linear correlation between these two distance matrices. A significant correlation would suggest concordant population and community structures across the region.

RESULTS

Overall, I found 20 species in the Chironomidae community and 13 haplotypes of the mitochondrial COI marker in *P. neomacropyga* populations across the five localities. Local species richness varied from 9-14 (mean 13.2), and genetic diversity varied from 3-6 haplotypes (mean 5.2) per locality. Local species richness and genetic diversity were positively correlated, although not significantly ($r = 0.37$; $p = 0.42$; Fig. 2.2).

AMOVA suggested a significant degree of spatial structuring (beta diversity) both at the community ($F_{ST} = 0.16$; $p < 0.0001$) and the population ($F_{ST} = 0.23$; $p < 0.0001$) levels at the regional scale. The spatial pattern of haplotype frequencies among local populations was qualitatively interpretable under neutral molecular evolution theory given the most obvious dispersal barriers (high ridges, Fig. 2.1). There was no correlation, however, between the distance matrices for population and community-level Slatkin's pairwise linear F_{ST} values ($r = -0.18$; Mantel $p = 0.54$; Fig. 2.3). Therefore, there was no evidence for spatial concordance of patterns of population-level isolation by distance and community-level dissimilarity.

DISCUSSION

Results demonstrated some general spatial patterns expected under neutral community theory in the RMNP alpine stream metacommunity. Genetic diversity and species richness were positively correlated across localities, and high beta diversity was confirmed at both the population and community levels by the detection of significant regional spatial structure using AMOVA. However, there was no evidence for concordance between community-level distance decay and population-level isolation by distance.

Given that dispersal-limited alpine streams may be "ideal" communities for the detection of neutral spatial pattern, an important question to address is: why the lack of evidence for community-level neutrality at the more spatially-explicit level? The answer is likely to fall in either or both of the following categories, which I discuss in detail below: 1) this novel approach to test for spatial patterns expected under neutral theory had drawbacks that did not allow detection even if communities were in fact assembled neutrally; 2) neutral theory is not sufficient to explain spatial pattern in these communities (i.e., selective pressures outweigh neutral effects).

Potential drawbacks of current approach

My method was based on the mathematical unity of neutral theories at the community and population genetic levels and the corresponding analogous entities (species and alleles), patterns (e.g. distance decay and isolation by distance), and processes (e.g. speciation and mutation) at the two levels. Despite the direct theoretical link, however, there are biological differences between levels that may cause the two neutral theories to be incompatible under some circumstances (see also Nee 2005). In particular, the temporal scale of two key processes may be quite different at the population and community levels of organization.

First, the mutation/speciation analogy may be problematic. Speciation is typically a slower process than mutation by orders of magnitude. This may be especially relevant in the current study system, where I chose a mitochondrial marker that was known to evolve quite rapidly (i.e. point mutations appear frequently within local populations) for greater power of resolution at the fine spatial scale. Hence, many haplotypes may have arisen *in situ* at individual sites, but new species did not arise this way in the respective communities, certainly not within an ecologically meaningful time period. Thus, in this alpine stream region, dispersal limitation alone would be expected to be the primary influence driving neutral patterns at the community level, while both dispersal limitation and local mutation would contribute to neutral patterns at the population genetic level.

Second, genetic drift and ecological (community) drift probably occur on very different time scales that may preclude uncovering neutral community patterns. Turnover in local community composition is often rapid, occurring on the order of species' generation times, whereas population genetic drift is usually a slower process, especially for large population sizes. Indeed, in my study streams, community structure did not remain constant across the four years of replicate sampling, as can be seen in a graphical representation of how sites by sampling years fall out according to the first two axes of a correspondence analysis (Fig. 2.4). Over this short time period, local communities changed perceptibly (although they occupied the same general area of multivariate space through time). Rapid community change is akin to rapid rate of molecular evolution at the population level. Characters that evolve too rapidly are unreliable in uncovering patterns of molecular evolution because they are more likely to be homoplastic (i.e. identical characters in two populations are likely to have arisen by chance rather than by common ancestry; Swofford *et al.* 1996). Analogously, if community-level drift is too rapid, then predictable patterns of distance decay may be obscured, even if changes in community structure are indeed completely random (i.e. truly undergoing ecological "drift").

Selective pressures on community structure

In addition to the many previously-discussed analogies between the population and community levels, selection is also an analogous process that refers to non-neutral influences that favor some species/alleles over others (cf. Vellend & Geber 2005). A second potential explanation for the inability of the current approach to detect concordance of population- and community-level spatial patterns, then, is simply that local selective pressures may have been more important at the community than the population level (i.e. population differences may have been primarily neutrally-driven, given the use of a neutral molecular marker, but communities were not assembled neutrally). Certainly the most heated debate regarding neutral community theory to date has concerned the "ecological equivalence" assumption (e.g. Chave & Leigh 2002, Chave 2004, Maurer & McGill 2004, Hubbell 2005), which seems to ignore common ecological knowledge about differences between species. A more important issue, however, is whether such differences are strong enough to have a significant effect on community structure (cf. Hubbell 2001, 2005). Could differences among the "ecologically-equivalent" species in the Chironomidae community have been strong enough to allow selective pressures to outweigh neutral effects in determining community structure, thereby disrupting spatial patterns expected under dispersal limitation? To address this, I briefly discuss how these species may differ with regard to biotic interactions, dispersal behavior, and response to environmental conditions.

Biotic interactions (such as competition and predation) occur in aquatic insect communities, just as in any other type of assemblage; however, it is generally agreed that such interactions do not strongly structure whole stream communities, especially when food resources are not limiting (Allan 1995). Due to the minimal apparent effects of biotic interaction, neutral assembly has in fact already been suggested in some aquatic insect communities. McPeek & Brown (2000), for example, invoked neutral theory to explain the remarkable diversity of damselflies that occur within individual eastern North American lakes, despite a lack of ecological differentiation among species. Similarly, among the ecologically similar, detritivorous Chironomidae at my study sites, it is also unlikely that competition is strong enough to have controlled community structure.

Large dispersal differences among species may influence the outcome of the simple relationship between species richness and genetic diversity (Vellend 2003) and thus may also alter the spatial patterns of distance decay expected under neutral theory. High dispersal by some species between localities will potentially overcome random effects of drift. Ecological differences in dispersal ability among the species of the alpine Chironomidae community are little-understood; however, past distributional studies using a variety of arthropod taxa suggest that dispersal barriers likely act similarly for many species, regardless of potential dispersal differences (Ferrier *et al.* 1999, Bouchard & Brooks 2004). Bouchard & Brooks (2004), for example, found that both winged and flightless insects showed a similar pattern of population divergence and speciation rates among localities in Queensland. These results suggest that all species, regardless of dispersal potential, still tended to move very little across areas of inhospitable habitat and further imply that slight differences in dispersal ability that may exist among Chironomidae species are unlikely to have a significant influence on community structure in isolated alpine streams.

A final potential difference among chironomid species that could weaken the spatial pattern expected under neutral theory is their selective response to local environmental conditions. In streams, it is widely accepted that many local habitat conditions act as strong “filters” of the regional species pool (e.g. Poff 1997). For small headwater streams in particular, it is possible that even slight differences in local environment can drive noticeable differences in community structure because these streams are more intimately connected with and therefore respond more sensitively to local environmental conditions (Lowe & Likens 2005). Although I controlled for some important local conditions (e.g. altitude, slope, stream size), localities in this study likely varied to some extent with respect to other potentially important habitat characteristics (e.g. temperature regime, organic matter, riparian vegetation). Some of these characteristics have previously been shown to have a detectable influence on stream communities in this area, although their influence was more apparent in lower-elevation (sub-alpine) streams more likely to be connected by dispersal (Finn & Poff 2005, D.S.

Finn unpublished data). Variation in species responses to local environmental conditions may therefore represent important non-neutral selective pressures occurring in RMNP alpine stream communities. These local selective pressures also are likely to disrupt patterns expected under strictly neutral dispersal assembly because differences in local environment in the study area are not correlated with geographic distance.

Synthesis and future directions

I have presented a novel approach to testing for neutral patterns at the community level that avoids the most common pitfalls of past approaches (see Introduction). The approach was based on the directly analogous neutral theories at the community and population genetic levels and their concordant predictions of regional spatial pattern of allele/species frequency distributions under dispersal limitation. Results supported the more general, non-spatially-explicit predictions of significant beta diversity at both levels and a positive correlation between local genetic diversity and species richness. However, the more spatially-explicit expectation of concordance between patterns of community-level distance decay and population-level isolation by distance was not met. Because this was a first empirical attempt at this type of spatially-explicit empirical approach (but see Chave and Leigh 2002 for a spatially-explicit theoretical approach), I offer a few thoughts on how some problematic issues could be targeted and more easily resolved in future studies of this type.

In this study, ecological (community) drift probably occurred much more rapidly (see Fig. 2.4) than genetic drift. If communities change so rapidly that spatial patterns of distance decay break down, this approach may be more appropriate for use in communities that are temporally more stable, such as the tropical tree communities that influenced Hubbell's thinking about neutral assembly processes. Using more temporally stable communities across a broader spatial scale may also enable the use of more conservative molecular markers, which could ameliorate the potential problem created by differences in mutation and speciation rates. Further, spatially-explicit simulation modeling of communities/populations with various rates of random drift and speciation/mutation could be employed to identify the types of systems in which my approach would be most valuable.

Another potential problem with my study system was that local environmental differences may have acted as important selective forces that induced variation in community structure. Such confounding of selective pressures acting concurrently with neutral effects of dispersal limitation is likely to be the case in almost any ecological system (cf. Gilbert & Lechowicz 2004, Leibold *et al.* 2004) and may be a general cause for failure of either neutral or niche theory alone to explain community structure in many cases. Because apparently even very small differences in local environment in the current study system may have an influence on local community structure, an ideal model would partition the variance caused by the combination of all forces, neutral and niche-based. In fact, some past studies have begun to approach this problem using a variety of statistical tests, including partial Mantel tests and canonical ordination techniques, to separate influences of space and local environment (e.g., Borcard, Legendre & Drapeau 1992, Gilbert & Lechowicz 2004).

Still, neutral theory is the simplest of community-assembly models, and its direct link to neutral theory at the population genetic level provides a strong starting point to identifying the relative contribution of various other influences on community structure.

Further, looking for parallels between spatial patterns (neutral or otherwise) at community and population levels may be a promising approach to understanding the interplay between various ecological influences (cf. Vellend & Geber 2005). For example, in my alpine stream communities a next step may be to compare local species richness with *non*-neutral genetic variation (e.g., quantitative genetic traits) for representative species. A positive correlation between species richness and non-neutral genetic diversity is often an indicator of parallel effects of local environmental differences at both levels (Vellend & Geber 2005); therefore, such comparisons may provide insight into the relative influences of local selective pressures vs. neutral effects in this metacommunity.

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Figure 2.1 Schematic map of alpine study sites in northern RMNP, Colorado. Inverted V's indicate major ridgelines that extend 400-600 m above stream elevation. Streams were sampled at the approximate locations of the pie graphs. Pies represent genetic structure at each location, with different shades signifying unique haplotypes. Individual haplotypes representing less than 5% of total genetic diversity were lumped into the white pie slices.

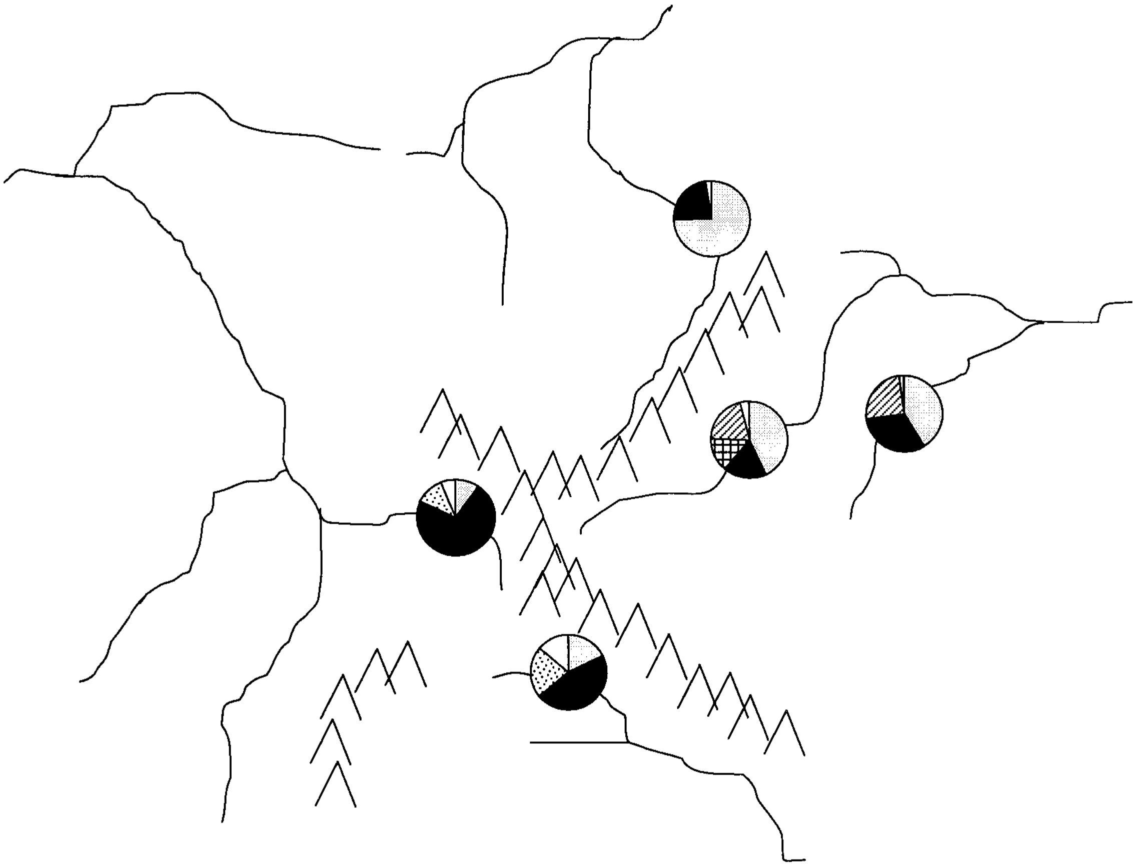


Figure 2.2 Positive relationship between *P. neomacropyga* mitochondrial haplotype richness and chironomid species richness at seven alpine stream localities (r = 0.37; p = 0.42). Note that 2 localities additional to the 5 main study sites have been added to the correlation and are noted by small dashed circles (see text for explanation).

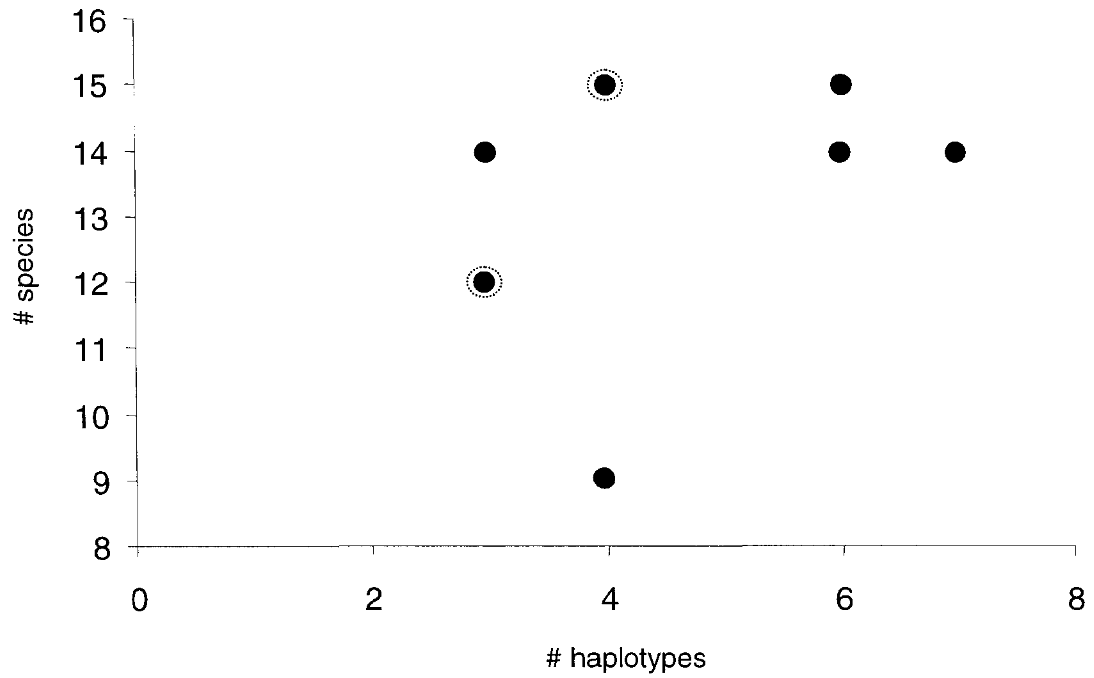


Figure 2.3 Plot of population genetic distance and community distance between each of the 10 pairs of alpine stream localities in the study region. Both distances were calculated as Slatkin's linear F_{ST}. There is no correlation between the population- and community-level distances ($r = -0.18$; Mantel test $p = 0.54$).

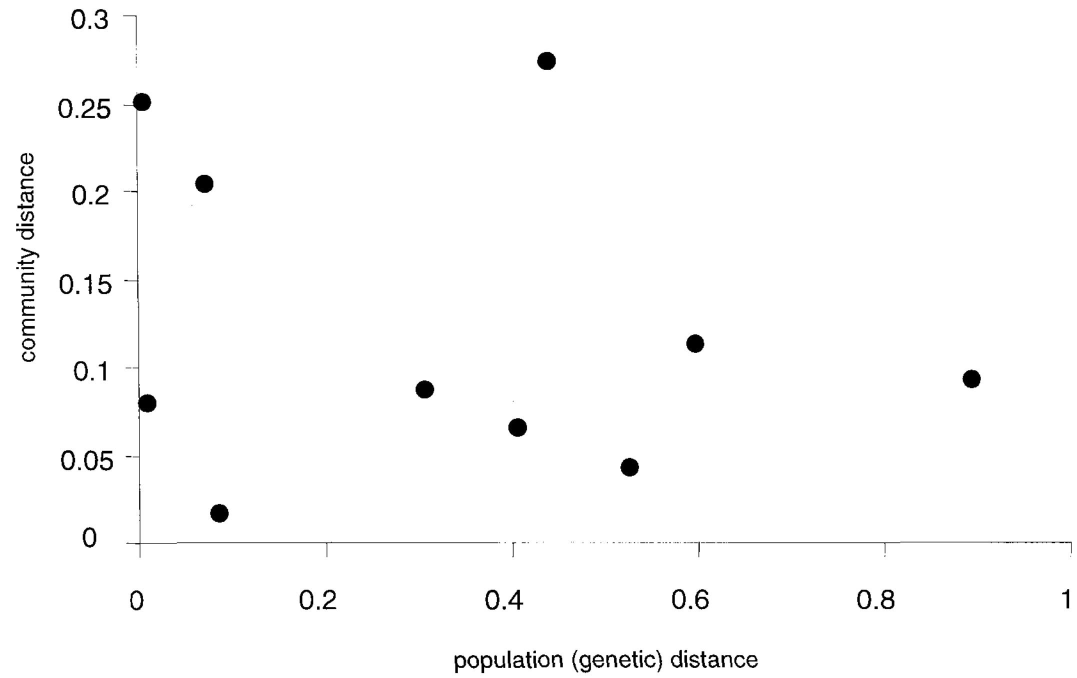
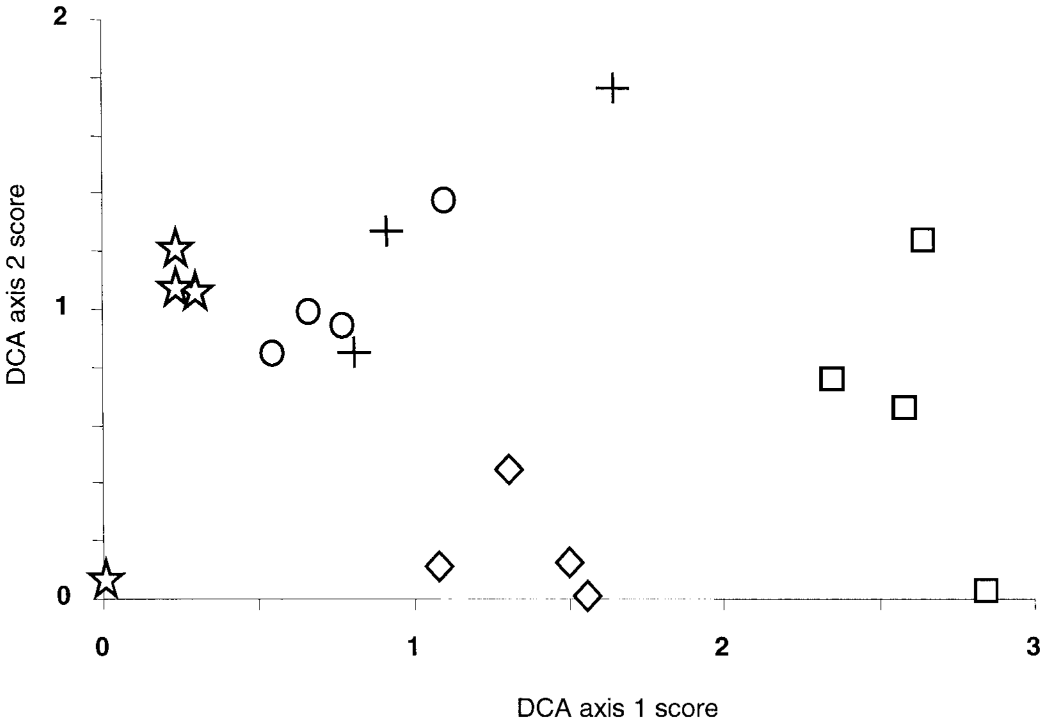


Figure 2.4 Plot of the five alpine stream sites with respect to axes 1 and 2 scores from a detrended correspondence analysis (DCA) using CANOCO 4 (ter Braak and Smilauer 1998). Each of the five sites is represented by a unique symbol; replicate symbols indicate different sampling years (2000-2003) for the same sites. Total numbers of alpine stream insects collected across three Surber samples per year were used as input, with no data transformation.



CHAPTER 3

SPATIAL POPULATION GENETIC STRUCTURE AND LIMITED DISPERSAL IN A ROCKY MOUNTAIN ALPINE STREAM INSECT

ABSTRACT

Using a rapidly-evolving fragment of the mitochondrial COI gene I assessed the phylogeographic structure of *Prosimulium neomacropyga*, a black fly (Simuliidae) that occurs only in alpine tundra streams at the southernmost extent of its range in Colorado, USA. Given high habitat specificity, lack of hydrological connectivity between streams, and the harsh alpine environment, I hypothesized limited gene flow. A spatially nested sampling design showed that grouping populations according to high-elevation "islands" of alpine tundra (which typically include headwater streams of >1 watershed) explained a significant proportion of genetic variation while grouping streams according to major watershed (across islands) did not. Within an individual alpine island, there was strong evidence for isolation by distance (IBD) using each of four increasingly complex alternative models of pairwise landscape connectivity for flying insects. Results of all landscape models were positively correlated, suggesting that straight-line distance is an acceptable surrogate for more biologically meaningful connectivity measures in this system. IBD was significantly weaker across the entire study area, comprised of three separate islands. These results and molecular clock estimates suggested higher gene flow during the last major glacial advance of the Pleistocene followed by allopatric fragmentation on mountaintop islands associated with climatic warming. Overall, population structure was significant with a large F_{ST} (0.38), suggesting strong dispersal limitation for an alpine stream insect across a small spatial extent.

INTRODUCTION

Movement of animals across the landscape is an important but oft-overlooked process influencing population (e.g. Wright 1940, Macdonald & Johnson 2001) and community (e.g. MacArthur & Wilson 1967, Palmer *et al.* 1996, Hubbell 2001) dynamics. Understanding animal movement should therefore be an important goal for both basic ecology and conservation biology. However, two important obstacles to reaching this goal have been 1) landscapes are naturally heterogeneous, different elements having different and sometimes unpredicted effects on movement (Moilanen and Hanski 1998, Roland *et al.* 2000, Wiens 2001); 2) animal movement patterns are difficult to quantify by direct observation, especially across long distances (Slatkin 1985, Turchin 1998). If the movement of organisms across the landscape also results in gene flow, however, then analysis of spatial population genetic structure can be a robust method to address both these issues (Bohonak 1999). Relative levels of dispersal within and among different spatially-structured groups of populations can be compared using selectively neutral genetic markers, and components of the landscape that impede dispersal can be identified using phylogeographic principles (cf. Avise 2000).

Stream-dwelling species present an ideal opportunity for assessing spatial genetic structure because streams are clearly-defined habitat patches embedded in an uninhabitable terrestrial matrix. Distinct populations are therefore easy to identify in this spatial context. Many stream species are insects that spend the majority of their lives as fully-aquatic juveniles and then emerge as mature, often winged adults that mate and lay eggs back into a stream. Studies of population genetic structure of stream insects have often suggested that adult flight dispersal between drainages is relatively common due to a lack of broad-scale genetic differentiation (e.g., Schmidt *et al.* 1995, Bunn & Hughes 1997, Hughes *et al.* 1998, 2000, Baker *et al.* 2003), and stream invertebrates have generally been assumed to have the ability to disperse widely in flight (but see Bohonak & Jenkins 2003). Exceptions to this generalization have been found in some mountain stream insect species, where dispersal may be limited between watersheds due to steep drainage divides (Hughes *et al.* 1999, Monaghan *et al.* 2002, Wishart & Hughes 2001, 2003, Hughes *et al.* 2003b), and further population differentiation may result when a species specializes on a rare and stable instream habitat type thereby increasing the risk associated with dispersal (Wishart & Hughes 2001, 2003; also see Roff 1990). We therefore may expect lower levels of gene flow among stream insect populations when 1) there are strong dispersal barriers between them, and/or 2) the species of interest occupies a larval habitat that is spatially rare along the stream continuum.

In the highest-altitude areas of the Southern Rockies ecoregion of the U.S. (Omernik 1987), streams originate in the alpine ecological zone, which occurs above permanent treeline (>ca. 3300 m a.s.l. in northern Colorado). Alpine streams in this area have a distinct assemblage of insect species, some of which are confined to these highest-elevation reaches (Allan 1975, Ward 1994, Finn & Poff 2005). It is likely that these alpine specialists were more widespread during Pleistocene glaciations (ca. 10,000-100,000 years ago), when the spatial extent of the alpine zone would have been much more extensive than it is at present, consisting now only of small "islands" of alpine tundra in a "sea" of trees (Elias 1996, DeChaine & Martin 2004). As the climate gradually warmed, species adapted to tundra conditions either left lower latitudes completely in favor of Arctic regions or were forced to the highest peaks where the tundra still remains. For some terrestrial insect species such remnant alpine populations have been shown to have a high level of genetic structure, suggesting isolation between alpine islands (e.g. Knowles 2001, DeChaine & Martin 2004). Such analyses have not been done on alpine stream insects, which provide a particularly interesting case in that even streams occupying a single alpine island will often occupy different major watersheds, the boundaries of which have been shown in many cases to be important dispersal barriers (e.g. Hughes *et al.* 1999, others cited above).

An important feature of alpine areas is the extreme spatial heterogeneity in terrestrial microclimates, driven by high topographic relief (Bowman 2001). Such heterogeneity may have significant effects on dispersing animals, such that the straight-line distance between two points is not likely to be a biologically realistic route. Several studies outside of the alpine zone have used high resolution geographic information systems (GIS) to model important landscape features and predict animal movement (e.g. Schippers *et al.* 1996, Moilanen & Hanski 1998). More recently, similar models have been used in conjunction with spatial population genetic patterns to address various questions about dispersal pathways (Michels *et al.* 2001, Coulon *et al.* 2004, Spear *et al.* 2005). A key assumption of these studies is that physically closer populations will exchange migrants more often than more distant ones; therefore, there will be a positive correlation between genetic distance and physical distance. This pattern is referred to as genetic "isolation by distance" (IBD), and the more biologically realistic the measure of physical distance, the tighter the expected fit between these two variables.

For stream insects, some studies have tested for IBD using both straight-line and stream-course distances to reveal whether adult vs. larval dispersal is more important in generating genetic patterns (e.g. Schultheis *et al.* 2002, Hughes *et al.* 2003b); however, to date none has attempted to test more biologically realistic measures of flight dispersal across the terrestrial landscape. The rapidly-developing field of population genetics in stream ecology is increasing our understanding of broad-scale influences on insect dispersal; therefore, testing alternative models of landscape connectivity is an essential next step.

For this study, I addressed several questions regarding gene flow and population genetic structure of alpine stream insects using a representative black fly species (Simuliidae: *Prosimulium neomacropyga* Peterson) that occurs only in streams well above treeline in the Southern Rockies (Adler *et al.* 2004). Within these environmental limits, *P. neomacropyga* is relatively common, occupying several headwater streams on multiple alpine islands in my study area. However, given potentially strong dispersal barriers between populations in the form of mountainous topography, in addition to the confinement of this species to a rare habitat type (the uppermost extent of the longitudinal stream continuum), I hypothesized that gene flow would be limited.

Beyond assessing the overall spatial genetic structure, I asked whether the most important dispersal barriers were major drainage divides (as suggested in previous genetic studies of mountain stream insects) or lowlands separating islands of alpine tundra (as suggested in studies of alpine terrestrial insects). Further, at the finer scale of a single alpine island containing multiple headwater populations of *P. neomacropyga*, I developed several biologically realistic alternative models of dispersal connectivity given various elements of the terrestrial landscape. I then confronted each model with genetic data to assess which terrestrial features were most influential in determining spatial population structure, given an IBD assumption.

Because watershed divides often have been shown to limit gene flow in stream insects, I predicted that the particularly steep and high-elevation divides in the study region would be important dispersal barriers at the within-island scale. However, since *P. neomacropyga* is limited to the highest headwater streams, I hypothesized that there would be limited gene flow among alpine islands as well, even between streams on different islands located within the same major watershed.

MATERIALS AND METHODS

Study organism

P. neomacropyga is distributed extensively in Alaska and the Yukon but is patchily distributed as far south as Colorado, where it is limited to alpine streams. This species is unusual among black flies in that it is obligately autogenous (i.e., mouthparts are incapable of piercing flesh; females do not need a bloodmeal for egg maturation). Long-distance flights in search of blood are thus unnecessary, and plant nectar may be obtained by adults as an energy source. *P. neomacropyga* is univoltine, overwintering as eggs and emerging as adults from mid-August to early September in Colorado. This narrow window for adult emergence and breeding probably prevents temporal reproductive isolation among populations (see West & Black 1998). Little is known about mating behavior, and therefore little has also been presumed about between-stream dispersal. This species is, however, closely related to other alpine/arctic species within the *Prosimulium macropyga* group, some of which have evolved reproductive strategies that avoid leaving the vicinity of the natal stream presumably due to the harsh terrestrial environment (Downes 1965).

Study sites and data collection

I collected *P. neomacropyga* from 11 alpine streams just east of the continental divide in the RMNP area, Colorado (Fig. 3.1a). The alpine zone here extends from ca. 3300-4200 m a.s.l., and much of the area below treeline is coniferous forest, with *Pinus*, *Picea*, *Abies*, and *Pseudotsuga* species dominant. Each of the 11 sample streams occupies one of three distinct alpine islands separated by valleys and other lower-elevation areas. Each stream also occupies one of three major watersheds, defined by U.S. Geological Survey cataloging units having 8-digit hydrologic unit codes (http://water.usgs.gov/GIS/huc.html), including the Cache la Poudre, Big Thompson, and St. Vrain basins, all tributaries of the South Platte River. Watersheds are not geographically correlated with alpine islands because high alpine areas often divide the headwater reaches of multiple drainage basins (Fig. 3.1a).

Five of the 11 sample streams occupy an alpine island in the vicinity of Hague's Peak (hereafter termed the "Hague's region") in northern RMNP; four streams occupy an alpine island in southern RMNP in the area surrounding Long's Peak; and the final two streams are found on opposite sides of Niwot Ridge, ca. 12 km south along the continental divide of the RMNP boundary (Fig. 3.1a). North Boulder Creek (NBC, Fig. 3.1a) is the southernmost of the sample sites as well as the southernmost site from which *P. neomacropyga* has previously been recorded (Adler *et al.* 2004). Most streams between the northernmost and southernmost of the 11 sites were sampled but no *P. neomacropyga* found; therefore, I am confident that there were no unsampled intervening "ghost" (Beerli, 2004) populations that may affect statistical analyses of population genetic subdivision.

I collected *P. neomacropyga* in early-mid August 2003, the time of year when larvae are mature, just prior to pupation. Larvae were collected from the bottoms of cobbles and boulders in areas of fast flow and immediately preserved in 75% ethanol. These were transferred back to the laboratory, taxonomically verified, and stored at -20°C prior to DNA extraction.

Genetic typing

Total genomic DNA was isolated from 50-60 individuals per sample stream using a basic salt extraction method and ethanol precipitation (Black & DuTeau 1997). Pellets were resuspended for storage in 200 μl Tris-EDTA (50mM Tris-HCl, 5 mM EDTA, pH 8.0). Polymerase chain reaction (PCR) amplified a 307 bp fragment comprising the extreme 3' end of the mitochondrial cytochrome oxidase subunit I (COI) gene. Primers followed Lunt *et al.* (1996), including a version of their forward primer UEA9 modified for *P. neomacropyga* (5'-GTAAACATCACATTCTTCCCACAACA-3'), and their unmodified reverse primer UEA10, which includes 22 bp of the 5'end of the adjacent tRNA-leucine and the stop codon for COI. Although 307 bp is a relatively short fragment, it includes the most rapidly-evolving section of the insect COI gene (Lunt *et al.* 1996); therefore, I reasoned that it would likely discriminate population differences across even the small spatial extent of the study system. Additionally, fragments ca. 100-400 bp in length are ideal for single-stranded conformation polymorphism (SSCP, Orita *et al.* 1989), the primary method I used to screen for sequence variation.

Each PCR reaction was run in a 51 µl total volume. I mixed a master solution containing 4350 µl ddH_2O, 500 µl 10X buffer (with 15mM $MgCl_2$), 10 µl 20 mM dNTPs, and 10 µl each of 500 µM primers. 49 µl of this solution per reaction were covered in 25 µl autoclaved light mineral oil and placed under a UV lamp for 10 min to destroy any contaminant DNA. Two µl of template DNA were then added and the reaction mixture was heated to 94°C for 5 min. I added 0.2 µl *Taq* polymerase at 80°C after this initial denaturation step. PCR consisted of 35 cycles of 94°C for 40 sec, 55°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 6 min.

I used SSCP to assess sequence variation among amplified PCR products following the procedures outlined by Hiss *et al.* (1994) and Black and DuTeau (1997). For each PCR product, 3 µl were added to 4 µl denaturing loading buffer (95% formamide, 1% 1M NaOH, 4% ddH_2O, and 0.05% each of xylene cyanol and bromphenol blue), and this mixture was heat-denatured at 95°C for 5 min then immediately plunged into an ice bath. After at least 5 min on ice, the full sample volume was loaded onto a 38x50 cm nondenaturing polyacrylamide gel and allowed to run at room temperature until the bromphenol blue dye band was ca. 15 cm from the end of the gel. Depending on acrylamide and glycerol concentration of the gel, running time was 22-26 h at a constant current of 15 mA.

I ran all PCR products on each of two gels of varying acrylamide and glycerol concentrations. A 5% acrylamide/5% glycerol gel was prepared by combining 16.7 ml 30% acrylamide, 20 ml 5X TBE (54 g Tris base, 27.5 g boric acid, 20 ml 0.5 M EDTA pH 8.0 to make 1 liter), 58.3 ml ddH_2O, and 5 ml glycerol. An 8.5% acrylamide/4% glycerol gel was prepared using 28.4 ml 30% acrylamide, 20 ml 5X TBE, 47.6 ml

ddH_2O, and 4 ml glycerol. Just prior to pouring, 100 μl of TEMED and 100 μl of 25% ammonium persulfate were added to the gel mixture. I used both gel mixtures for all samples because this combination allowed detection of all sequence variation among the sampled individuals.

SSCP bands were visualized using standard silver staining (Hiss *et al.* 1994, Black & DuTeau 1997) and scored according to variation in banding pattern. In order to control against false positives (different banding but same sequence) and false negatives (same banding but different sequence), I directly sequenced the purified PCR products (QIAquick PCR purification kit, Qiagen) of at least three individuals showing each distinct banding pattern per population using an ABI3100 Genetic Analyzer with the PCR primers. For less common banding patterns, all individuals were sequenced. I assessed sequence differences using BioEdit 5.0.9 (Hall 1999). I then ran PCR products of known individuals representing the more common sequences as reference standards in both outside and inside lanes of future SSCP gels. Validation of concordance between SSCP banding pattern and DNA sequence as such revealed neither false positives nor false negatives for any individuals in any populations.

Analyses

All sequences were aligned manually using BioEdit (Hall 1999), and I used Arlequin 2.000 (Schneider *et al.* 2000) for exploratory analyses of sequence variation. For each sample stream, genetic diversity (H) was calculated as

$$H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

where n is number of individuals in the sample population, k is the number of haplotypes, and p_i is the frequency of the i-th haplotype. H is analogous to expected heterozygosity for diploid data and equals the probability that two randomly-chosen haplotypes in a sample are different. I also used Arlequin to create a matrix of all pairwise F_{ST} values using Kimura's 2-parameter model (Kimura 1980) among streams for a preliminary assessment of geographic structure.

Spatial structure of genetic variation was further assessed using a nested analysis of molecular variance (AMOVA) in Arlequin. Nesting was imposed in one of two ways. First, I tested the null hypothesis that population genetic structure was not associated with major watershed by grouping streams according to their associated watershed. Second, I tested the null hypothesis that genetic structure was not associated with alpine island by grouping streams as such. Fixation indices (as per Weir and Cockerham 1984) were tested for significance using 100 000 permutations of the data.

I constructed a haplotype network using the software TCS 1.21 (Clement *et al.* 2000) based on the statistical parsimony method described by Templeton *et al.* (1992). Networks are often more realistic than bifurcating phylogenetic trees at the intraspecific level, especially across a small geographic extent (e.g. Posada and Crandall 2001). Reticulations (loops) in the network can arise due to homoplasy or recombination; the former is most likely in this study because it is unlikely that the mitochondrial genome experiences frequent recombination (but see Lunt & Hyman 1997); therefore, I followed common theoretical predictions about haplotype network structure (Posada & Crandall 2001, Crandall & Templeton 1993) to choose the most likely of the alternate network topologies resulting from broken network loops (specifically in this case, singletons are more likely to be tip haplotypes and connected to nonsingletons than to other singletons). In order to visualize differences between geographically grouping streams according to watershed vs. alpine island, the network was coded in two ways, each depending on the geographic structure of interest (see Fig. 3.1b, c).

MEGA (Kumar *et al.* 2004) was used to calculate net nucleotide divergence using the Kimura 2-parameter model between all individual populations and between alpine islands. Significance was assigned by bootstrapping with 10 000 replicates. I compared divergence estimates to a mean divergence rate of 2.2% Myr^{-1} for the arthropod COI gene (Gaunt & Miles 2002) to approximate between-group divergence times.

I tested for IBD following Rousset (1997) using matrices of pairwise genetic distances [as $F_{ST} / (1 - F_{ST})$, Slatkin 1995] and log spatial distances in a Mantel test using Bohonak's (2002) IBD program. Initially, I used straight-line (Euclidean) distance to compare IBD patterns between two spatial scales, the broader including all pairs of sample sites (55) and the finer including only pairs in the most site-rich alpine island (Hagues region, 10 pairs). I included both scales because strong dispersal barriers have been suggested to cause a breakdown of isolation by distance patterns due to the combined effect of random genetic drift and lack of migration (Slatkin 1993); however, if dispersal is indeed extremely limited, IBD ought to be evident across a smaller spatial extent (e.g. Keyghobadi *et al.* 2005).

In addition to testing for genetic isolation by Euclidean distance, I also tested for IBD among populations in the Hagues region using three other biologically realistic distance measures. I calculated these distances by estimating pairwise least-cost pathways between populations according to spatial models incorporating landscape elements important to aquatic insect flight dispersal. Number of landscape elements included increased additively in each successive model as listed in Table 1. A 10x10 m digital elevation model (DEM) was the base layer for these models, and I used ArcGIS (ESRI 2005) to assign costs to each cell based on predicted relative importance of associated landscape elements to insect flight. Resulting pairwise least-cost distance values and genetic distances were then used in a Mantel test of IBD as described above, and I compared fit and significance of the relationship for each competing model.

In the most complex model (#4, Table 3.1), per-cell costs of elevation difference and slope were linearly normalized 0-1, and cells on ridge-tops were assigned an additional two cost units; for each cell the sum of all costs gave its total cost (see Fig. 3.2). The ridge-top cost was doubled for two reasons: 1) slope cost decreases substantially on ridge tops (making these apparently more preferred locations for movement), but at the same time 2) ridge-tops were assumed to have higher wind speed than any other location on the grid (see Liston & Sturm 1998). Model #4 included all of the landscape factors thought to be influential to insect flight in the alpine zone; however, each individual factor was given a normalized and linear effect on flight cost in order to minimize complex and potentially unrealistic assumptions.

RESULTS

I identified a total of 26 unique haplotypes among the 11 populations, with final sample size 47-51 individuals per population for a total of 539 individuals analyzed (Table 3.2). Twenty-three of the 307 nucleotide sites were variable due to substitutions; of these, there were 20 transitions and three transversions, and six were nonsynonymous substitutions. As is common for insect mtDNA, nucleotide frequencies were AT-biased (freq. $T = 0.34$, $C = 0.23$, $A = 0.29$, $G = 0.14$).

Population structure

Genetic diversity values ranged from 0.194 (GLC, Longs region) to 0.735 (NBT, Hagues region) across the 11 sample streams (Table 3.2). Average diversity varied but was not significantly different either between alpine islands (0.60, 0.51, 0.50 for Hagues, Longs, and Niwot, respectively) or between major watersheds (0.43, 0.60, 0.54 for Poudre, Big Thompson, and St. Vrain, respectively). Private haplotypes (those found in a single location) occurred in eight of the 11 sample populations; indeed, 16 of the 26 total haplotypes identified were private (Table 3.2). Additionally, all pairwise F_{ST} values were significant (α=0.05) except for two (NBT-ELK and HAG-SDL, see Appendix 3.1), and these were pairs of populations that were geographically proximate without major topographical barriers.

AMOVA revealed that grouping streams by major watershed did not explain a significant proportion of genetic variation ($F_{CT} = 0.014$, $p = 0.33$, Table 3.3a). Grouping streams by alpine island, however, explained a significant 13.6% ($p = 0.01$) of total variation (Table 3.3b), implying that dispersal has been more limited across extensive low-elevation forested areas than across high-elevation watershed boundaries. In the significant model, a further 24.6% of genetic variation was distributed among streams within alpine areas, and the remaining 61.8% was due to within-stream variation. Overall F_{ST} was significant ($p < 0.0001$) and relatively large at 0.38.

Statistical parsimony analysis yielded a starburst-type haplotype network in which there were five mutational steps between the two most distant haplotypes (Fig. 3.1b, c). Haplotype 2 was the postulated ancestral haplotype due to its representation in a significant proportion of individuals in all populations (see Table 3.2). Only a single unsampled haplotype was suggested in the network, this linking haplotype 2 to a singleton (Fig. 3.1b, c). I have presented the same tree in two ways: first, by representing haplotype distribution proportionally according to alpine island occupied (Fig. 3.1b), and second, according to major watershed occupied (Fig. 3.1c). The 16 (of 26) private haplotypes were fully represented in a single alpine island or watershed, and, conversely, the presumed ancestral haplotype was found in all populations and therefore was present in all alpine islands and watersheds. Of the nine remaining haplotypes, seven were distributed across >1 watershed (Fig. 3.1c) and only four across >1 alpine island (Fig. 3.1b). Furthermore, the second most common haplotype (#1) was dominant in a single alpine island (Hagues) but was shared nearly equally between two watersheds (Poudre and Big Thompson). These observations bolster the AMOVA results (Table 3.3), suggesting that alpine islands are more meaningful partitions of the landscape than major watersheds in explaining genetic variation in this region.

Isolation by distance

Genetic isolation by Euclidean distance was evident at both spatial scales analyzed (Fig. 3.3). At the broad scale (across all pairs of sites), the relationship was weak ($r = 0.34$) but significant ($p = 0.006$, Fig. 3.3a). At the finer scale including only populations in the Hagues region, the relationship was strong ($r = 0.80$; $p = 0.04$), despite the much smaller sample size (Fig. 3.3b). Indeed, pairs of sites occupying the same alpine island may be driving positive IBD at the broad scale, as evidenced by comparing the closer vs. more distant sections of the broad-scale plot (Fig. 3.3a). There is a positive relationship among sites sharing an alpine island but no relationship for pairs occupying different islands.

Effective pairwise distances estimated by each of the four models of landscape connectivity produced similar IBD results (Table 3.4, Figs. 3.3b, 3.4). Euclidean distance showed stronger correlation to genetic distance than did any of the distances determined by more biologically realistic connectivity models. Estimated effective dispersal distances were highly correlated between all models (Table 3.4), however, and each model produced significant ($\alpha = 0.05$) Mantel results with r having a narrow range among them ($r = 0.71$ for Model 2 to $r = 0.80$ for Model 1).

Net divergence between populations

Net nucleotide divergence between pairs of streams ranged from 0.0023% - 0.33%; however, standard errors associated with these values were large and none were significant at $\alpha = 0.05$. Net nucleotide divergence between pairs of alpine islands ranged from 0.06% - 0.09% (Table 3.5), suggesting a coalescence time approximately 27,000–41,000 ybp. Associated standard errors here are also relatively high, however, and no divergence estimates were significant at $\alpha = 0.05$ (Table 3.5).

DISCUSSION

Results demonstrate a high degree of genetic structure for *P. neomacropyga* among 11 alpine streams in the RMNP area, Colorado. Across the study region, this structure is significantly associated with high-elevation "islands" of alpine tundra, suggesting that dispersal between populations is more limited across extensive lower-elevation areas than across major drainage divides for this strictly-alpine species. These results are similar to those from studies of terrestrial alpine insects in the Rocky Mountains (e.g. Knowles 2001, DeChaine & Martin 2004) and probably reflect the isolation of patches of alpine habitat during climatic warming following the most recent glacial recession (see Elias 1996).

The suggested divergence time between alpine islands was 27,000-41,000 ybp based on a molecular clock for the arthropod COI gene (Gaunt & Miles 2002). Although a rough estimate (as is common when relying on a molecular clock, cf. Hillis *et al.* 1996), this coalescence timing overlaps with estimates of the most recent major glacial advance in the RMNP area beween 30,000 and 13,750 ybp (Elias 1996). During this time, alpine conditions would have been widespread extending 14-15 km down the eastern slope of the continental divide (Elias 2001), and *P. neomacropyga* and other alpine populations were likely more connected, particularly within major watersheds. Climatic warming led to fragmentation of the extensive alpine tundra and its associated species assemblages, and my results provide evidence that dispersal is now limited between the isolated fragments.

Still, although allopatric fragmentation probably explains a significant proportion of genetic differences between alpine islands, I found some indication of isolation by distance across the sample range, a pattern that could be interpreted as limited ongoing dispersal between populations. Typically, if strong dispersal barriers exist, IBD is not detected (e.g. Slatkin 1993, Keyghobadi *et al.* 2005). Given the weak vs. strong relationships at the respective broad vs. fine spatial scales however (Fig. 3.3), the broad-scale IBD pattern more likely reveals the imprint of historical gene flow (see Garnier *et al.* 2004), probably when the climate was cooler and alpine populations were more widespread. Furthermore, the broad-scale IBD pattern appears to be controlled by the stronger relationship of genetic to geographic distance at the finer, within-island scale (Fig. 3.3a).

At the finer spatial scale, *P. neomacropyga* populations within the single most populated alpine island (Hagues region) indeed demonstrated strong IBD (Fig. 3.3b, Table 3.4). The only two pairs of populations (NBT-ELK and HAG-SDL) that did not have a significant pairwise F_{ST} occupied four streams all within the Hagues region, and they were geographically proximate (Figs. 3.1, 3.2). The stronger IBD pattern across this shorter spatial extent bolsters the AMOVA results implicating significant genetic subdivision between alpine islands. Both results suggest some dispersal occurring at the within-island scale but little occurring among islands.

Given the evidence of fine-scale genetic isolation by Euclidean distance, I anticipated that distances generated by the more biologically realistic spatial models would improve the fit of the IBD relationship. Of the four models compared, Euclidean distance surprisingly provided the best fit. Importantly, however, pairwise distance values obtained by each of the other models were strongly correlated with Euclidean distance. As such, the implication is that Euclidean distance may be a reliable surrogate for more biologically meaningful measures of connectivity across a heterogeneous landscape. However, the correlation between Euclidean and more meaningful distances is likely system- and scale-specific and cannot be expected to hold in all cases. In relatively small areas of steep mountainous terrain, it makes sense that sites that are more geographically distant are also more likely separated by landscape features that may inhibit flight dispersal, such as high ridgelines and more extensive areas lacking stream and riparian dispersal corridors (e.g., see Fig. 3.2). As spatial extent increases, however, coarse-grain heterogeneity is also likely to increase (Wiens 1989) and the positive relationship between Euclidean distance and biologically realistic dispersal routes is less likely to hold.

In general, my results are comparable to several other studies of stream insect population genetic structure that have suggested some movement among streams within a reasonably small spatial extent, often accompanied by a break in the pattern associated with various types of stronger dispersal barrier encountered as spatial extent increases (Smith & Collier 2001, Wilcock *et al.* 2001, 2003, Wishart & Hughes 2003, Monaghan *et al.* 2002, Schultheis *et al.* 2002). These are in contrast to studies showing the other common pattern identified in stream insects: a strong differentiation of populations within individual streams resulting in lack of significant spatial structure among streams (Bunn & Hughes 1997, Schmidt *et al.* 1995, Hughes *et al.* 1998, Hughes *et al.* 2000, Monaghan *et al.* 2002, Hughes *et al.* 2003a). This type of pattern has been attributed to different sample "populations" along a stream corridor being the results of only a few matings ("patchy recruitment," Bunn & Hughes 1997), combined with minor larval dispersal barriers along the stream. This pattern is unlikely to occur (and impossible to test) for *P. neomacropyga* alpine populations because of its extremely limited longitudinal distribution (only a single spatially continuous population was identified on each sample stream). Furthermore, the more temporally synchronous emergence of this (and most other) species in the U.S. Rocky Mountains makes the kind of nonrandom mating necessary for patchy recruitment unlikely (cf. Hughes *et al.* 2003b).

Compared to other studies of stream insect mtDNA population genetic patterns across spatial scales broad enough to include >1 major watershed, this study of *P.*

neomacropyga suggests an unusually strong pattern of genetic subdivision (overall F_{ST} = 0.38). Typically, F_{ST} values have fallen in the range from insignificant (e.g., for a widely-dispersing caddisfly in southeastern Australia, Baker *et al.* 2003) to significant but low values ranging from ca. 0.08-0.15 (baetid mayflies in both southeastern Queensland and the US Rocky Mountains, Hughes *et al.*2003a, 2003b; and a stonefly in the Appalachians, Schultheis *et al.* 2002). One exception has been a highly local-habitat-specific South African blepharicerid, in which overall F_{ST} was 0.94 and time since divergence was estimated at 2-3.5 Myr (Wishart & Hughes 2003) between groups of populations separated by <100 km. Aside from this notable exception, however, *P. neomacropyga* occupying alpine islands distributed across a relatively small spatial extent (50 km) has one of the most significant levels of population structure yet recorded for a stream insect. Both strong dispersal barriers and habitat specificity to patchily-distributed alpine streams probably contribute to the lack of gene flow. Additionally, flight activity is probably limited in this species relative to other black flies due to its autogenous nature.

Isolation among high-alpine stream reaches likely has had a similar effect on other strictly-alpine stream insects with limited dispersal potential. Confronted with potential broad-scale anthropogenic disturbances such as rapid climate change and atmospheric deposition in high-elevation Southern Rockies ecosystems (e.g. Baron *et al.* 2000, Welker *et al.* 2001), such species may have little capacity to respond and may be faced with local extinction. Oddly, despite the importance of headwater streams to whole-watershed biodiversity and ecosystem function (cf. Lowe & Likens 2005), there have been relatively few studies focused on the dispersal potential and genetic diversity of headwater species. The results of this study point to the need for further research on alpine stream species distributions, on their potential for evolutionary response to change, and on methods to understand dispersal between isolated habitat patches.

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Table 3.1. List of model parameters and assumptions of four models used to estimate pairwise distances between sites, with reasoning behind each and supporting references. Models 1-3 yield minimum distance under respective assumptions; see text for further explanation of Model 4.

model #	assumptions/costs of landscape features	reasoning	references
1	Euclidean distance (straight line on map)	simplicity	most prior IBD studies
2	minimum surface distance	flight does not occur at great distances above the earth	Adler *et al.* (1983) Choe *et al.* (1984)
3	travel along stream = 0 cost + minimal surface distance	flight of stream insects is concentrated along the stream, rather than inland	Thompson (1976) Flecker & Allan (1998) Petersen *et al.* (1999, 2004) Briers *et al.* (2002) Macneale *et al.* (2005)
4	travel along stream = 0 cost	"	"
	difference in elevation from stream increases cost; related to:		
	air temperature	flight activity is positively correlated to air temperature, ceasing in many cases below ca. 7ºC	Shipp *et al.* (1988) Waringer (1991) Kuusela & Huusko (1996) Briers *et al.* (2003)
	wind speed	flight activity is negatively correlated with wind speed	Roberts & Irving-Bell (1996) Briers *et al.* (2003)
	steeper slope of terrain increases cost; related to:		
	vegetation density	flight activity is positively correlated with vegetation density, especially in dry climates where vegetation may increase local relative humidity	Jackson & Fisher (1986) Myers *et al.* (2001) Briers & Gee (2004)
	ridge tops = double cost	dramatic increase in wind	Roberts & Irving-Bell (1996) Briers *et al.* (2003)

Table 3.2. Sample size (*N*), genetic diversity, and numbers of each haplotype found in each population. Shaded haplotypes are private.

alpine area:	Hagues					Longs				Niwot	
site:	IPC	HAG	ELK	NBT	SDL	FRZ	GLC	CHM	SHP	ISV	NBC
N:	48	50	48	49	50	50	49	49	48	47	51
diversity:	0.393	0.465	0.680	0.735	0.716	0.669	0.194	0.624	0.547	0.429	0.566
haplotype											
1	36	5	20	22	9		2				
2	11	36	15	9	23	14	44	11	25	35	23
3	1	6			11						
4					3			1			
5				1							
6				7							
7				1							
8						1					
9		1			1						
10		1									
11			1								
12					2					2	25
13		1			1						
14			12	9							
15						1	2	27	21		
16								9	2	6	
17								1			
18							1				
19											2
20						25					
21						6					
22						1					
23						1					
24											1
25						1					
26										4	

Table 3.3. AMOVA variance components, percent variation explained at each hierarchical spatial level, and fixation indices for (a) grouping populations according to major watershed occupied, and (b) grouping according to alpine "island."

(a)

source of variation	d.f.	variance components	percentage variation	fixation indices
among watersheds	2	0.008 (Va)	1.36	F_{CT} [Va/Vt] = 0.014 (p = 0.33 **NS**)
among streams within watersheds	8	0.20 (Vb)	34.41	F_{SC} [Vb/(Vb + Vc)] = 0.35 ($p < 0.0001$)
within streams	528	0.38 (Vc)	64.23	
total	538	0.58 (Vt)		F_{ST} [(1 – Vc)/Vt] = 0.36 ($p < 0.0001$)

(b)

source of variation	d.f.	variance components	percentage variation	fixation indices
among alpine "islands"	2	0.083 (Va)	13.64	F_{CT} [Va/Vt] = 0.14 (p = 0.010)
among streams within "islands"	8	0.15 (Vb)	24.58	F_{SC} [Vb/(Vb + Vc)] = 0.28 ($p < 0.0001$)
within streams	528	0.38 (Vc)	61.78	
total	538	0.61 (Vt)		F_{ST} [(1 – Vc)/Vt] = 0.38 ($p < 0.0001$)

Table 3.4. Summary of Mantel correlations (Pearson's *r*) and significance for the broad scale (all populations, Euclidean distance only) and the four alternate connectivity models at the fine scale (Hagues region only). Last column: correlations of respective model to Model 1 (Euclidean).

IBD model	Mantel *r*	 *p*	
Broad scale (Euclidean)	0.34	0.004	
Fine scale			
Model 1 (Euclidean)	0.80	0.036	
Model 2 (surface dist.)	0.71	0.018	$r = 0.933$
Model 3 (streams + surface)	0.72	0.025	$r = 0.942$
Model 4 (full model)	0.72	0.024	$r = 0.926$

Table 3.5. Net nucleotide divergence (below diagonal) and standard error (above diagonal) between alpine "islands." None are significant at $\alpha = 0.05$.

	Hagues	Longs	Niwot
Hagues		*0.00054*	*0.00057*
Longs	0.00089		*0.00057*
Niwot	0.00086	0.0006	

Figure 3.1. (a) Map of study region. Thin broken line shows boundary of Rocky Mountain National Park, solid bold line is continental divide, broken bold lines are major watershed boundaries (watershed names are underlined), large shaded circles approximate "islands" of alpine tundra (labeled in white boxes), and small triangles are sample streams with three-letter codes. (b) and (c) Haplotype network indicating distributions among alpine islands (b) and watersheds (c). Size of each circle is proportional to total haplotype abundance, and pie slices represent relative frequencies in each island/watershed *except* for haplotype 2, the ancestral haplotype, which was very abundant and found in significant proportion in all sample sites (see Table 3.2). Small triangle represents a hypothesized unsampled haplotype.

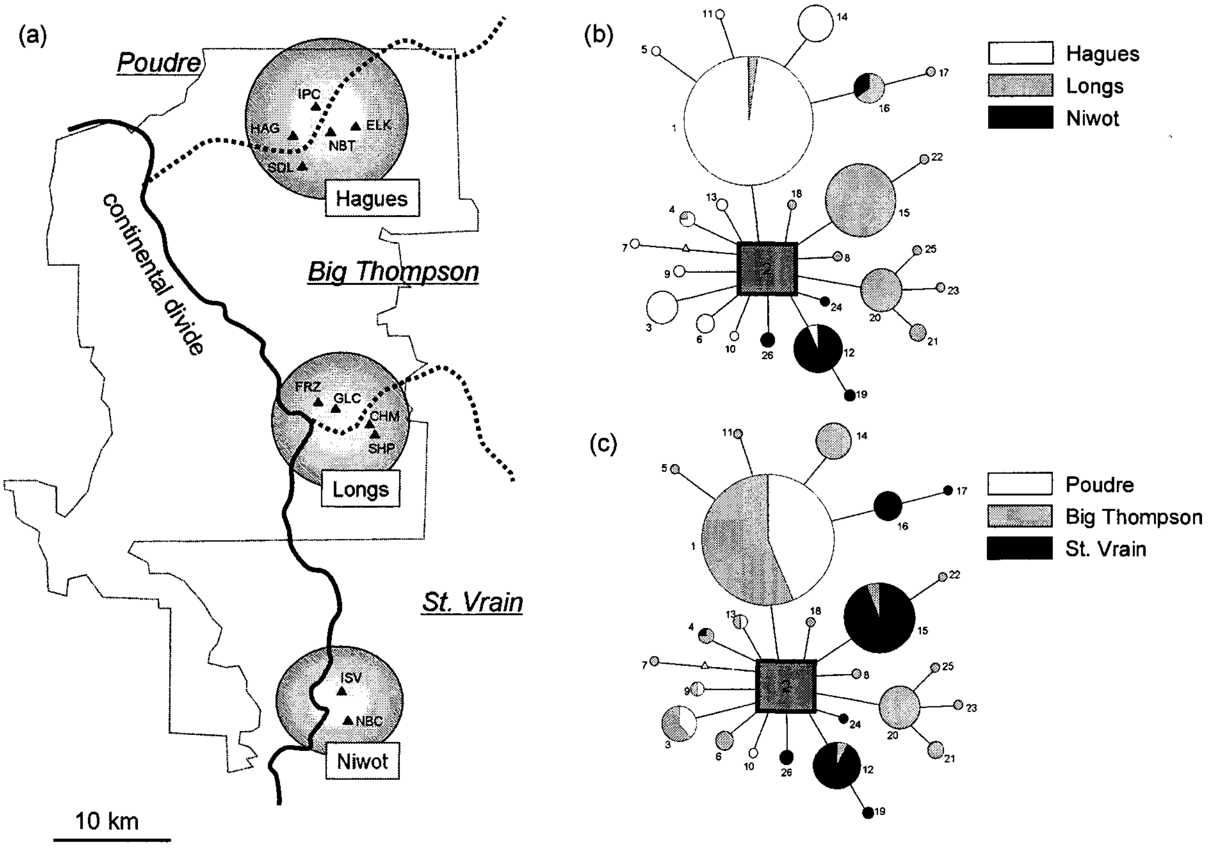


Figure 3.2. Map of the Hagues region alpine island showing the cost surface according to Model 4 (see Table 3.1). Cost increases along the color spectrum from blue (least cost) to red (greatest cost). Sample streams marked with + and three-letter code.

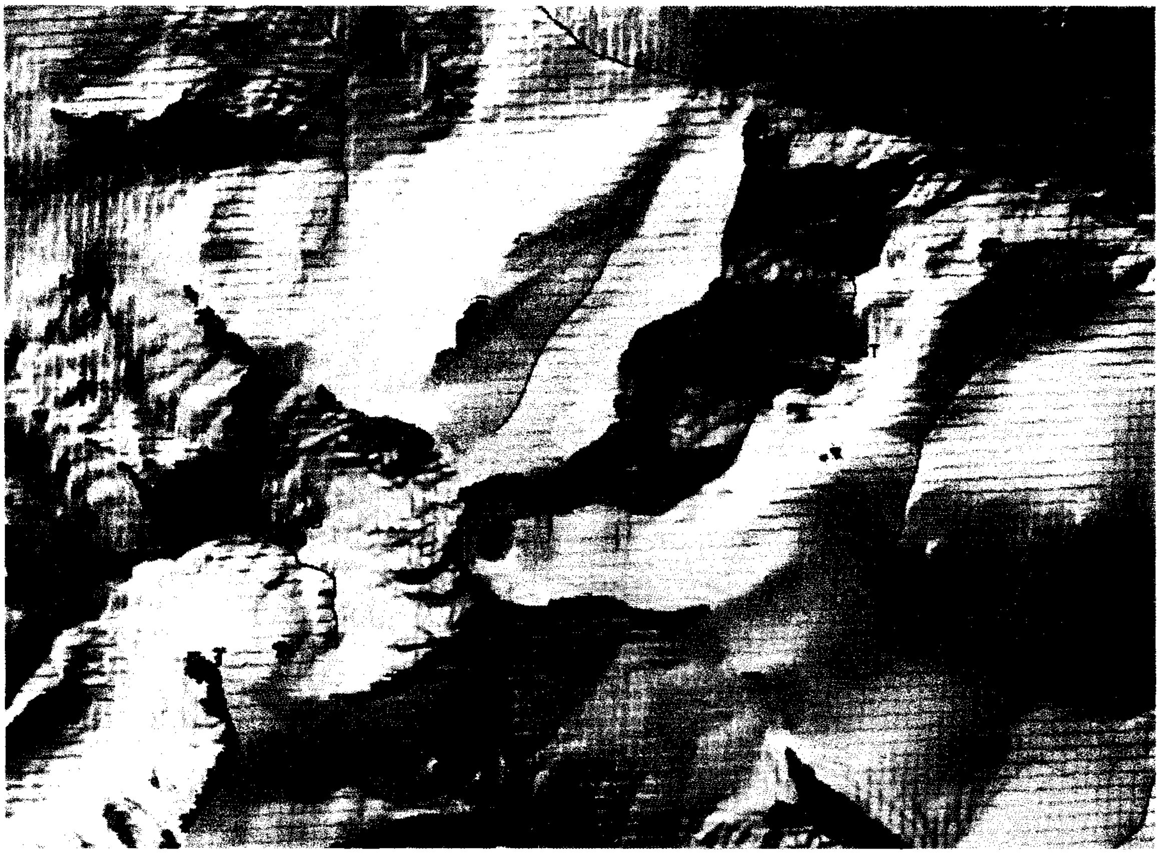


Figure 3.3. Isolation by [Euclidean] distance evident at two spatial scales. Each point represents one unique pair of populations; trendlines according to reduced major axis regression in IBD program (Bohonak 2002). (a) All populations included (55 pairs); vertical broken line indicates spatial break between pairs occupying same (left of line) vs. different alpine islands. (b) Only populations within the Hagues alpine region included (10 pairs). See Table 3.4 for correlation coefficients.

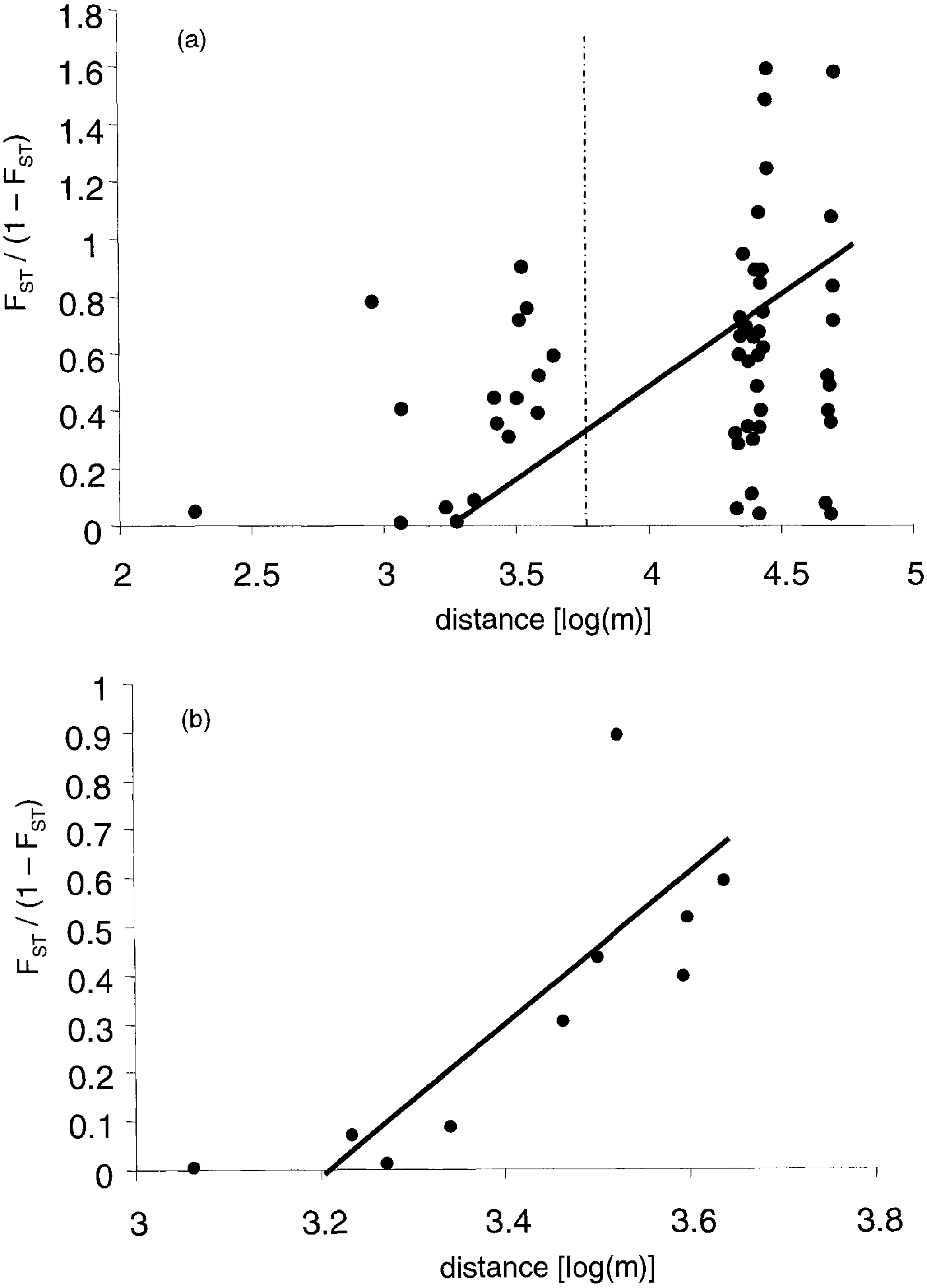
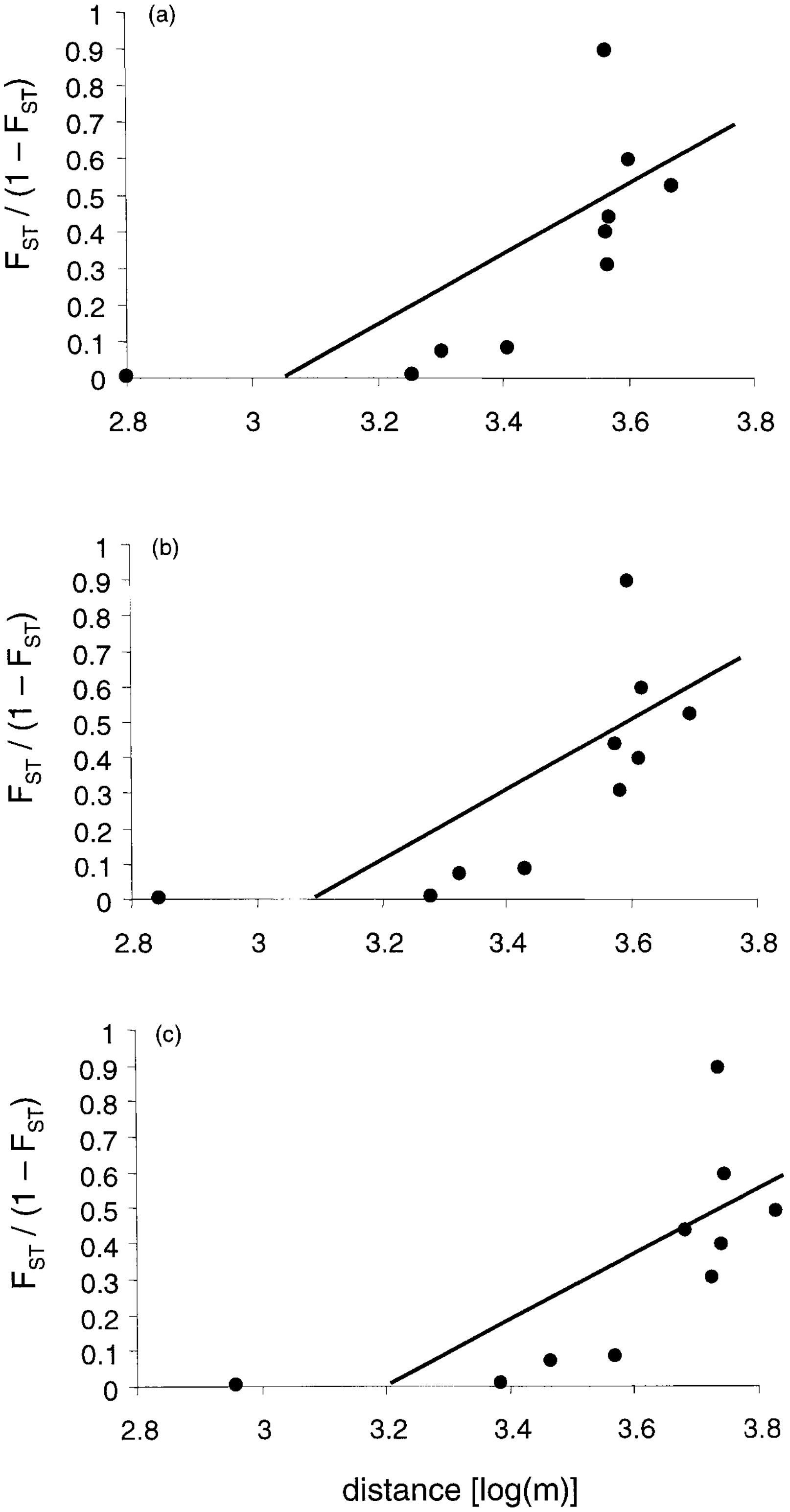


Figure 3.4. IBD plots using effective pairwise distances generated by (a) Model 2, (b) Model 3, and (c) Model 4 (see Table 3.1); trendlines according to reduced major axis regression. See Table 3.4 for correlation coefficients.



Appendix 3.1. F_{ST} values for all pairwise population comparisons. All values were significant at $\alpha = 0.05$ except those in bold.

	ELK	HAG	IPC	SDL	NBT	CHM	SHP	FRZ	GLC	NBC
ELK										
HAG	0.3742									
IPC	0.0769	0.4729								
SDL	0.2861	**0.0101**	0.3445							
NBT	**0.0044**	0.3042	0.0657	0.2348						
CHM	0.3697	0.2863	0.4262	0.2573	0.3266					
SHP	0.4632	0.2532	0.5568	0.2391	0.3999	0.0460				
FRZ	0.5239	0.3943	0.5994	0.3631	0.4716	0.4188	0.4312			
GLC	0.4724	0.0369	0.6157	0.1014	0.3836	0.3012	0.2588	0.4421		
NBC	0.5216	0.3419	0.6134	0.2877	0.4595	0.4118	0.4194	0.4858	0.4001	
ISV	0.3305	0.0461	0.4170	0.0709	0.2688	0.2364	0.2279	0.3729	0.0546	0.2875

CHAPTER 4

POPULATION GENETIC STRUCTURE OF A RARE HIGH-ELEVATION BLACK FLY, *METACNEPHIA COLORADENSIS*, OCCUPYING COLORADO LAKE OUTLET STREAMS

SUMMARY

1. Using a portion of the mitochondrial COI gene, I evaluated the population genetic structure of a rare black fly species (*Metacnephia coloradensis*) that is a habitat specialist in outlet streams of large, productive, alpine lakes in Colorado, USA. Given its rarity and life history traits that are restrictive to dispersal, I hypothesized that genetic structure would show a historic signature of allopatric fragmentation associated with climatic warming since Pleistocene glaciations.
2. I tested for genetic isolation by distance (IBD) and applied nested clade analysis (NCA) to ask whether current genetic structure is primarily a consequence of historic fragmentation or if there is evidence of ongoing gene flow.
3. Only four populations were located despite a thorough sampling of potential sites. Among these, there was a significant degree of population structure ($F_{ST} = 0.17$). However, there was also significant direct evidence of IBD, and NCA further supported IBD and restricted ongoing gene flow in clades at all nested levels. Compared to a more widespread Colorado alpine black fly (*Prosimulium neomacropyga*), *M. coloradensis* demonstrated a significantly lower level of population structure.
4. Although these results counterintuitively implicate limited ongoing gene flow driving current population structure, significant IBD may be a signature of historic gene flow, especially if migration-drift equilibrium has not been reached since the fragmentation event. Extraordinarily dense local populations probably allowed *M. coloradensis* to maintain large effective population sizes and minimize genetic drift since the Pleistocene.

5. Despite large local populations, *M. coloradensis* is vulnerable to continued rapid environmental change due to its high habitat specificity and small range.

INTRODUCTION

Many species that are currently confined to high-elevation "islands" of alpine habitat are believed to have been pushed to these extremes after the most recent glacial recession and associated climatic warming (Elias 1996, Hewitt 2000). There is much evidence that climatic shifts during the Quaternary drove the contraction and expansion of populations along altitudinal gradients (e.g. Knowles 2001, DeChaine & Martin 2004). Today, the entire planet is in the midst of a warm interglacial period, and the earth has probably not experienced average temperatures this warm in over 100 000 years (e.g. Williams *et al.* 1998). Species presently restricted to high alpine areas likely were considerably more widespread in the cooler past. Decreasing gene flow associated with presumed allopatric fragmentation of alpine populations is expected to lead to an increase in spatial population structure, especially in species with low dispersal ability, and at its extreme may result in speciation (e.g. Hewitt 2000).

Population genetic studies using neutral variable markers can elucidate the degree of population subdivision among isolated habitats such as those occupying alpine areas. However, it can be difficult to tease apart the relative importance of past allopatric fragmentation versus current isolation by distance in driving present-day patterns (e.g. Garnier *et al.* 2004, Smith & Farrell 2005). For example, in a recent study of a Colorado, USA alpine black fly species (Simuliidae: *Prosimulium neomacropyga* Peterson) a high degree of population structure was suggested among "islands" of alpine area surrounded by sub-treeline habitat (Chapter 3). There, I concluded that the extensive low-elevation "sea" surrounding the alpine islands acts as a strong dispersal barrier, and allopatric fragmentation was the primary driver of spatial genetic structure. However, isolation by distance was also detected, which may implicate a low level of ongoing gene flow between alpine islands.

Metacnephia coloradensis (Peterson & Kondratieff) is a rarer black fly known only from the outlet streams of large, productive lakes in high-elevation alpine areas of northern Colorado (Adler *et al.* 2004). This high level of habitat specialization may minimize its dispersal potential (Wishart & Hughes 2003, Chapter 3), particularly because these lake outlets provide permanent, stable habitat, which is probably a key determinant of traits conferring reduced dispersal in insects (see Roff 1990).

Indeed, *M. coloradensis* has an additional suite of life-history traits that make long-distance dispersal unlikely. It mates on the ground immediately after emergence, is incapable of blood feeding (and therefore is obligately autogenous), and makes only infrequent short flights (Peterson & Kondratieff 1995). These traits also characterize some aquatic insects in the environmentally similar Arctic tundra and are presumably adaptations to avoid risky dispersal into the harsh terrestrial environment (Downes 1965). Given this combination of traits, *M. coloradensis* is among the least likely of stream insects in this region to undergo long-distance dispersal. Hence, this species may provide a useful population genetic model for investigating patterns of postglacial allopatric fragmentation of alpine stream populations in the southern Rocky Mountains.

Prior to this study, *Metacnephia coloradensis* was known from only two locations, both in Colorado: the type locality (a lake outlet 3558 m a.s.l. in the North Boulder Creek drainage south of Rocky Mountain National Park in Boulder County) and an unspecified site near Mosquito Pass in Park County (based on a single pupa, Adler *et al.* 2004). Although its geographic distribution is rare, population density at the type locality is extremely high (350,000 late larval instars/m^2, Adler *et al.* 2004), and it has been reported as the most abundant arthropod in the drainage (Bushnell *et al.* 1987). In late summer, larvae at this site coat all substrates so that the streambed itself appears to be an undulating organism. I refer to this extreme population density as the "Medusa effect" because of the writhing, snakelike movements of the large larvae (final-instar length: 7.7-10.0 mm) covering stones removed from the stream. Because of its success at the type locality, I believed that *M. coloradensis* would be found in other characteristic local habitats in the region.

Detailed studies of simuliid population genetics are largely lacking, perhaps due to a lack of reliable, highly variable molecular markers. Microsatellites have been difficult to isolate (but see Dumas *et al.* 1998), and few nuclear or mtDNA markers have been variable enough to reveal population genetic patterns (e.g. Xiong & Kocher 1993, Tang *et al.* 1995, Moulton 2000, Pruess *et al.* 2000), instead being more useful in resolving higher-level phylogenetic relationships. Inferences regarding broad patterns of intraspecific population subdivision within the Simuliidae have mostly been derived from inversion patterns in the polytene chromosomes (e.g. Bedo 1975, Rothfels 1981, Spironello & Hunter 2004). Recently, however, a portion of the mitochondrial COI gene was used successfully to reveal population genetic patterns in *P. neomacropyga* across a fine spatial scale (Chapter 3, as cited above). I apply this marker in the current study.

The primary objective of the current study was to assess the degree of genetic structure among populations of *M. coloradensis*, based on sequence variation in the mitochondrial COI fragment. Additionally, I used two complementary methods to assess the weight of evidence for allopatric fragmentation versus ongoing gene flow in contributing to observed population genetic patterns. Finally, I compared aspects of population structure between *M. coloradensis* and *P. neomacropyga*, which is also confined to alpine streams in Colorado but is not limited to lake outlets. I expected a higher level of population structure for *M. coloradensis* than for *P. neomacropyga* due to its relative rarity, habitat specialization and presumed lower dispersal capabilities. Given these characteristics, I also hypothesized that the population structure of *M. coloradensis* would be driven primarily by post-glacial allopatric fragmentation and subsequent genetic drift in local populations, and that evidence for ongoing gene flow would be minimal.

MATERIALS AND METHODS

Study area and data collection

I focused collection efforts for *M. coloradensis* at lake outlets in alpine areas patchily distributed to the north and south of the species' type locality (N. Boulder Creek, Fig. 4.1). Collections were made throughout August to early September 2003 and included a collection from N. Boulder Creek. Simuliid larvae were collected from cobbles, boulders, and occasional bedrock at each site. They were fixed in 75% ethanol in the field, sorted to species, and stored in fresh ethanol at -20°C prior to DNA extraction.

Genetic typing

Total genomic DNA was isolated from individuals of *M. coloradensis* using a basic salt extraction method followed by ethanol precipitation (Black & DuTeau 1997). I used primers UEA9 and UEA10 (Lunt *et al.* 1996) to PCR-amplify a fragment of the mitochondrial cytochrome oxidase I (COI) gene comprised of 307 bp (primer sequences included) at the rapidly evolving extreme 3' end of the gene. PCR protocols followed those in Chapter 3 and included an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 94°C for 40 sec, 51°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 6 min.

I used single-stranded conformation polymorphisms (SSCP, Orita *et al.* 1989) to assess sequence variation among PCR products, following the specific procedures outlined in Chapter 3. SSCP is a simple, inexpensive, and sensitive way to assess sequence differences among a large number of individuals that therefore facilitates analysis of the large sample sizes often essential for population genetics analysis (*cf.* Hiss *et al.* 1994, Sunnucks *et al.* 2000). The procedure is based on the principles that a denatured (single-stranded) DNA molecule will conform into one or more stable shapes due to secondary base-pairing, that variation in sequence yields variation in these shapes, and that mobility of a molecule in a nondenaturing gel is determined by both its size and shape. The sensitivity of SSCP is due to the sensitivity of the 3-D shapes to even single-point mutations in a DNA sequence. Despite these sensitivities, false positives (different banding but same sequence) and false negatives (same banding but different sequence) can occur (Chakravarty *et al.* 1996) but can be avoided by using a controlled analysis strategy.

I ran replicates of all SSCP products on each of three nondenaturing polyacrylamide gels, each with different acrylamide/glycerol concentrations. Initially, I ran all samples on a 5% acrylamide/5% glycerol gel prepared by combining 16.7 ml 30% acrylamide, 20 ml 5X TBE (54 g Tris base, 27.5 g boric acid, 20 ml 0.5 M EDTA pH 8.0 to make 1 liter), 58.3 ml ddH$_2$O, and 5 ml glycerol. I then directly sequenced at least three individuals showing any distinct banding pattern in each population, using an ABI3100 Genetic Analyzer (for banding patterns less common than three individuals per site, all individuals were sequenced). Due to some false negatives (ca. one in eight visually different banding patterns) on the 5%/5% gel mixture, all individuals were further run on both 7.5%/5% (25 ml 30% acrylamide, 20 ml 5X TBE, 55 ml ddH_2O, 5 ml glycerol) and 8.5%/4% (28.4 ml 30% acrylamide, 20 ml 5X TBE, 47.6 ml ddH_2O, 4 ml glycerol) gels. Using the combination of these three gel mixtures, I could resolve all sequence variation. No false positives were identified.

Analyses

All sequences were aligned manually using BioEdit (Hall 1999), and I used Arlequin 2.000 (Schneider *et al.* 2000) and MEGA 3.0 (Kumar *et al.* 2004) for exploratory analysis of sequence variation. For each population of *M. coloradensis*, basic genetic diversity was calculated as the probability that two randomly chosen haplotypes are different (analogous to heterozygosity for diploid loci, see Chapter 3). All pairwise F_{ST} values were calculated, and analysis of molecular variation (AMOVA), as per Weir and Cockerham (1984), was implemented in Arlequin to assess the level and significance (using 100 000 permutations of the data) of spatial genetic structuring within versus among populations.

I tested for evidence of isolation by distance (IBD), using a Mantel test in the program IBD (Bohonak 2002) regressing pairwise genetic distance (as Slatkin's linearized F_{ST}, Slatkin 1995) against geographic (Euclidean) distance. Although the effective "distance" between alpine streams might be more complex than simple geographic distance due to hydrological linkages and extremely variable topography, map distance in this area is positively correlated with more biologically meaningful measures for flying insects (Chapter 3). I therefore deemed Euclidean distance appropriate for an exploratory analysis of IBD in this species. IBD is expected under conditions of limited ongoing gene flow among populations; however, it breaks down under conditions of extreme dispersal limitation due to the overwhelming effect of local genetic drift against the lack of migration (Slatkin 1993). Hence, if population genetic structure has been driven primarily by past allopatric fragmentation, significant IBD should not be detected.

I looked for further evidence of processes influencing population genetic structure with a nested clade analysis (NCA, Templeton *et al.* 1995). NCA was developed to decipher between historical events (e.g. allopatric fragmentation) and ongoing ones (e.g. limited gene flow) given current population genetic patterns. Specifically, NCA uses a coalescent approach to inform such inferences, based on a haplotype network broken into nested hierarchical clades and associated geographic information. Although the method has been questioned mainly based on its inability to place statistical significance on results from an inference key (e.g. Knowles and Maddison 2002), it has been implemented in several phylogeographical studies and specifically found to give results concordant with expectations based on local biogeographic history (e.g. DeChaine and Martin 2004) and known population histories (e.g. Templeton 2004).

I constructed a haplotype network using the statistical parsimony method described by Templeton *et al.* (1992) implemented in the software TCS 1.21 (Clement *et al.* 2000). Networks are often more meaningful than bifurcating trees to show evolutionary relationships at the intraspecific level due to the concurrence of ancestral and derived haplotypes in extant populations (e.g. Posada and Crandall 2001). I resolved reticulations (loops) in the network using the common theoretical prediction that singleton haplotypes are more likely to be connected to nonsingletons than to other singletons or to unsampled haplotypes (Posada & Crandall 2001, Crandall & Templeton 1993).

For NCA, I delineated nested clades for the haplotype network according to rules given by Templeton *et al.* (1987). Given the topology of these clades and their proportional distribution among sample sites, the software GEODIS 2.4 (Posada *et al.* 2000) provides statistical tests of the correlation between phylogeny and geography. Rather than using geographic coordinates as GEODIS input, I created user-defined distances between sites specifically due to the close geographic proximity of two populations separated by a steep, high-elevation (>4200 m a.s.l.) ridgeline (see Fig. 4.1). In this case, geographic coordinates would suggest that these populations are much closer than they effectively are; they also occupy different major watersheds (defined by having 8-digit U.S. Geological Survey Hydrologic Unit Codes, see Chapter 3). Populations of *Prosimulium neomacropyga* on opposite sides of this type of topographic dispersal barrier are isolated, as are populations occupying different mountaintop "islands" (Chapter 3). To preserve relative degrees of pairwise effective distance between sites for GEODIS analysis, I defined all populations as equidistant, with the exception of those that shared neither a major watershed nor a mountaintop "island," in which case a pair of populations was given a distance twice that of the other pairs. As such, geographical locations were simplified so that pairs of sites occupied one of two categories: near or far. Because "true" effective distances among populations occupying a heterogeneous landscape are difficult to infer (e.g. Wiens 2001), simplified categorical classification is justified. Any nested clade for which the phylogeny-geography relationship was significant according to GEODIS ($p < 0.05$) was evaluated using the most recent inference key developed for NCA (Templeton 2004).

RESULTS

I identified populations of *M. coloradensis* only at the outlet streams of four relatively large lakes (including the type locality; Table 4.1, Fig. 4.1b). These four populations were distributed in the headwaters of two major watersheds: two in the St. Vrain and two in the Big Thompson. Three populations (NBC, GRL, CTL) occurred in extraordinarily high densities (the Medusa effect). The Chasm Lake population (CHM) had apparently lower densities but was more difficult to sample due to stream flow among and below a large boulder field.

Sample sizes for genetic analyses were 47 (NBC), 21 (CHM), 53 (GRL), and 50 (CTL). I identified 26 total haplotypes across the four populations, and local genetic diversity ranged from 0.67 at CTL to 0.90 at CHM, which had the highest diversity despite having the smallest sample size (Table 4.2). Twenty-two of the 307 nucleotide sites were variable due to substitutions, and two of these were hypervariable. There were 20 transitions and four transversions, with three of the substitutions nonsynonymous. As expected for insect mtDNA, nucleotide frequencies were strongly AT-biased (freq. T = 0.36, C = 0.20, A = 0.31, G = 0.13).

Several results indicated isolation among populations. First, all populations harbored private haplotypes (those found in only a single population, Slatkin 1985); indeed, 21 of the 26 total haplotypes were private (Table 4.2). Second, all pairwise F_{ST}

values were highly significant ($p < 0.0001$). AMOVA suggested that differences among populations explained a significant ($p < 0.0001$) 17.3% of total genetic variation. The remaining proportion was explained by within-population variation (Table 4.3).

The haplotype network generated by TCS (Clement *et al.* 2000) was relatively shallow, with at most five mutational steps separating haplotypes, and it demonstrated an unambiguous ancestral haplotype (#6) due to its abundance and interior position in the network (Fig. 4.2). The ancestral #6 was also the only haplotype found in all four populations. Only three haplotypes (including the ancestral) were found in both major watersheds (Fig. 4.2), suggesting that watershed boundaries may have been important dispersal barriers for this species.

Despite a high degree of population genetic structure, both a Mantel test of genetic isolation by distance and nested clade analysis suggested limited ongoing gene flow. The Mantel results showed a positive correlation between genetic and geographic distance ($p = 0.04$, Fig. 4.3). The pair of sites with the closest geographic coordinates (CHM, GRL) but separated by a major dispersal barrier was a minor outlier in the analysis (Fig. 4.3).

A simplified diagram of the haplotype network (Fig. 4.2) is shown in Fig. 4.4, along with a delineation of each nested clade used in NCA. Because of the relatively shallow genetic structure, two-step clades were the most inclusive with the exception of the total cladogram level. Nonetheless, one one-step clade (1-4), both two-step clades, and the total cladogram all produced significant results from GEODIS. Based on the inference key (Templeton 2004), both two-step (intermediate-sized) clades showed evidence of ongoing restricted gene flow with some long-distance dispersal. Restricted gene flow and IBD were inferred from the patterns in the smallest significant clade (1-4) and the total cladogram (see Table 4.4 for summary).

DISCUSSION

Despite a survey of 26 potentially suitable locations, I found only three previously-unidentified populations of *M. coloradensis* in alpine areas north and south of the type locality. The sparse distribution probably is attributable to the habitat specificity of this species; all four populations occupied the outlets of large lakes well above treeline (>3450 m a.s.l). Site characteristics such as high productivity also might be important habitat requirements for *M. coloradensis* (Adler *et al.* 2004).

Previous studies have suggested that habitat specificity of stream insects can contribute to natal site fidelity and, therefore, to low levels of overland dispersal, leading to high levels of genetic structuring (Wishart & Hughes 2001, 2003, Kelly *et al.* 2002, Chapter 3). The verified habitat specificity and sparse distribution of *M. coloradensis*, in addition to life-history traits that suggest limited dispersal ability (e.g. mating at the emergence site and obligate autogeny), bolstered the main hypothesis that populations should show a high degree of spatial genetic structure and reveal historical patterns of postglacial allopatric fragmentation.

Population genetic analyses demonstrated a significant degree of spatial structure in *M. coloradensis*. However, a direct test of IBD in addition to NCA implied a limited amount of ongoing gene flow and long-distance dispersal. Such results yield the impression that current population genetic structure in *M. coloradensis* is primarily driven by ongoing processes and does not retain a strong signature of the pattern of post-glacial allopatric fragmentation.

Furthermore, populations of the more widespread alpine black fly species *P. neomacropyga* showed a higher level of geographic structure than those of *M. coloradensis* (Table 4.5). Indeed, F_{ST} for *P. neomacropyga* based on the same mtDNA fragment was more than twice that for *M. coloradensis*. If a higher value of F_{ST} alone indicates lower dispersal among populations, as suggested by several studies directly comparing species biology to population genetic patterns (Bohonak 1999, Miller *et al.* 2002, Wilcock *et al.* 2003), then successful between-stream dispersal appears to be occurring significantly more often in *M. coloradensis* than in *P. neomacropyga*.

This conclusion, however, assumes near-equal effective population sizes (N_e) in both species. Larger N_e will negatively influence F_{ST} (Wright 1951). Table 4.5 compares several other population genetic statistics for *M. coloradensis* and *P. neomacropyga,* whose populations are distributed across overlapping spatial extents. Mean local genetic diversity is significantly higher for *M. coloradensis*, and I identified the same number of COI haplotypes in each species, despite unequal (4 vs. 11) numbers of local populations. These observations imply that, despite its sparse distribution across the landscape, genetic diversity has been well-maintained in *M. coloradensis*. The readily-observable local success of populations in terms of density of individuals (the Medusa effect) suggests large local N_e, which would help explain these patterns. Genetic drift occurs more gradually in populations of larger effective size, and lower-frequency haplotypes are less likely to drift to extinction. The more widespread *P. neomacropyga*, conversely, maintains relatively much lower local population densities in the study area.

Large populations are less likely than their smaller counterparts to have achieved a migration-drift equilibrium since historical fragmentation events, especially when gene flow is limited (Neigel 1997), as has been inferred in the current study populations. Time since isolation of *M. coloradensis* on mountaintop islands likely dates back only to the late Pleistocene, ca. 10,000 ybp (see Chapter 3). A large local N_e combined with comparatively recent fragmentation can cause current population structure to retain a signature of historical patterns of gene flow due to low drift rates. An imprint of historically important processes that indeed may be negligible in the present will not only affect basic population statistics such as F_{ST}, but also will influence the conclusions of more complex phylogeographic models, including IBD (Slatkin 1993, Garnier *et al.* 2004) and NCA (Knowles & Maddison 2002, Templeton 2004). Hence, the isolation by distance conclusion that these analyses suggested may be indicative of pre-fragmentation rather than current processes.

M. coloradensis was probably once a series of populations that were more widespread across cooler Pleistocene habitats. Limited gene flow among adjacent populations would have led to an IBD pattern. During the warming period, some populations persisted, retreating to the mountaintop "islands" where their preferred habitat still exists today. Although current gene flow between these isolated habitats is unlikely, extremely large local populations may have prevented drift so that across the range of the species, the historic IBD signal has been retained.

The haplotype network (Fig. 4.2) suggests some influence of major watershed boundaries in gene flow limitation. These divides extend to altitudes significantly lower than the terminal moraines of Pleistocene glaciers in RMNP (Elias 1996) and therefore probably influenced dispersal between aquatic populations even when climates were cooler and current alpine-restricted species were likely more widespread. Based on population genetic patterns of *P. neomacropyga* in Chapter 3, alpine populations that are closer to migration-drift equilibrium are not expected to be spatially structured according to major watershed; however, this pattern might be expected if large *M. coloradensis* populations have retained historical patterns. Watershed boundaries have been shown to restrict current gene flow in population genetic studies of stream insects (e.g. Hughes *et al.* 1999, 2003, Wishart & Hughes 2001, 2003).

Although the Medusa effect suggests large local effective population sizes for *M. coloradensis* and provides a robust argument to refute this study's analytical inferences as well as those from the comparative study with *P. neomacropyga*, there are further uncertainties. Especially for analyses comparing multiple species, it is desirable to pair gene flow estimates with behavioral and life history observations (Bohonak 1999). In this study, I have provided a controlled comparison (using the same molecular marker) between two species with presumably similar life histories and occupying the same range. However, a more detailed understanding of the comparative biology of the two species is necessary to bolster the inferences made above. Although *M. coloradensis* is somewhat better-known (e.g. Peterson & Kondratieff 1995), mating behavior, site fidelity, and other bionomic details of *P. neomacropyga* are lacking. Rothfels (1981) found evidence for natal site fidelity in another *Prosimulium* species based on site-specific chromosomal polymorphism profiles. If site fidelity or other life history characteristics conferring low dispersal are more common in *P. neomacropyga* than in *M. coloradensis*, this could further explain observed population genetic differences between the two species.

Even in the absence of significant life history and behavioral differences, passive dispersal mechanisms are likely to be more important for *M. coloradensis* given its extraordinarily dense local aggregations. A denser population provides a greater number of potential dispersers, and although adult behaviors suggest limited activity, passive dispersal has been speculated to be important in many aquatic invertebrates (e.g. Bilton *et al.* 2001). Massive silk production of *M. coloradensis* larvae (Adler *et al.* 2004) could provide an exceptionally sticky means of attachment to birds or other more widely dispersing macrofauna (see Figuerola & Green 2002). Also, the strong winds characteristic of alpine areas could be an important passive dispersal mechanism for adults. In general, the number of dispersers from a site is thought to be correlated positively with local population size (*cf.* Hanski 1982, Stenseth 1983). For these reasons and because of its probable effect on rates of genetic drift, the Medusa effect is apparently more than just an interesting natural history side note for *M. coloradensis*!

Further comparative studies on *M. coloradensis* and *P. neomacropyga* are also called for because they appear to share similar phylogeographic histories across a much broader spatial scale. *P. neomacropyga* in Colorado is at the extreme southern periphery of its range; indeed, its distribution is centered in Beringia (Russian Far East, Alaska, and the Yukon). The sister species of *M. coloradensis* is *M. sommermanae* (Stone), which has a similar distribution, except it does not extend south to the patchily distributed alpine habitats in the southern Rocky Mountains. *M. coloradensis* is found here instead and thus may have diverged enough from *M. sommermanae* in isolation to now be recognized as a separate species. The two species differ chromosomally by a single inversion that is fixed in *M. sommermanae* but linked to the Y chromosome in *M. coloradensis* (Adler *et* *al.* 2004), and morphologically in characteristics of the eyes (Peterson & Kondratieff 1995). Similar distributions and presumably similar biology probably led to similar responses to glacial advances and retreats in *P. neomacropyga* and *M. sommermanae.* A question is whether these two species left southern isolate populations in response to the same glacial episode. A comparative study of neutral population genetic structure across the entire range would provide clues.

Metacnephia coloradensis has two of three important factors leading to rarity (Rabinowitz 1981): narrow habitat tolerance and restricted geographic range. It does not have the third, small local population size. Only one of the three, however, is sufficient to produce rarity and possible conservation concern (Rabinowitz 1981). Faced with potential broad-scale disturbance such as climate change and atmospheric deposition (e.g. Baron *et al.* 2000, Welker *et al.* 2001), *M. coloradensis* will likely have little capacity to respond if the conditions of their local habitat are altered. Understanding the specific environmental characteristics influencing current and past distribution will be necessary to predict the response of this and other alpine-stream species to changing conditions.

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Table 4.1. Alpine lake outlets sampled for *Metacnephia coloradensis*, early August – early September 2003. For those sites where the species was present, the lake name is followed in parentheses by the three-letter code used for the site throughout the paper.

alpine lake sampled	drainage	elev. (m)	latitude (N)	longitude (W)	sample date	*M. colo.* present?
unnamed	S. St. Vrain (main)	3426	40º 03.719'	105º 38.135'	1 Aug	no
Summit Lake	Bear Creek	3840	39º 35.785'	105º 38.212'	2 Aug	no
Pass Lake	N. Fork Snake R.	3551	39º 39.267'	105º 52.603'	2 Aug	no
Oliver Twist Lake	Mosquito Creek	3654	39º 17.650'	106º 09.819'	3 Aug	no
Crystal Lake **(CTL)**	Roaring River	3450	40º 28.249'	105º 38.750'	6 Aug	**yes**
Unnamed	Hague Creek	3414	40º 28.570'	105º 40.761'	7 Aug	no
Unnamed	N. Fork Big Thompson	3486	40º 29.745'	105º 37.399'	12 Aug	no
Unnamed	N. Fork Big Thompson	3465	40º 29.893'	105º 37.375'	12 Aug	no
Lake Dunraven	N. Fork Big Thompson	3378	40º 30.207'	105º 37.294'	12 Aug	no
Lake Louise	N. Fork Big Thompson	3306	40º 30.466'	105º 37.017'	12 Aug	no
Green Lakes #3	N. Boulder Creek	3396	40º 03.046'	105º 36.834'	14 Aug	no
Green Lakes #4	N. Boulder Creek	3438	40º 03.345'	105º 37.040'	14 Aug	no
Green Lakes #5 **(NBC)**	N. Boulder Creek	3558	40º 03.215'	105º 37.618'	14 Aug	**yes**
Frozen Lake	Glacier Creek	3477	40º 15.568'	105º 38.492'	15 Aug	no
Green Lake **(GRL)**	Glacier Creek	3462	40º 15.320'	105º 37.928'	15 Aug	**yes**
Blue Lake	Glacier Creek	3345	40º 16.034'	105º 37.914'	15 Aug	no
Chasm Lake **(CHM)**	Roaring Fork	3537	40º 15.564'	105º 36.117'	16 Aug	**yes**
Keplinger Lake	Hunters Creek	3506	40º 14.496'	105º 37.500'	18 Aug	no
Pipit Lake	Ouzel Creek	3423	40º 11.520'	105º 40.009'	21 Aug	no
Lake Powell	N. Inlet Creek	3465	40º 15.397'	105º 39.691'	22 Aug	no
Snowbank Lake	N. St. Vrain	3456	40º 14.404'	105º 38.586'	22 Aug	no
Lion Lake #2	N. St. Vrain	3429	40º 14.246'	105º 38.488'	22 Aug	no
Lake of Many Winds	N. St. Vrain	3489	40º 13.447'	105º 39.898'	23 Aug	no
unnamed	S. St. Vrain (N. trib.)	3396	40º 04.503'	105º 37.432'	26 Aug	no
Blue Lake	Mitchell Creek	3387	40º 05.266	105º 36.970'	27 Aug	no
Square Top Lakes	Duck Creek	3614	39º 35.439'	105º 44.298'	7 Sep	no

Table 4.2. Sample size (*N*), genetic diversity, and frequency of 26 haplotypes at each of four Colorado sites occupied by *Metacnephia coloradensis*, early August – early September 2003. Shaded haplotypes are private alleles.

site:	NBC	CHM	GRL	CTL
N:	47	21	53	50
diversity:	0.802	0.900	0.676	0.673
haplotype:	haplotype frequencies:			
1	0.34		0.23	
2	0.26	0.10		
3	0.02			
4	0.09			
5	0.02			
6	0.15	0.19	0.51	0.50
7				0.04
8		0.05	0.04	0.22
9			0.15	
10			0.02	
11	0.04			
12				0.02
13				0.20
14		0.05		
15		0.24		
16		0.10		
17		0.05		
18			0.04	
19			0.02	
20				0.02
21	0.02			
22	0.02	0.14		
23		0.05		
24		0.05		
25	0.02			
26	0.02			

Table 4.3. Analysis of molecular variation for *Metacnephia coloradensis* at four sites in Colorado, early August – early September 2003.

source of variation	d.f.	variance components	percentage variation
among streams:	3	0.13	17.3
within streams:	167	0.62	82.7
total	170	0.75	100

$F_{ST} = 0.173$ ($p < 0.0001$)

Table 4.4. Results of nested clade analysis for *Metacnephia coloradensis* from northern Colorado, early August – early September 2003. Only clades with significant geographical relationships are included. IBD = isolation by distance; refer to Fig. 4.4 for clade topology.

nesting	inference key steps	conclusion
one-step clades		
1-4	1,2,3,4, no	restricted gene flow with IBD
two-step clades		
2-1	1,2,3,5,6,7,yes	restricted gene flow with some long-distance dispersal
2-2	1,2,3,5,6,7,yes	restricted gene flow with some long-distance dispersal
total cladogram	1,2,3,4, no	restricted gene flow with IBD

Table 4.5. Population genetic statistics for two autogenous, alpine black flies distributed across overlapping spatial extents in northern Colorado. Results for both are based on the same molecular marker; all statistics for *P. neomacropyga* are from Chapter 3.

species	habitat	# streams occupied	# COI haplotypes	# private alleles	F_{ST}	mean genetic diversity (st. dev.)	IBD slope (*p*-value)
Metacnephia coloradensis	lake outlets	4	26	21	0.17	0.76 (0.11)	0.0051 (0.04)
Prosimulium neomacropyga	lake outlets and other streams	11	26	16	0.38	0.55 (0.16)	0.0068 (0.04)

Figure 4.1. (a) State of Colorado, illustrating approximate extent of sampling for *Metacnephia coloradensis* (solid rectangle) along the north-south oriented cordillera. All sample sites are listed in Table 4.1. Dashed line represents Rocky Mountain National Park (RMNP) boundary. (b) Magnified view of RMNP area (thin dashed line), with locations of *M. coloradensis* populations (triangles) and major watershed boundaries (thick dashed lines). Watershed names are underlined. The divide between sites GRL and CTL is a ridgeline that reaches >4200 meters a.s.l.

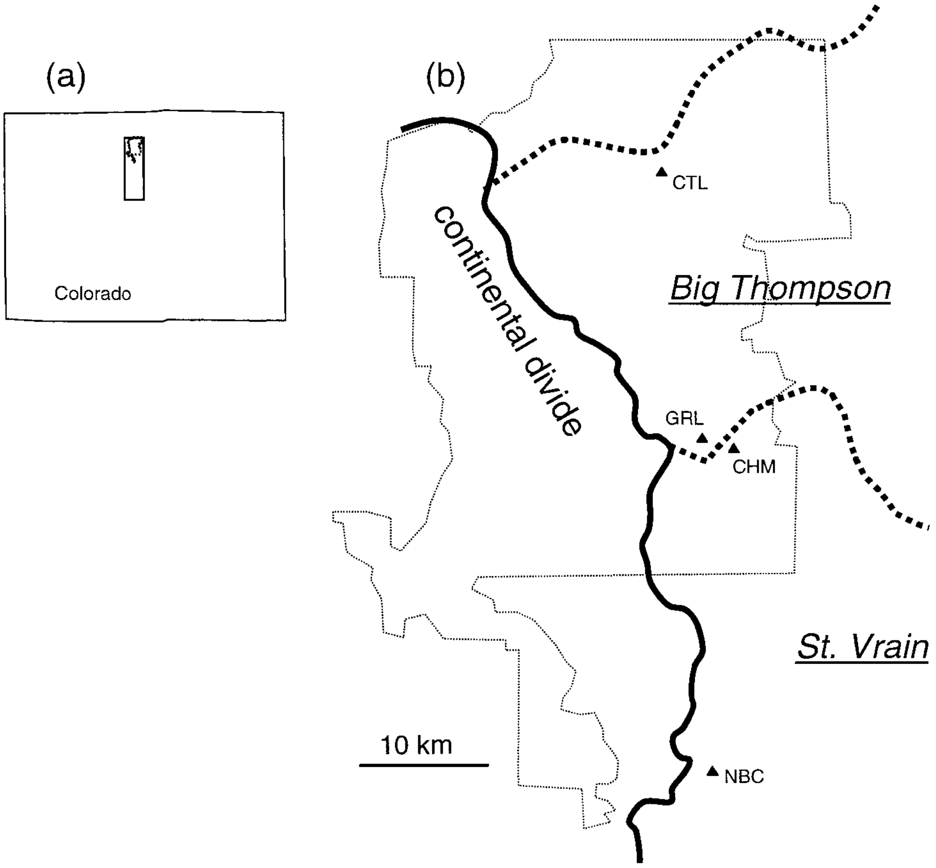


Figure 4.2. Haplotype network indicating proportional representation of each haplotype (circle) in each of four populations (indicated by color/fill pattern) of *Metacnephia coloradensis* in northern Colorado, early August – early September 2003. Solid fill represents haplotypes found in Big Thompson watershed; hatched fill indicates St. Vrain watershed (see Fig. 4.1). Each line is one mutational change. Size of circle is proportional to total number of individuals possessing each haplotype. Small triangles represent hypothesized unsampled haplotypes. Numbers are haplotype numbers as in Table 4.2, and haplotype 6 is the unambiguous ancestral haplotype.

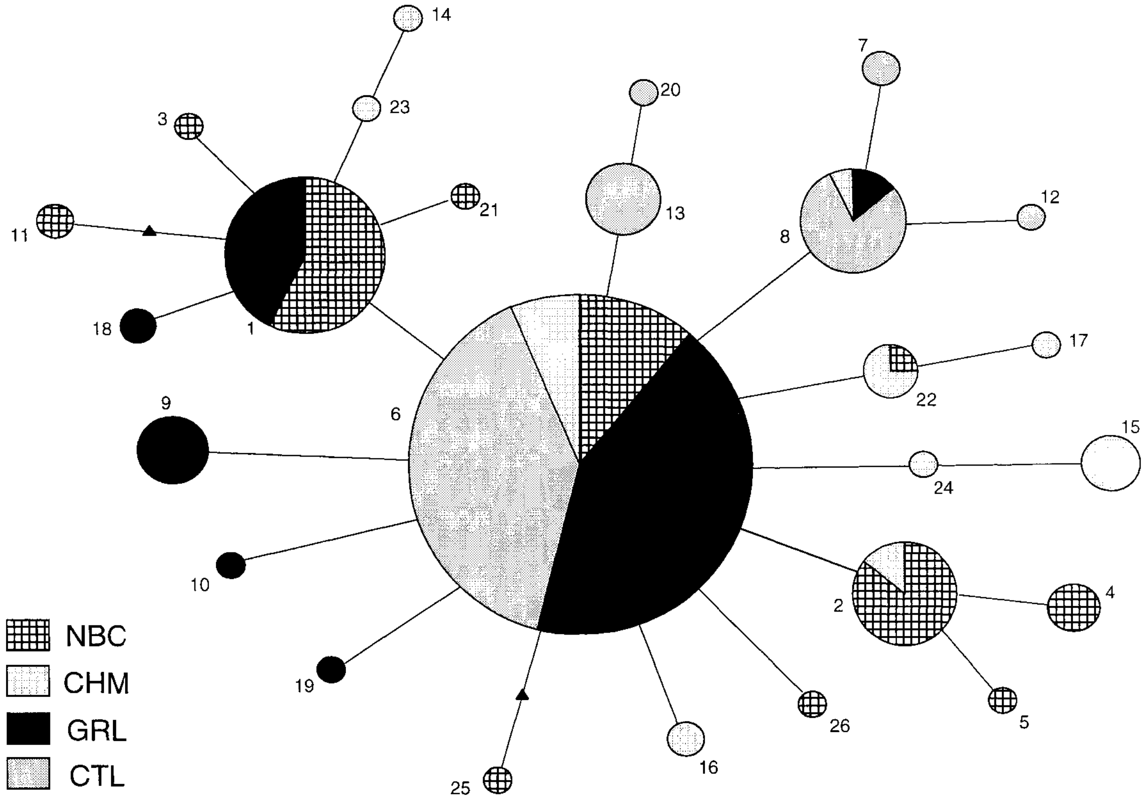


Figure 4.3. Evidence for isolation by distance (IBD) among populations of *Metacnephia coloradensis* in northern Colorado indicated in a plot of genetic distance (as Slatkin's linearized F_{ST}) vs. geographic distance. Each point represents a unique pair of populations. Slope = 0.0051; $p = 0.04$. Broken circle indicates the CHM-GRL pair, which is separated by a major topographic dispersal barrier.

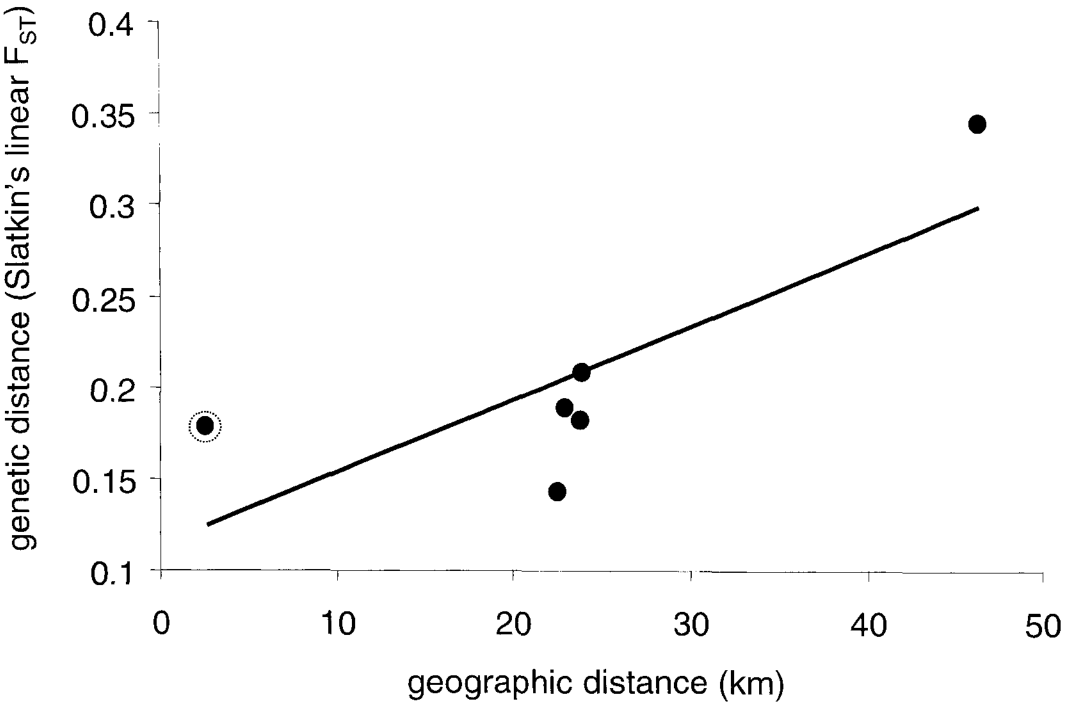
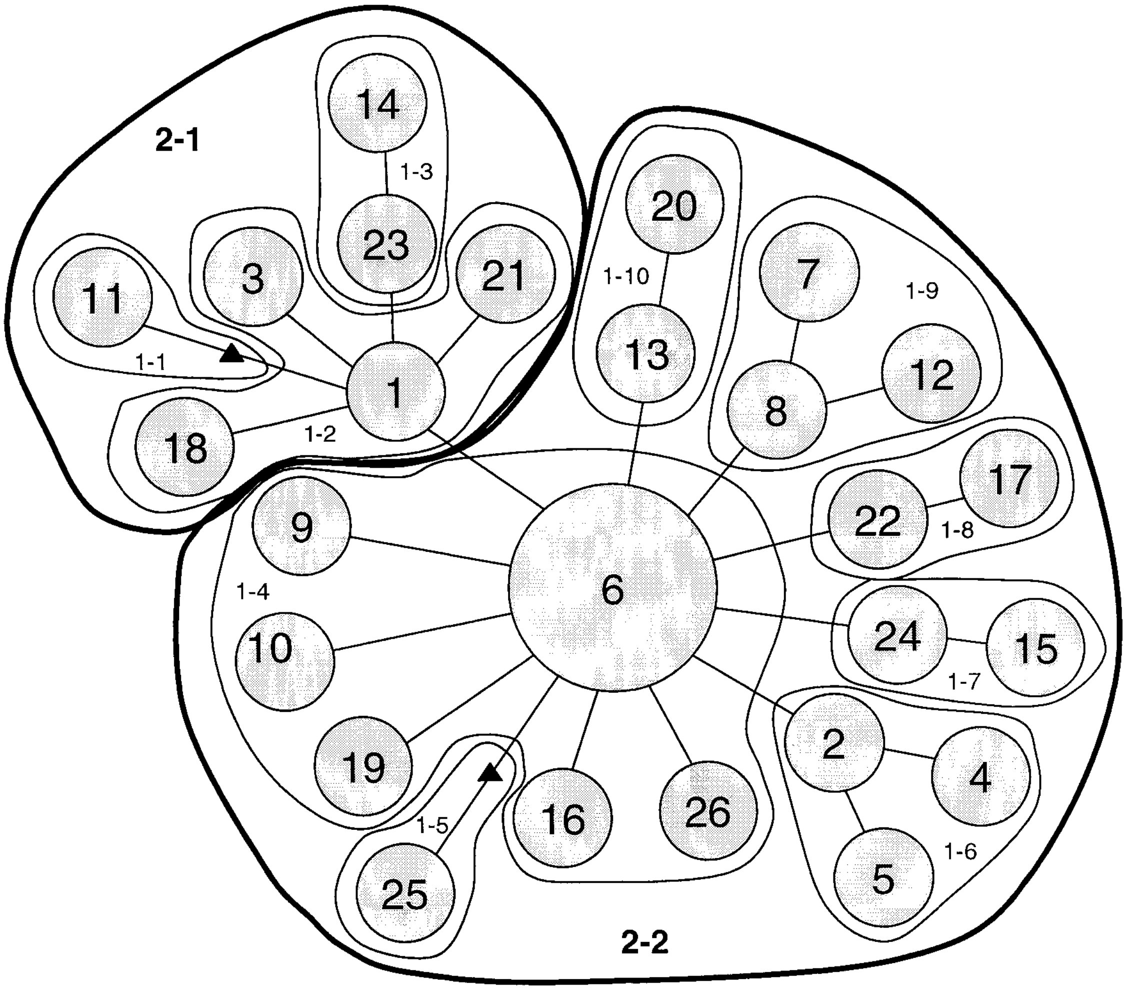


Figure 4.4. Simplified haplotype network for *Metacnephia coloradensis* in northern Colorado, from Fig. 4.2, with clade nestings used in NCA. Thin lines enclose one-step clades; thick lines enclose 2-step clades.



CHAPTER 5

LATERAL FLIGHT ACTIVITY OF INSECTS FROM A ROCKY MOUNTAIN ALPINE STREAM.

SUMMARY

1. Little is known about the lateral movement of adult stream insects in harsh terrestrial environments such as the alpine tundra, which is characterized by a short, cool growing season and high winds. I investigated the lateral flight activity of Ephemeroptera, Plecoptera, Trichoptera, and Simuliidae emerging from a first-order alpine stream in 2002 and 2003 in Rocky Mountain National Park, Colorado, USA.
2. I measured flight inland from the stream using Malaise traps set in perpendicular transects at distances of 1, 10, 30, and 60 meters. Only a downwind transect was set in 2002, with downwind and upwind transects set in 2003.
3. Overall, patterns at this spatial extent were similar to those reported in lower-elevation streams. Insect flight and species richness were concentrated near the stream. Abundance decreased as an inverse power function for Ephemeroptera and Plecoptera, as a negative exponential function for Trichoptera, and it did not decrease with distance for anautogenous Simuliidae. Generalizations could not be made across all species in a higher taxon. The mayfly *Ameletus celer*, for example, did not decrease in abundance away from the stream. Very few alpine stream specialists were caught, possibly because habitat generalists are more likely to be active dispersers.
4. Most taxa were unexpectedly more abundant on the upwind than downwind side of the stream. A flatter, wetter terrestrial habitat on the upwind side probably explains this pattern.

5. Sex ratios were heavily male-biased for Ephemeroptera, Plecoptera, and Trichoptera, female-biased for Simuliidae, and they did not vary with lateral distance. This suggests that common biological differences in flight activity between sexes were intensified in the alpine zone.
6. Increased snowpack caused significantly later emergence in 2003 than 2002 and explained several differences in adult population parameters between years. In the future, snowfall is expected to increase with global climate change, and an important implication of this may be a delayed and shortened emergence season for alpine stream insects. Lateral dispersal of late-emergers in particular may be impacted because colder air temperatures during their adult phases may limit flight activity.

INTRODUCTION

Adult flight is the primary means by which stream insects can actively disperse between hydrologically unconnected headwater streams. Such movement is important for a variety of reasons, not the least of which is that dispersal rates will influence local population and community dynamics of interest to both basic (e.g. Palmer *et al.* 1996) and applied (e.g. Fuchs & Statzner 1990, Briers & Gee 2004) ecological problems in streams. Flight behavior of stream insects can additionally influence the degree to which aquatic resources subsidize the energy budgets of terrestrial organisms such as birds, bats, and spiders (*sensu* Nakano & Murakami 2001).

Despite the importance of inland dispersal, this is a subject that has remained relatively understudied in streams. Comparatively, stream ecologists have studied finer-scaled movements of insects along the stream corridor much more extensively, both with respect to larval movements within the stream (especially drift, see reviews by Sheldon 1984, Mackay 1992) and adult flight along the stream especially as it pertains to Müller's (1954) "colonization cycle" (e.g. Bird & Hynes 1980, Flecker & Allan 1998, Winterbourn & Crowe 2001, also see Bilton *et al.* 2001 and Malmqvist 2002 reviews). Improved efficiency and cost of population genetics techniques have also now led to a number of indirect studies of much broader-scale patterns of movement in stream insects. Jane Hughes and colleagues have led the way among stream ecologists using this method (e.g. Bunn & Hughes 1997, Hughes *et al.* 1999, Wishart & Hughes 2003). At the intermediate scale, however, direct studies of lateral dispersal from streams remain relatively few and far between (and I will cite most of these below).

A few broad generalizations about flight activity away from the stream can be gleaned from this small collection of lateral dispersal studies. First, for most taxa, a high proportion of flight activity tends to be concentrated near the stream. This pattern is often more pronounced in taxa with traits that restrict flight. Among stoneflies (Plecoptera), for example, members of the Euholognatha tend to move farther inland than the Systellognatha (Kuusela & Huusko 1996). Most adult Systellognatha have mouthparts incapable of feeding and they have shorter lifespans compared to the Euholognatha, which disperse inland to feed. Although there are a few notable exceptions to this generalization (see Sheldon 1984), published studies of stonefly dispersal have shown euholognaths to comprise the vast majority of species caught in significant numbers away from the stream (Kuusela & Huusko 1996, Griffith *et al.* 1998, Petersen *et al.* 1999, 2004, Briers *et al.* 2002, 2004, Macneale *et al.* 2005). Even these cases usually demonstrated a rapid decrease in abundance with lateral distance, however.

Slightly more lateral dispersal studies have focused on caddisflies (Trichoptera), probably because they are among the strongest flying stream insects (some Diptera excepted – see below). Larger-bodied caddisflies, especially members of the families Limnephilidae and Hydropsychidae, for example, have been caught consistently in light traps >1 km from the nearest aquatic habitat (Svensson 1974, Kovats *et al.* 1996). Kovats *et al.* (1996) combined observations of average lateral dispersal distance with body size measurements among species and found a positive correlation between the two. Indeed, larger-bodied insects in general have been hypothesized to be relatively better dispersers (Roff 1977); and relative wing length has also been suggested to be important to aquatic insect flight (e.g. Corkum 1987, Malmqvist 2000). Still, although caddisflies as a group (especially the larger-bodied ones) have been caught in greater abundances at greater distances from streams than stoneflies or mayflies (Ephemeroptera), the general pattern of decreasing abundance with increasing lateral distance holds in most cases (Svensson 1974, Jackson & Resh 1989, Sode & Wiberg-Larsen 1993, Kovats *et al.* 1996, Collier & Smith 1998, Griffith *et al.* 1998, Petersen *et al.* 1999, 2004).

Little work has been done on lateral dispersal of Ephemeroptera, perhaps because it is often simply assumed that they remain near the stream due to the adults' short life span and inability to feed. Nonetheless, inland movement of mayflies is potentially important because they undergo a final terrestrial molt that probably takes place at some distance from the natal stream (Kovats *et al.* 1996). In the few studies that have included mayflies, they have consistently shown a significant decrease in abundance with lateral distance inland (Jackson & Resh 1989, Petersen *et al.* 2004); however, a species of *Hexagenia* has been light-trapped 5 km from its source despite demonstrating a greater than exponential rate of lateral decrease (Kovats *et al.* 1996).

Overall, representatives of Diptera are thought to be among the most mobile aquatic insects in their adult stages (Sheldon 1984). This notion seems to hold particularly true for the stream-dwelling black flies (Simuliidae), which have been recorded at extremely long distances (up to >30 km) from the nearest larval habitat (Bennett & Fallis 1971, Baldwin *et al.* 1975, Choe *et al.* 1984). Nevertheless, there are probably exceptions. The black flies in the above studies were anautogenous (requiring a blood meal for egg maturation). Conversely, it has been speculated that autogenous simuliid species are likely much poorer dispersers since they needn't leave a natal stream habitat in search of a blood meal (e.g. Rothfels 1981, Snyder & Linton 1984). Indeed, population genetic studies of two autogenous species have revealed an extremely high level of population structure across a small spatial extent, suggesting limited flight dispersal (Chapter 3, 4). To my knowledge, however, no direct trapping studies have been completed on autogenous species, and no research has focused specifically on lateral dispersal functions of distance from natal stream for any Simuliidae.

Given results of lateral dispersal studies thus far, then, we can broadly generalize that most adult stream insects stay near the natal stream while few individuals are more wide-ranging (although anautogenous black flies probably break this overall "rule"). Beyond this generalization, different types of terrestrial habitat may influence movement behavior. It has been suggested, for example, that emerged insects may be less abundant but fly farther on average in open than in forested landscapes (Delettre & Morvan 2000, Collier & Smith 1998). This type of response may help explain the distant but rare catches reported by Kovats *et al.* (1996) since their study area was primarily agricultural. Higher abundance in the trees vs. open has been attributed to a more desirable microclimate (low wind, increased relative humidity, Myers *et al.* 2001, Briers & Gee 2004). Comparative studies of lateral dispersal in watersheds having different land cover have found ambiguous results (Briers *et al.* 2002, Petersen *et al.* 2004), but species-specific responses to structure of the terrestrial landscape may vary and help resolve some ambiguity.

Weather conditions are also probably important factors influencing insect flight activity and lateral movement tendencies. Wind has been speculated to be an important passive dispersal mechanism (Cushing & Gaines 1989, Crosskey 1990, Kovats *et al.* 1996, Kuusela & Huusko 1996, Briers *et al.* 2004), although many insects have lower flight activity in higher wind speeds probably because they are less likely to initiate flight (Choe *et al.* 1984, Roberts & Irving-Bell 1996, Briers *et al.* 2003). Strong-flying Trichoptera provide at least one exception however, their activity having been shown to be unaffected by ambient wind speeds (Waringer 1991). In one case caddisflies were even reported to orient flight *into* the wind (Usseglio-Polatera 1987, as cited by Sode & Wiberg-Larsen 1993). However, wind is probably less influential in the forested landscapes where many lateral dispersal studies have taken place because dense trees increase the depth of the atmospheric boundary layer (e.g. Jackson & Resh 1989, Briers *et al.* 2003). Thus, the influence of wind speed on lateral movement patterns is little known but may be significant in wind-prone locations given its differential influence on flight activity.

Air temperature also affects flight, and in most insects both flight activity and distance traveled tend to increase with increasing air temperature (see Roff 1977). Even for caddisflies, who are little affected by wind, air temperature is apparently quite important. Collier & Smith (1998) reported low light trapping success on a night when air temperature dipped to 0°C and cited unpublished work that showed a minimum flight threshold of 9°C for caddisflies in their region. Similarly, Waringer (1991) found air temperature to be the most important meteorological influence on caddis flight, which ceased below 7°C. Similarly, most black flies cease flight at air temperatures 8-10°C (Crosskey 1990), and stoneflies have a similar response (Briers *et al.* 2003). Some stoneflies cease flight and move by crawling along the ground in "cool" temperatures (Kuusela & Huusko 1996). Indeed, crawling rather than flying seems to be a ubiquitous response of insects to cold temperatures (see Downes 1965).

As hinted above, most prior studies of lateral flight dispersal from streams have been in forested landscapes and all have been in low-elevation, subarctic regions. Little is known about insect dispersal away from streams in harsher climates that may be more prone to wind, low air temperatures, and low humidity, although much has been speculated. For example, many insects in arctic and alpine regions have evolved flight-limiting mechanisms, such as aptery or brachyptery, parthenogenesis to eliminate the need for mating flights, and autogeny in typically blood-feeding taxa (Downes 1965). Such traits are probably adaptations to avoid exposure and energy loss to the cold and dry terrestrial environment, especially when local breeding habitats are stable (e.g. Roff 1990). It is a reasonable hypothesis therefore that flight activity lateral to the stream is limited in these environments (see Nilsson *et al.* 1993). Arctic and alpine regions in particular are also treeless and windy, however, so it may be expected that at least some species have the potential to disperse long distances, perhaps passively in the wind.

My objective was to evaluate lateral flight from a permanent first-order alpine stream in Rocky Mountain National Park (RMNP), Colorado, USA. Physically, alpine regions differ from the Arctic in their high degree of topographic relief which contributes to the development of high spatial variation in snowpack depth (see Bowman 2001). Many alpine streams occupying somewhat protected valleys accumulate a thick layer of snow that remains for a large proportion of the year. Water temperatures remain just above freezing under the snowpack (D.S. Finn, unpublished data), and the insect emergence period is limited to a short growing season.

In Rocky Mountain streams, insect diversity is low in alpine reaches compared to further downstream (Allan 1975, Ward 1986, Finn & Poff 2005); therefore, I did not limit my investigation to only one or two taxa as have many other lateral dispersal studies in higher-diversity streams. I broadened taxonomic focus to include all Ephemeroptera, Plecoptera, Trichoptera (EPT) and Simuliidae in order to obtain as many taxa as possible for comparison. I used traps designed to intercept flying insects only, the catches from which were used to reveal flight activity as a function of distance from the stream bank, and I report results from two consecutive years of collection.

I hypothesized that, due to the short emergence and breeding season and relatively harsh terrestrial climate, overall insect flight would be concentrated near the stream and show a steep lateral decrease in abundance. An additional expectation, however, was that weaker fliers (e.g. some mayflies and stoneflies) would be passively dispersed by the wind and therefore would be caught in greater numbers and at greater distances in downwind vs. upwind traps. Given broad taxonomic differences suggested in prior studies, I also hypothesized that there would be predictable differences in lateral flight between each of the four higher taxa in traps set upwind of the natal stream. Thus, anautogenous Simuliidae should show the most extensive lateral dispersal, followed by Trichoptera, and that Ephemeroptera, Plecoptera, and autogenous Simuliidae would show the steepest declines in abundance with distance.

MATERIALS & METHODS

Study site.

Adult stream insects were collected at an alpine headwater reach of the S. Fork Cache la Poudre River near the northern boundary of RMNP. At this location, the stream has an average width of 1.6 m and bankfull depth 34 cm. At an altitude of 3416 m a.s.l., the reach lies above the permanent treeline in a north-facing valley, but it is unusual among alpine streams in the area in that the valley it occupies is relatively wide and low-gradient with a broad riparian zone dominated by low-lying willow (*Salix* spp., Fig. 5.1). Just below treeline, the stream plunges into a steep and confined section that is more representative of high-elevation streams in the area.

The terrain extending from the east bank of the study reach slopes gradually upwards to ca. 45 m perpendicular distance from the stream, after which the riparian zone ends at a short slope to more barren terrain. The west side of the stream is flat and remains moist to ca. 65 m distance from the bank and houses several snowmelt pools throughout most of the summer growing season. A steep, rocky slope extends up to a ridgeline beyond 65 meters west.

Flow regime is driven by snowmelt (sensu Poff & Ward 1989), and stable summer flow is maintained via a permanent snowfield (see Fig. 5.1). In a typical year, the stream is completely covered in snow from late October to mid June, with snow depths probably exceeding three meters in mid-winter. Indeed, snow depth was two meters on 21 May, 2003 (D.S. Finn, personal observation). In the summer emergence season (late June to late September) water temperature averages ca. 5°C (range 0.5-13.5°C), and mean air temperature is ca. 9°C (range from below freezing to 20°C). In the Rocky Mountains, the prevailing wind is from the west, and high winds are the norm above treeline (Greenland & Losleben 2001).

Data collection.

On 8 July 2002, I set a transect of four Malaise traps as an exploratory study to test if 1) traps could be maintained through the entire emergence season despite strong winds above treeline, and 2) enough insects of any species could be trapped to gain statistical power for analyses of dispersal potential. Malaise traps were used because they are non-attracting and thus their collections better represent the natural spatial occurrence of flying insects than do collections using light or other attractive traps. The traps were two meters in both length and height with walls black and roof white nylon (similar to those described in Sode & Wiberg-Larsen 1993), and each had attachment points for six wooden support poles 3-4 cm in diameter. I also rigged several guy lines on each trap using parachute cord and tent stakes for added support against wind. The roof sloped up to a single collecting head half-filled with a 50% propylene glycol solution for preservation of insects in the field.

I made the 2002 collections on the east side of the stream due to the prevailing western winds and my hypothesis that more insects would be caught on the downwind side after emergence and passive transport. Traps were set in a perpendicular transect from the stream at distances of 1, 10, 30, and 60 meters from the bank. The long axis of each trap was set parallel to the stream, and traps were slightly offset from a direct perpendicular to prevent interference between them and to respect interfering elements of the landscape (such as large willow shrubs and boulders).

I transferred collections from each trap when heads became full; therefore, collections were made more frequently during parts of the season with greater insect flight activity. In the laboratory, insects were transferred to 95% ethanol, separated, and sexed. Species assignments were made for all stoneflies (B.C. Kondratieff, Dept. of Bioagricultural and Pest Management, Colorado State University), mayflies (B.C. Kondratieff), caddisflies (D.E. Ruiter, Lakewood, CO), and blackflies (P.H. Adler, Division of Entomology, Clemson University). Females of the mayfly genus *Cinygmula* could not be identified to the species level and were therefore recorded as *Cinygmula* spp.

After successful trapping in 2002, I repeated the design in 2003 including the original east-side transect as well as a replicate transect on the west side of the stream. In 2003, I set all traps immediately after snow had melted enough to expose the stream surface (on 23 June) with the intent to begin trapping as soon as insect emergence was possible. In order to start collections at this time, it was necessary to dig out a large snow bank for placement of the 60 m west trap, which was then set in saturated soil. That trap blew down sometime during the next three days and its stability remained a problem until 3 July. Future analyses accounted for this potential data loss (see below).

In 2002, collections continued until 22 September and in 2003 until 21 September. By this time, the first snows of the season have fallen, temperatures consistently fall well below 0°C at night, willows have begun winter defoliation, and stream insects have completed their emergence periods.

Analyses.

For this study, there were 12 total traps distributed evenly among three different transects: one on the east side in 2002 (hereafter termed "2002"), one on the east side in 2003 ("2003E"), and one on the west side in 2003 ("2003W"). I compared overall species richness, sex ratios, and emergence phenology of aquatic insects between years using total catches for 2002 (one transect only) and 2003 (2003E + 2003W). Species richness was calculated as the total number of species caught in each year for each of the EPT and Simuliidae. Sex ratios were determined for species common in both collection years (at least 25 individuals per year) and also for each of the four higher taxonomic levels. Significant deviations from 1:1 were determined for each taxon/year using *G* log-likelihood statistics (Zar 1996). Emergence phenology was also compared between years for common species by pooling the total catch for each year and plotting cumulative proportion of total caught vs. date.

Most of the remaining analyses were concerned with various biological trends with distance from the stream and with comparisons of these patterns between years and between the east and west sides of the stream. Hereafter, comparisons between years use only 2002 and 2003E transects; and comparisons between sides of the stream use only 2003E and 2003W transects. Furthermore, east-west comparisons use only data from after 3 July, the date after which the 60 m west trap was fully functional (see above). I tested for differences in total abundance (across all traps) of each taxon between the east and west sides using *G* log-likelihood statistics (as with sex ratios, above). For the remaining lateral distribution analyses, data used were per trap total abundances pooled across the whole emergence season.

I looked for relationships between distance from the stream and 1) species richness, 2) sex ratios (arcsin-squareroot percent females) of common species and Simuliidae, and 3) numbers of individuals of common species and higher-level taxa. For each of these relationships, I fit linear, exponential, and power functions, each of which reveals a different rate of change with linear distance from the stream. To compare lateral patterns of abundance (#3 above) for each taxon, I chose the model that demonstrated the best fit across all three transects and across all species in a higher-level taxon. I then used the best-fit model to compare slopes and intercepts of fitted models using analysis of covariance (ANCOVA, SAS Institute, Inc. 2003) with distance from stream as the covariate, and either side or year as the main factor, depending on the analysis. Thus, a significant ($\alpha = 0.05$) main effect indicates a difference in intercept, and a significant interaction with distance indicates different slopes. I also used ANCOVA to compare patterns of species richness and percent females with distance between years.

RESULTS

Overall catch statistics.

See Appendix for a complete list of Ephemeroptera, Plecoptera, Trichoptera, and Simuliidae males and females caught per trap in 2002 and 2003.

Ephemeroptera. In 2002, I collected 125 total mayfly individuals representing four species. *Cinygmula par* Eaton and *C. ramaleyi* Dodds males were identified; however, these two genera were pooled for all further analyses because females were not distinguishable. Thus, *Cinygmula* spp. represented 56% of total mayfly abundance in 2002, followed by *Ameletus celer* McDunnough, which represented 38% of the total. In 2003, I caught 201 total mayflies representing 5 species. Again, *Cinygmula* spp. were numerically dominant comprising 52% of the total catch, and *A. celer* followed at 46%.

Plecoptera. I found a total of 213 individual stoneflies distributed among nine species in the four 2002 traps. A single dominant species (*Zapada haysi* Ricker) accounted for 56% of the catch. *Alloperla pilosa* Needham & Claassen was the next most abundant species, comprising 18% of the catch, and other species individually accounted for <7% of the total. In 2003, 879 individual stoneflies across 17 species were caught in eight traps. Again, *Z. haysi* was the dominant in 2003, making up 76% of the total. *A. pilosa* was second most abundant species, comprising 7% of the 2003 catch. All other species made up ≤5% of total 2003 abundance.

Trichoptera. I caught 155 individual caddisflies representing 12 species in 2002. The numerically dominant species was *Asynarchus nigriculus* Banks (54%), followed by *Limnephilus picturatus* MacLachlan (17%) and *L. abbreviatus* Banks (10%). The genus *Limnephilus* comprised 36% of all caddisflies caught; however, many of these may have arisen from lentic snowmelt pools in the area (B.C. Kondratieff, personal communication), so I did not use this genus for dispersal analyses. *A. nigriculus* has also been shown to breed predominantly in ephemeral lentic habitats in the southern Rockies >200 km southwest of this study area (e.g. Wissinger *et al.* 2003); however, its larvae have been found in the current study reach (D.S. Finn, unpublished data) and in another permanent alpine stream in RMNP (Finn & Poff 2005) so I included this species in lateral distribution analyses.

In 2003, I caught 317 caddisflies, a number only slightly greater than double the total from 2002 in double the number of nets. Despite this neat doubling of the totals between years, there were several differences in relative abundance. *A. nigriculus* was again the dominant species, making up 51% of the total. The second most abundant in 2003 was *Allomyia chama* Denning (at 18%), a species that comprised only slightly >1% of total 2002 catch, followed by *L. coloradensis* Banks at 10% of the catch. The second-most-abundant species in 2002, *L. picturatus*, made up only 3% of the 2003 catch.

Simuliidae. In 2002, there were 69 black flies caught across the four traps. Nine species were present, but a single species (*Stegopterna acra* Currie, Adler & Wood) comprised 77% of the total individuals. The next most abundant species [complex] was *Simulium aureum* at 6%. In 2003, I caught 904 individuals across the eight traps, and 18 species were represented. *St. acra* was again the numerical dominant, although not to the extent in 2002 (27.4%), and *S. silvestre* Rubtsov, a species not encountered in 2002, made up 25.9% of the 2003 catch. Although simuliid richness in Malaise traps doubled from 2002-2003, there were three species (*S. pugetense* Dyar & Shannon, *S. venustum* complex, and *Prosimulium daviesi* Peterson & DeFoliart) caught in 2002 but not in 2003. A female and several males of an undescribed species of *Prosimulium* were identified from 2003 catches; additionally, several *Greniera denaria* Davies, Peterson & Wood females were discovered and are a state record for Colorado and only the third record for the US (see Appendix).

Comparisons of overall catch between years.

Species richness of emerged aquatic insects caught in Malaise traps was consistently higher in 2003 than 2002 for each of the Ephemeroptera, Plecoptera, Trichoptera, and Simuliidae (Table 5.1). For mayflies, richness varied between years by only a single species (which was represented by a single individual, see Appendix), but it doubled or nearly-doubled for stoneflies and Simuliidae and increased by 50% for caddisflies. Similarly to species richness patterns, total individuals caught increased dramatically from 2002 to 2003 for stoneflies and blackflies, but not for mayflies or caddisflies (after accounting for the doubling in number of traps in 2003).

Sex ratios were consistently male-dominated in both collection years for the EPT (Table 5.1). In each case of sex ratios differing significantly from 1:1, males outnumbered females except in the black flies, where there was a significant excess of females caught in each year. In some cases, the male-biased sex ratio was extreme; for example, only 3 females were caught overall for *Ameletus celer*, compared to 138 males (see Table 5.1).

A comparison of emergence phenologies between years demonstrated that for five of the six most common species across the EPT and Simuliidae (*Asynarchus nigriculus*, *Ameletus celer*, *Cinygmula* spp, *Stegopterna acra*, and *Alloperla pilosa*) a higher proportion of total emergence was achieved at earlier dates in 2002 vs. 2003 (Fig. 5.2). For the single remaining common species (*Zapada haysi*), the data showed no difference in temporal emergence patterns between years. *Z. haysi* is the commonest early-emerging species, leaving the stream as soon as melting ice and snow allow an exit.

Lateral patterns.

Ephemeroptera, Plecoptera, and Trichoptera each demonstrated a significant decrease in numbers of individuals caught with distance from the east stream bank in at least one of the two collection years (Table 5.2, Figs. 5.3-5.5). The best-fit model for Ephemeroptera and Plecoptera was an inverse power function (Table 5.2), which demonstrates the most rapid rate of decline with distance of the three models tested. In comparing these functions between years for Ephemeroptera (Fig. 5.3a) and Plecoptera (Fig. 5.4a), there was a significant difference in intercepts, reflecting differences in abundance between years; however, there was no difference in slope in either case. For Trichoptera (Table 5.2, Fig. 5.5a), there was a significant decrease with distance only for 2003, and a negative exponential model gave the best fit. In 2002, there were many fewer individual caddisflies caught at the farthest distance (60 m) but similar numbers caught at each of the three traps closer to the stream (1, 10, and 30 m). The Simuliidae showed no evidence of a decrease in numbers caught with distance for either collection year (Table 5.2, Fig. 5.6). Indeed, there were far more black flies caught in the 60 m east trap than any other in 2003 (Fig. 5.6a).

The most common species within each of the EPT and Simuliidae (*Cinygmula* spp., *Zapada haysi*, *Asynarchus nigriculus*, and *Stegopterna acra*) showed east-side lateral abundance patterns similar to the overall average for each of the respective higher-level taxa (Table 5.2, Figs. 5.3b-5.6b). The less-common species (that were still common enough for analysis) within the Ephemeroptera and Plecoptera, however, revealed differences from higher-level patterns. The mayfly *Ameletus celer* did not significantly decline in abundance with distance from the stream in either year (Fig. 5.3c). *Cinygmula* spp., conversely, had the steepest slopes of any species (Table 5.2), showing that they declined rapidly with distance in both years (Fig. 5.3b) and probably accounted for the overall significantly declining function in the total mayflies despite the opposing influence of *A. celer*. The stonefly *Alloperla pilosa* showed a significant decrease with distance from stream (as an inverse power function) in both years as did stoneflies as a whole (Table 5.2, Fig. 5.4c); however, intercepts did not vary for *A. pilosa* between years ($p = 0.97$). For all taxa studied, >50% of total abundance caught was recorded from within 10 meters of the east side of the stream except for the mayfly *Ameletus celer* (Table 5.2).

Species richness declined significantly with distance from the east stream bank in both years (Fig. 5.7). A negative linear function gave the best fit when species richness was measured across all four higher-level taxa (Fig. 5.7a), in which case there was a significant difference in intercept between years (reflecting temporal differences in total richness) but no difference in slope. When total species richness was plotted vs. distance without including Simuliidae species (abundances of which had no relationship to distance from the stream, as above), the best-fit model for both 2002 and 2003 collections was an inverse power function (Fig. 5.7b). Neither slope nor intercept was significantly different between years when simuliid species were excluded.

Sex ratios did not change significantly with distance from the stream for any taxon at any transect. Fig. 5.8 shows the proportional distribution of males and females with distance from the stream along representative transects for the five common species in the EPT and for Simuliidae as a whole. The plots demonstrate the overall patterns in sex ratio (Table 5.1) but no distance effect.

In 2003, there were differences between the east and west-side transects with respect to total abundance and lateral distribution patterns for several of the common taxa. Total numbers caught on east vs. west sides varied significantly from 1:1 for *Asynarchus nigriculus*, *Ameletus celer*, and *Stegopterna acra* (Table 5.3). In each case, there were significantly more individuals caught on the west vs. the east side of the stream. The extreme differences in distribution between sides for *A. celer* probably drove the significant overall pattern for Ephemeroptera (Table 5.3).

Lateral distribution patterns for Trichoptera (Fig. 5.9a) and Plecoptera (Fig. 5.10a) did not vary between sides in slope or intercept. For the most common species within these orders, patterns were similar (Figs. 5.9b & 5.10b); however, the less common stonefly *Alloperla pilosa* showed a significant difference in intercepts between sides as well as a near-significant difference in slopes (Fig. 5.10c). Both slope and intercept were greater on the east side.

For Ephemeroptera, richness declined as an inverse power function on the east side (as in Fig. 5.3a); however, there was not a significant decrease in number with distance on the west side of the stream, and the 60 m west trap caught the highest number of any of the west-side traps (Fig. 5.11a). This pattern was driven by *Ameletus celer*, which showed no evidence of decreasing with distance on either side of the stream, indeed reflecting an unexpected increase in numbers with distance on the west side (Fig. 5.11c). The slightly more common *Cinygmula* spp. decreased as a power function of distance away from both sides of the stream (Fig. 5.11b); however, differences in slope and intercept were both significant and suggested the same general differences between sides of the stream as in *Alloperla pilosa* above.

As with the 2002-2003 comparisons for the Simuliidae, there was no evidence of a decrease in numbers caught with distance from the stream, and patterns with distance did not vary consistently between sides (Fig. 5.12a). Figure 5.12b, however, reflects the significantly greater proportion of the common species *Stegopterna acra* on the west side.

DISCUSSION

Overall lateral distribution patterns.

In general, results support the main hypothesis that most insect flight is concentrated near the stream in the alpine environment, thereby also matching generalizations about lateral movement suggested in previous studies. Furthermore, average dispersal patterns compared between each of the four major higher-level taxa (Ephemeroptera, Plecoptera, Trichoptera, and Simuliidae) met hypothesized expectations. Mayflies and stoneflies on average focused most activity near the stream and steeply decreased in abundance with distance (as a power function); caddisfly flight activity decreased with distance also, but at a slower rate (exponential function); and anautogenous black flies showed no evidence of a lateral decrease. Additionally, total species richness significantly decreased with distance from the stream, suggesting that most species that I encountered followed the pattern of decreasing lateral abundance, thereby leading to the absence of rare species except near the stream.

Interestingly, the lateral decrease in species richness took different forms depending on whether Simuliidae were included. Their exclusion showed richness as an inverse power function of distance in both years, suggesting an extreme decay in diversity with distance from the stream. When black flies were included in the species richness analysis, there was still a significant decrease with distance, but only as a much less steeply declining linear function. Most of the black flies caught did not show a lateral decrease in abundance (and they were quite speciose); thus, they so drove the differences in overall richness patterns.

A probable explanation for their widespread flight is that all of the simuliid species that I collected were anautogenous. I expected autogenous species to disperse much less widely because they do not need to look for a blood meal. Autogenous species are known to breed in RMNP alpine streams, and their larvae have been found in a stream 1 km from the current study site (Chapter 3). Thus, my failure to capture any in this study was likely either because they are absent or too rare to be detected in the study stream or they do not actively fly as adults. The only way to resolve this issue is to set flight traps at streams where autogenous species have substantial known populations.

Significant differences between taxa with respect to lateral flight dispersal support a contention that broad generalizations about dispersal behavior in aquatic invertebrates are typically unwarranted and that, rather, specific information about species differences may be ecologically more meaningful (Bohonak & Jenkins 2003). The mayfly *Ameletus* *celer* provided a compelling example of the potential for high variation among species even within the same higher taxon. Other mayflies in which lateral dispersal has been studied (see Kovats *et al.* 1996, Petersen *et al.* 2004), including the other common species here (*Cinygmula* spp.), have shown rapid (greater than exponential) decrease in abundance with distance from stream. *A. celer* on the contrary showed no evidence of lateral decrease and even demonstrated an increase in abundance with distance in one transect (Fig. 5.11c).

A. celer is a relatively large-bodied mayfly (see Pritchard & Zloty 1994) in which the male also has exceptionally large eyes, both traits associated with active flight. Species of *Ameletus* in general may be more resistant to various environmental disturbances (e.g. see Rosemond *et al.* 1992, Rader & Belish 1999) and also may be broadly generalist feeders as nymphs (e.g. Mihuc & Minshall 1995). Indeed, the genus *Ameletus* also occupies a wide elevation range (e.g. Ward 1986, Finn & Poff 2005) although little is known about how much of that range is occupied by *A. celer* specifically. Generalism typically promotes higher dispersal capacity because the short-term costs of dispersal are more likely to be outweighed by the longer-term benefits of the high probability of encountering locations of suitable habitat. Specialists on a rare (and stable) habitat type, conversely, tend to be poor dispersers (see Roff 1990, Wishart & Hughes 2003). Essentially then, traits that may influence flight activity vary from one species to the next and may cause large and unexpected variation around the mean behavior within a higher taxon.

Secondary to the general support for the first hypothesis, I have little evidence that insect flight activity and lateral dispersal were somehow limited to a greater extent at this alpine stream reach than at lower-elevation, forested streams that have climatic conditions more conducible to flight. The generalist vs. specialist argument may apply here to a broader extent. Almost none of the flying species that I trapped were alpine zone specialists. Certainly, several of them were species known to occupy the upper extents of the longitudinal gradient in Rocky Mountain streams (e.g. *Allomyia chama*, *Prosimulium travisi* Stone, Ward 1986; *Megarcys signata* Hagen, *Zapada haysi, Cinygmula* spp., Finn & Poff 2005), and others may be limited to high-elevation aquatic habitats, given their high densities there (including *Asynarchus nigriculus*, Mihuc & Toetz 1996, Wissinger *et al.* 2003). Very few, however, were species thought to be alpine specialists, and these were caught in low abundance (e.g. *Prosimulium frohnei* Sommerman and one of the two species in the *Simulium aureum* complex, see Appendix).

Alpine areas are typically small and patchily distributed, allowing for extensive ecotonal areas at the timberline and leading to the presence of many generalist species (see Nilsson *et al.* 1993). This condition applies along the longitudinal gradients of streams originating in alpine areas as well (Ward 1994). Perhaps the reason I trapped primarily generalist species has to do with both 1) they are more abundant than specialists at the study site, and 2) specialists may be expected to fly less (and thus not be caught by Malaise traps). Indeed, in a previous study of the distribution of species traits along the longitudinal gradients of mountain streams in the RMNP area, Finn & Poff (2005) found that higher dispersal abilities tended to be associated with higher elevations. This finding may reflect greater proportions of generalist species in the higher-elevation reaches.

The current study took place across a relatively short spatial extent in an alpine stream occupying an unusually broad valley with extensive riparian lowlands, and all traps were either within or just beyond the riparian zone. Despite the overall "normal" (compared to other lateral dispersal studies) patterns demonstrated by the generalist taxa that I caught, it is likely that dispersal barriers that exist at a broader spatial scale are more effective in limiting flight. The extreme topographic heterogeneity characteristic of alpine zones leads to drainage divides that are significantly higher in elevation than streams. Vegetation thins, air temperatures decrease and winds increase with increasing altitude. Indeed, in Chapter 3 I demonstrated that these barriers act predictably to limit gene flow in an autogenous alpine specialist black fly. Therefore, even for generalists that are more active in flight, these topographic barriers may limit between-stream movement. Further research is needed to assess flight dispersal of these species across greater spatial extents.

Distributions on east vs. west side of stream.

The prevalent western wind had no apparent effect on flight activity at the scale of this study. In many cases, abundance of flying insects was significantly greater on the *upwind* (west) side of the stream, even for taxa traditionally considered to be weak fliers possibly passively transported by wind (mayflies and stoneflies, Corkum 1987, Kuusela & Huusko 1996, Briers *et al.* 2004). Indeed, for some mayfly (*Cinygmula* spp.) and stonefly (*Alloperla pilosa*) taxa that showed a significant decrease in abundance with distance on both sides of the stream, the relationship was significantly steeper on the downwind east side, suggesting directed upwind flight.

The terrestrial habitat on either side of the stream had differences that probably outweighed the influence of wind direction. Due to the western winds, a large amount of snow is deposited each winter on the leeward east sides of ridges. Thus, the ridge that begins just beyond the study area on the west side of the stream maintains a snowpack that creates several small, unmapped snowmelt streams that flow for most of the summer season. The west side also maintains several small snowmelt pools in greater abundance than the east side, which is drier (although still willow-covered). These differences may affect differences in flight activity between sides in one of two ways: 1) some aquatic insects may have emerged from the snowmelt pools or streams on the west side, thereby increasing abundances there; 2) adult insects that emerged from the study stream may prefer the moister habitat on the west side.

Although some of the species (primarily caddisflies) that I trapped may have emerged from the lentic habitats created by snowmelt pools on the west side, I argue that the effects of these were probably minimal in this study for two reasons. First, differences in Trichoptera abundance between sides were not as extensive as differences for other strictly lotic taxa (see Table 5.3). Second, in other lateral dispersal studies that had a similar situation, authors have demonstrated that >95% of all trapped flying insects originated from the study stream rather than the smaller lentic habitats scattered across the landscape (Svensson 1974, Sode & Wiberg-Larsen 1993).

It is also possible that some of the trapped species emerged from the temporary snowmelt streams west of the main study stream. For example, larvae of the black flies *Greniera denaria* and *Simulium curriei* Adler & Wood are known to occur in small seeps or rills (Adler *et al.* 2004). Other lotic species that can overwinter as resistant eggs and survive on the available resources may also have emerged from these temporary streams. However, any of these streams that were within 100 m of any Malaise trap in the current study were of an extremely small size and ephemeral nature; therefore, I argue that the numbers of individuals emerging from these habitats were probably greatly outweighed by the numbers emerging from the main study reach and thus their influence on lateral distribution patterns is probably negligible.

It is more likely that the moister terrestrial habitat on the west side of the stream influenced the asymmetrical distributions of most of the common taxa by being attractive to the newly-emerged adults. Adult aquatic insects previously have been suggested to be more abundant in areas with standing water and/or higher local relative humidities (e.g. Shipp *et al.* 1987, Myers *et al.* 2001, Briers *et al.* 2002, Macneale *et al.* 2005), and relative humidity has been demonstrated to significantly increase the lifespan of some caddisflies (Collier & Smith 2000). Within the small spatial extent of the study area, the flatter and wetter areas on the west side of the stream were probably detected by emerging insects, and flight was oriented towards them.

Although the mean condition at the study site included strong winds from the west, local weather patterns are heterogeneously distributed in time, and calm conditions do exist, often in the morning (D. Finn, personal observation). Many insects are not likely to attempt to fly when the wind is strong (e.g. Briers *et al.* 2003), and most flight activity is concentrated in these calm periods of little or no wind, thereby allowing local habitat choice to trump the prevailing wind direction in determining the spatial distribution of insects at this spatial scale.

Sex ratios.

Overall sex ratios were significantly different from 1:1 for all higher taxa in 2003, and for all but Plecoptera in 2002 (see below for an explanation of this discrepancy). In most cases, significance was strong ($p < 0.001$, Table 5.1). Males dominated the catch in all but the Simuliidae, where females dominated. These patterns did not change predictably with lateral distance from the stream for any taxon.

Within the EPT, several studies using non-attractant traps have demonstrated a higher proportion of female activity near the stream, and increasing male activity with lateral distance (Svensson 1974, Jackson & Resh 1989, Sode & Wiberg-Larsen 1993, Kuusela & Huusko 1996, Collier & Smith 1998, Petersen *et al.* 1999, 2004). This pattern has been explained in terms of reproductive costs in females. The more energy used for flight, the less is available to invest in egg production (cf. Rankin & Burchstead 1992); hence females are less likely than males to pursue active flight and to disperse long distances. Indeed, Hughes *et al.* (2003) found indirect genetic evidence for restricted female dispersal in a Rocky Mountain mayfly, *Baetis bicaudatus* Dodds, which also occurs at my study site.

In anautogenous Simuliidae, the expected pattern is reversed. Although males are still relatively active in flight because it is a good investment for them to locate females and form mating swarms, it is only the female that requires a blood meal for full maturation of her eggs. Once a blood meal has been searched for and located, further flight is typically necessary to locate an oviposition site. Hence, males are more often found associated with plants near their natal streams while females may undergo long-distance dispersal (Crosskey 1990).

The sex ratios that I observed in Malaise traps are from a sample of the population that included only actively-flying insects lateral to the stream. Certainly, then, this sample does not accurately represent the actual sex ratios of the entire emerging population – only the actively flying portion. Because there was no difference in sex ratio with lateral distance, the inference is that at this stream, *all* inland flight activity is dominated by one sex (males in EPT; females in Simuliidae), and these unbalanced sex ratios make sense in light of the biology of these taxa. The harsher terrestrial conditions of the alpine zone may have influenced the extreme unbalance in sex ratios in many species, potentially driving the more weakly-flying sex to become even more sedentary.

2002-2003 comparisons.

I collected a greater abundance of several species as well as higher species richness in 2003 vs. 2002. Certainly, my intention in setting traps just after snowmelt in 2003 was to capture a greater portion of the total emergence period, and I seem to have achieved this goal. Nonetheless, the differences between years are striking since there was only a span of two weeks between 2002 and 2003 start dates. Furthermore, total species richness for 2003 does not change even if the first two weeks of data are excluded. In 2003, species that emerged during the first two weeks of trapping continued to emerge well beyond the 2002 start date. Therefore, even when holding start date constant, species richness was significantly greater in 2003 than 2002, and differences in total abundance were affected only by a few early-emerging species.

These patterns may be explained at least partially by delayed emergence in 2003 vs. 2002. Fig. 5.2 shows that for all common species except one, similar proportions of total emergence (as of the late Sept. termination date) were achieved up to one month earlier in 2002. Therefore, emergence of adult insects from the study stream may have begun up to a month earlier that year. Unfortunately, I did not visit the study stream in 2002 until 21 June, at which time it and the riparian zone were completely free of snow; however, another alpine stream in RMNP that is higher in elevation (3538 m a.s.l.) and similar in aspect (northeast-flowing) was snow-free on a 5 June 2002 sample date (D. Finn, personal observation). Thus, despite similar start dates for Malaise trapping, I likely captured most of the emergence period in 2003 (start date 23 June), while the early tail of the emergence distribution was probably left out in 2002 due to snowmelt and emergence beginning up to a month earlier.

The most abundant stonefly species *Zapada haysi* was among the earliest-emerging of all species collected, and it showed the most significant between-year differences in adult population parameters (according to my trapping regime). *Z. haysi* abundance increased by >5X (>2.5X when controlling for number of traps) and sex ratios shifted from near 1:1 to extremely male-dominated from 2002 to 2003 (Table 5.1). Both of these shifts can be explained by differences in trapping schedule relative to emergence timing between years. Greater abundance in 2003 was probably simply due to the inclusion of more of the total emergence period. Also, males emerge earlier than females in this species (Cather & Gaufin 1976), thereby providing an explanation for the increased male domination of the sex ratio in 2003.

Later-emerging species showed either no effect or an opposite trend in abundance of flying adults between years, with the exception of the black flies. After controlling for the difference in numbers of traps each year, total numbers caught of the two dominant mayfly species and the caddisfly *Asynarchus nigriculus* were lower in 2003 vs. 2002.

Peak emergence was shifted, especially for the mayflies, to a later part of the season with colder air temperatures. The negative effects of cold air on flight activity may help explain these between-year differences in flight activity for the late-emerging species.

As hinted above, differences in snowpack between years probably drove the differences in emergence timing. In 2002, total snowfall the previous winter at a nearby RMNP subalpine zone location was 3.7 m; in 2003, it was 7.6 m (RMNP Bear Lake Dispatch). A larger amount of snow yields a higher spring runoff, in addition to snow continuing to cover alpine streams for a longer duration, given similar air temperature and insolation in the spring. Fig. 5.13 shows the hydrographs during peak runoff times in 2002 vs. 2003 at a USGS gauge on the nearby Big Thompson River in RMNP (elev. 2402 m a.s.l.). Note not only the significantly greater peak runoff, but also the sustained high runoff that continued throughout July in 2003. The sustained runoff was driven by significant amounts of snow remaining for a longer period of time at the higher elevations, and the snow that remains the longest is usually in the relatively protected valleys where streams are located. In turn, the insulating snowpack keeps water temperatures in snow-covered streams just >0°C in spite of the increasing ambient air temperatures.

Water temperature is one of the most important determinants of timing in an aquatic insect's life cycle, and often a minimum temperature or accumulated number of degree-days must be reached to cue adult emergence (e.g. Vannote & Sweeney 1980). Indeed, in experimental manipulations of stream temperature, emergence timing was significantly earlier with only minor upward shifts in stream temperature (e.g. Sweeney *et al.* 1986, Hogg & Williams 1996). The effects of thermal regime on emergence timing has also been noted spatially, as conspecific populations often emerge later from colder streams in the same region (e.g. Collier & Smith 1998). Thus, a significantly greater snowpack in 2003 probably delayed emergence that year not only by physically covering the stream but also by keeping water temperature colder.

On average, annual snowfall has been increasing on the eastern slope of the Colorado Rocky Mountains over the past >50 years (Greenland & Losleben 2001), and this pattern is predicted to continue with continued global climate change (Welker *et al.* 2001). If this trend also leads to increased duration of snow cover, then a gradual decrease in length of emergence season for alpine stream insects would be expected to follow. The results of my study suggest that these changes may yield different effects on the lateral flight activity and dispersal potential for different species, dependent on whether they are late- or early-season emergers. Late-emerging species will likely decrease in dispersal potential because their flight periods would be pushed to a part of the season with colder air temperatures. Early-emergers, conversely, will likely feel the effects less strongly but may even increase their dispersal potential because their emergence peak will be shifted to the mid-summer when air temperature is warmest (see Greenland & Losleben 2001).

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Table 5.1. Total numbers of males (m) and females (f) caught for each common species and higher-level taxonomic group, and total species richness within higher-level groups across all Malaise traps in 2002 and 2003. Sex ratios marked with stars are significantly different from 1:1 (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). In all significant cases, males were more abundant than females.

taxon	2002 *N* (m/f)	2003 *N* (m/f)	2002 richness	2003 richness
Ephemeroptera	94/31***	152/49***	4	5
Ameletus celer	47/1***	91/2***		
Cinygmula spp.	44/26*	58/45		
(C. ramaleyi + C. par)				
Plecoptera	111/102	553/326***	9	17
Zapada haysi	55/64	447/223***		
Alloperla pilosa	29/10**	41/24*		
Trichoptera	127/28***	252/65***	12	18
Asynarchus nigriculus	82/2***	56/7***		
Diptera				
Simuliidae	23/46**	258/646***	9	18
Stegopterna acra	20/33	105/143*		

Table 5.2. Lateral dispersal statistics for each order and common species along each of the three transects (2002 east, 2003 east and west). R^2, slope and p-value are for the best-fit models for each order; "ND" = "not decreasing:" p-values >0.2 for decreasing slope. Last two columns indicate % of taxon caught within 1 and 10 m of stream, respectively. All 2003 data are from after 3 July only.

							% of total caught within:	
taxon	year	side	best model	R^2	slope	p-value	1m	10m
Trichoptera	2002	east	*exponential*	0.80	-0.03	0.10	33	64
	2003	east	*exponential*	0.99	-0.05	**0.003**	46	83
		west	*exponential*	0.86	-0.04	0.07	54	89
Asynarchus nigriculus	2002	east	*exponential*	0.66	-0.03	0.19	30	56
	2003	east	*exponential*	0.96	-0.06	**0.02**	49	81
		west	*exponential*	0.86	-0.06	0.08	61	95
Plecoptera	2002	east	*power*	0.93	-0.71	**0.02**	65	91
	2003	east	*power*	0.86	-0.31	**0.05**	47	72
		west	*power*	0.90	-0.24	0.07	47	66
Zapada haysi	2002	east	*power*	0.89	-0.64	**0.04**	63	89
	2003	east	*power*	0.79	-0.25	0.11	40	69
		west	*power*	0.84	-0.22	0.08	43	62
Alloperla pilosa	2002	east	*power*	0.80	-0.75	0.07	54	92
	2003	east	*power*	0.97	-0.51	**0.02**	57	82
		west	*power*	0.71	-0.22	0.14	38	68

Table 5.2. (continued)

							% of total caught within:	
taxon	year	side	best model	R^2	slope	*p*-value	1m	10m
Ephemeroptera	2002	east	*power*	0.99	-0.43	**0.006**	55	77
	2003	east	*power*	0.91	-0.49	**0.05**	66	78
		west	*ND*				28	39
Cinygmula spp.	2002	east	*power*	0.96	-0.92	**0.02**	79	93
	2003	east	*power*	0.97	-0.95	**0.02**	89	95
		west	*power*	0.68	-0.34	0.17	56	71
Ameletus celer	2002	east	*ND*				15	50
	2003	east	*ND*				18	41
		west	*ND*				7	13
Simuliidae	2002	east	*ND*				19	65
	2003	east	*ND*				26	52
		west	*ND*				18	31
Stegopterna acra	2002	east	*ND*				21	64
	2003	east	*ND*				31	76
		west	*ND*				14	23

Table 5.3. Total numbers caught on east (E) vs. west (W) side of stream in 2003 for each order and common species. Ratios marked with stars are significantly different from 1:1 (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Only data collected after 3 July are included.

taxon	*N* (E/W)
Trichoptera	139/168
Asynarchus nigriculus	63/99**
Plecoptera	374/357
Zapada haysi	282/253
Alloperla pilosa	28/37
Ephemeroptera	64/137***
Cinygmula spp.	44/59
Ameletus celer	17/76***
Simuliidae	404/371
Stegopterna acra	95/153***

Figure 5.1. Photo of the study site at the S. Fork Poudre River headwaters in July 2002. Note dense, low-lying riparian willows dominant in the watershed. The stream is snowmelt-fed from the permanent snowfield in upper right corner. East is left.

Figure 5.2. Cumulative proportions of the total catch of the six most common lotic species at different dates during the study period: a) *Asynarchus nigriculus*, b) *Ameletus celer*, c) *Cinygmula* spp, d) *Stegopterna acra*, e) *Alloperla pilosa*, f) *Zapada haysi*. White and black bars are 2002 and 2003 catches, respectively. Dates are not equally separated and are more densely distributed in July, a peak emergence period for several species. 2002 collections did not begin until 8 July; hence the 2002 lack of appearance of *Z. haysi* in early samples.

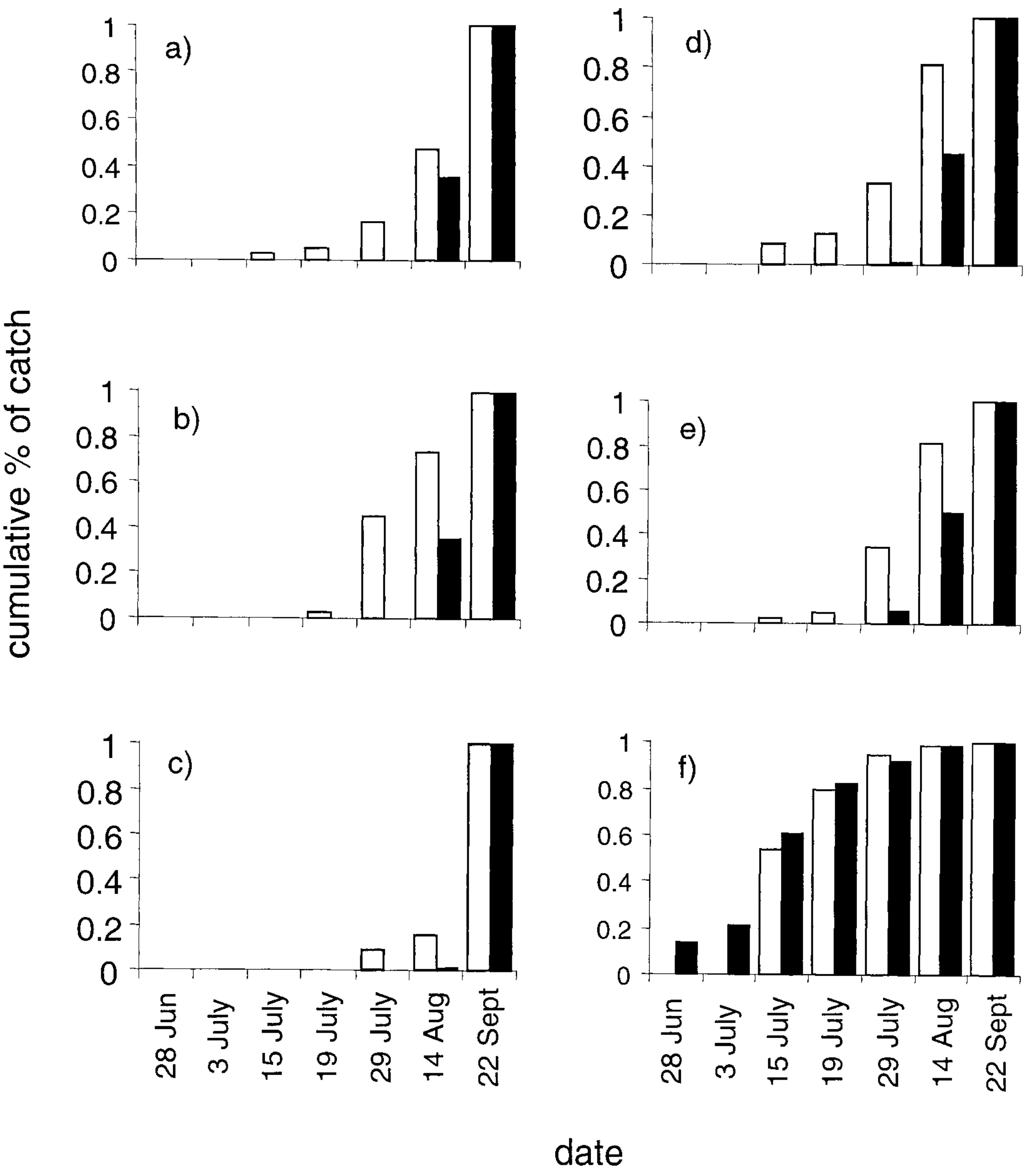


Figure 5.3. Relationship of Ephemeroptera abundance to distance from stream for two years, east transect only. a) all Ephemeroptera combined; b) *Cinygmula* spp.; and c) *Ameletus celer*. Closed diamonds and open squares are 2002 and 2003 catches, respectively. Fitted functions are significant decreasing power functions (see Table 5.2). Solid and broken lines represent 2002 and 2003, respectively. Slopes are not significantly different in any comparison; intercepts are different (p = 0.01) in a but not b.

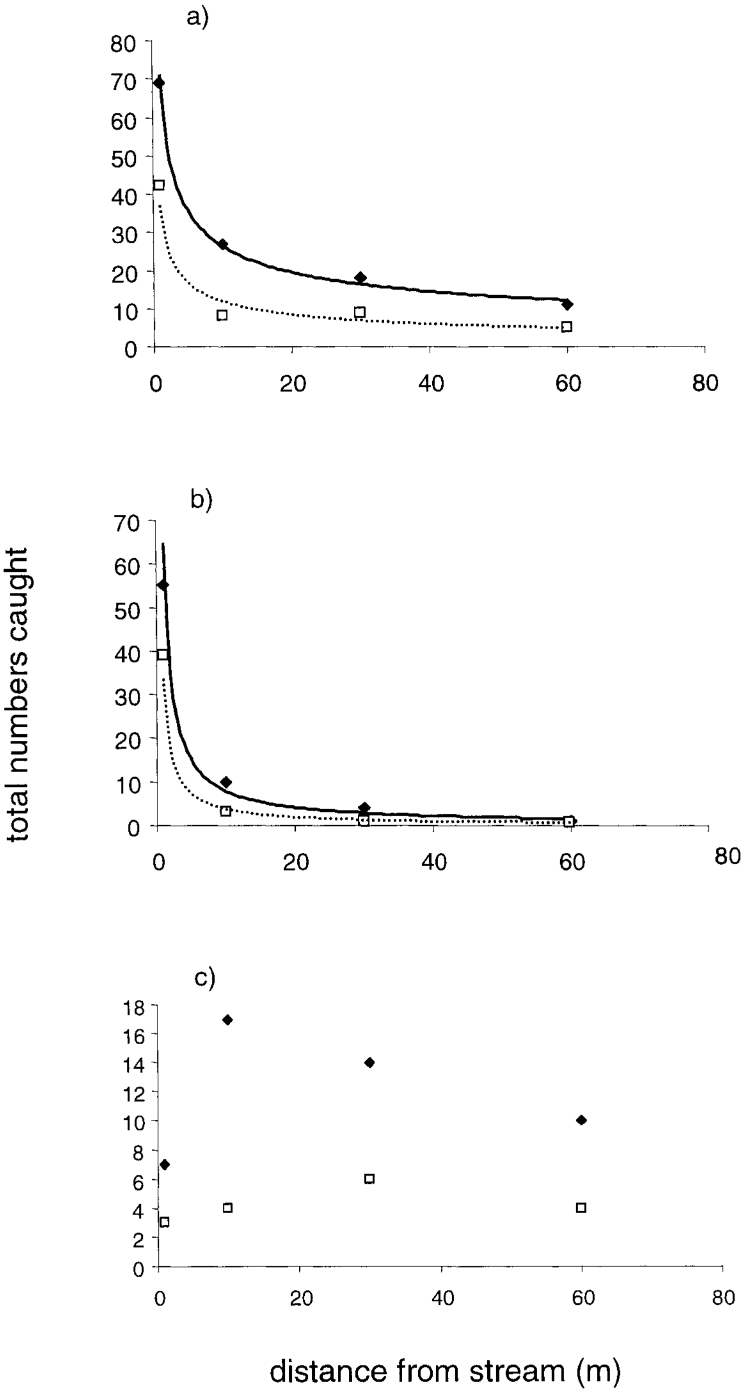


Figure 5.4. Relationship of Plecoptera abundance to distance from stream for two years, east transect only. Symbols and lines are same as in Fig. 5.3 for a) all Plecoptera combined, b) *Zapada haysi*, and c) *Alloperla pilosa*. See Table 5.2 for regression parameters and significance. Slopes are not significantly different in any comparison; intercepts are significantly different for a ($p = 0.007$) and b ($p = 0.005$) but not c.

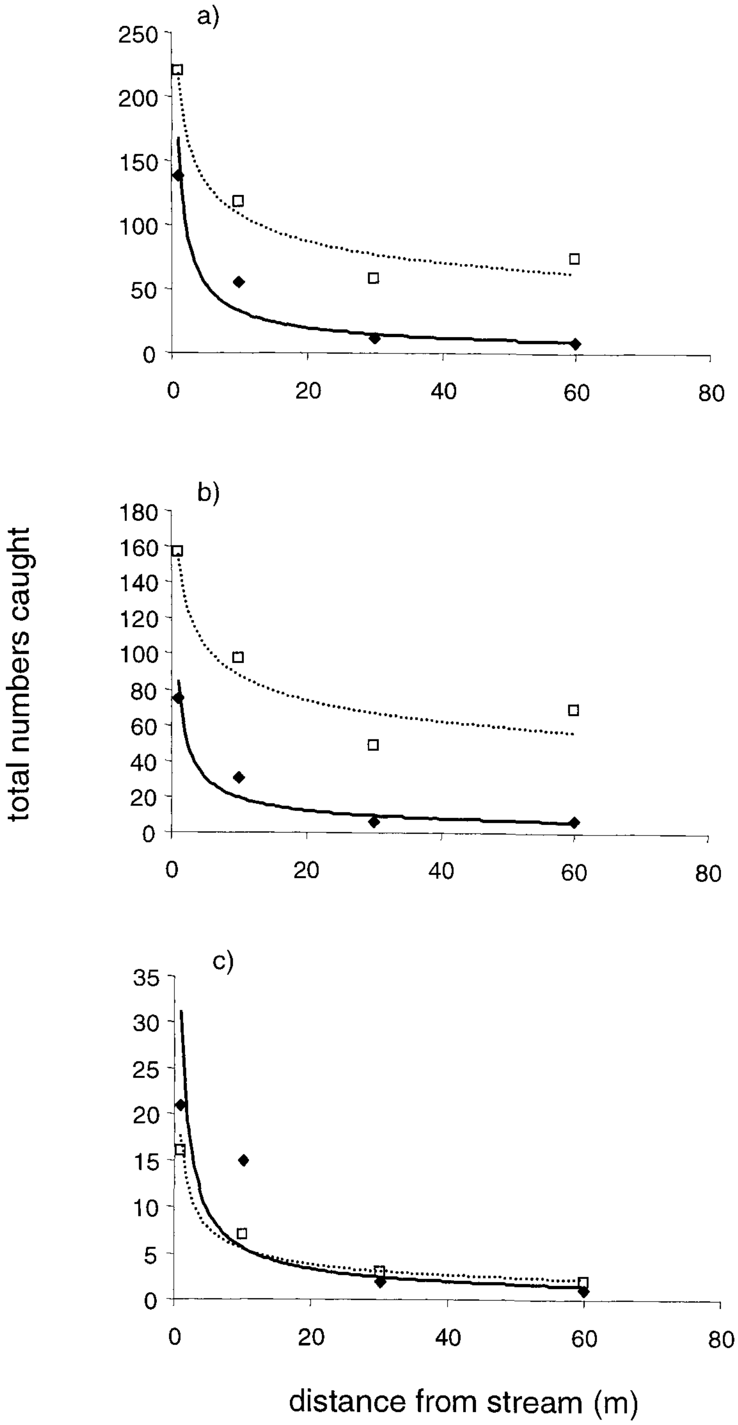


Figure 5.5. Relationship of Trichoptera abundance to distance from stream for two years, east transect only. Symbols are the same as in Figs. 5.3 & 5.4 for a) all Trichoptera combined; b) *Asynarchus nigriculus*. Broken line is significant negative exponential function (see Table 5.2) for 2003 catches; the models for 2002 were insignificant and therefore not shown.

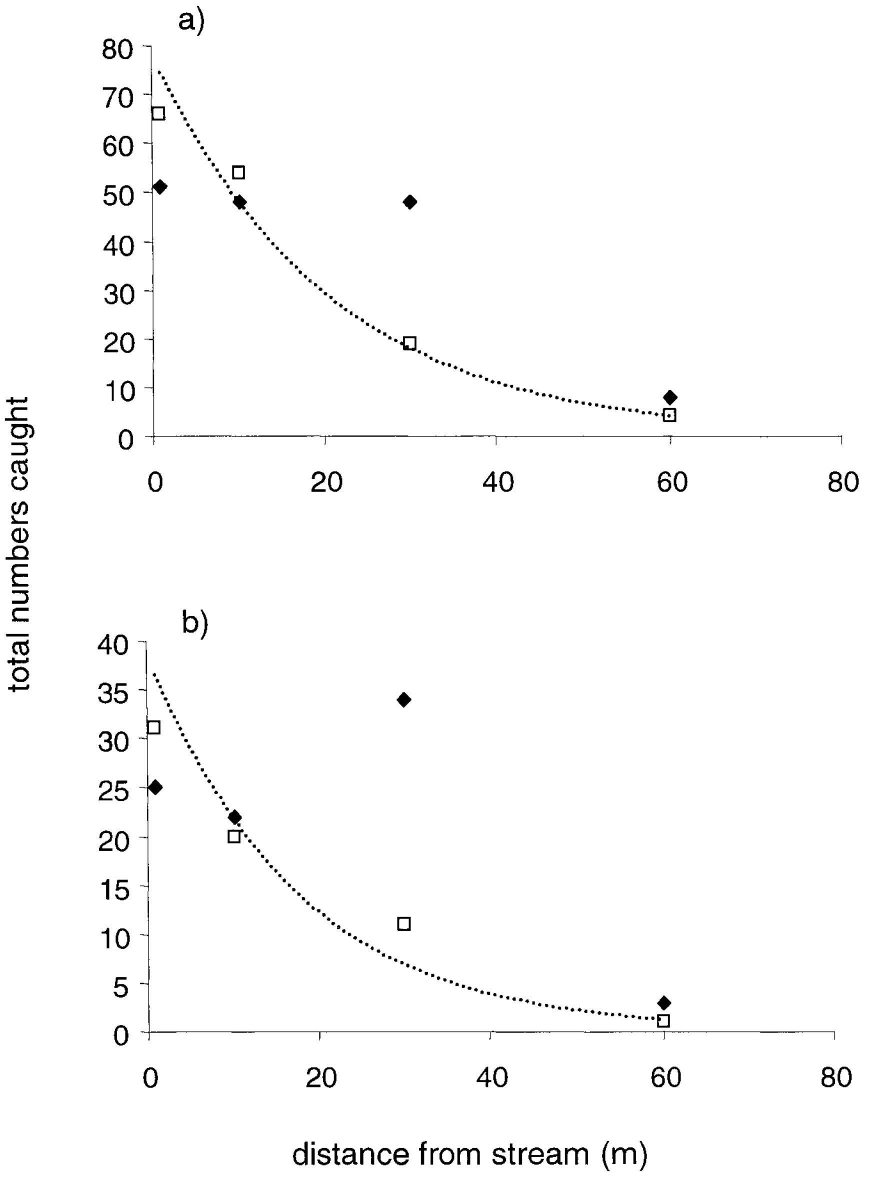


Figure 5.6. Relationship of Simuliidae abundance to distance from stream for two years, east transect only. Symbols are same as in Figs 5.3-5.5 for a) all Simuliidae combined, and b) *Stegopterna acra*. Numbers caught did not significantly decrease with distance in either year or taxon (see Table 5.2).

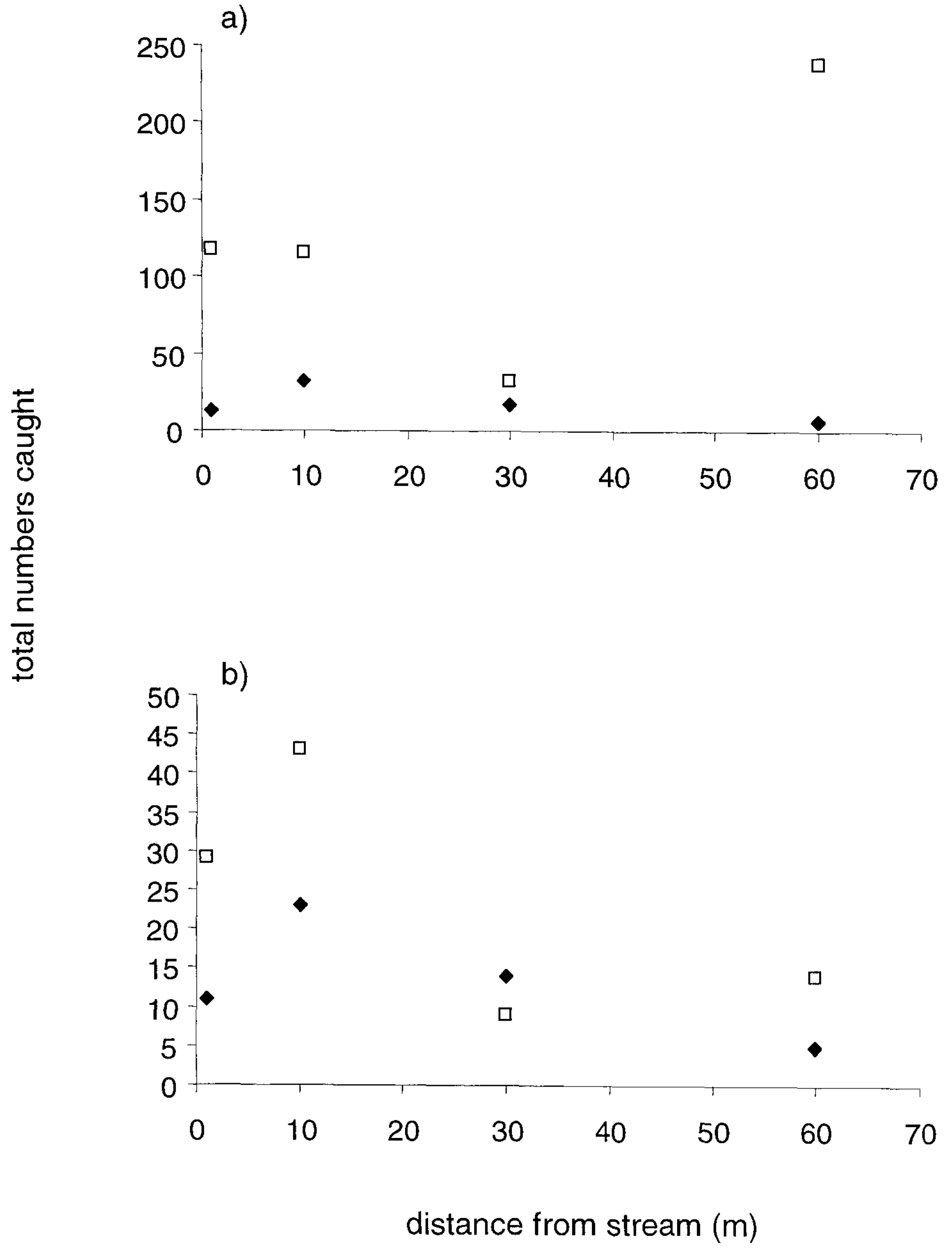


Figure 5.7. The relationship between distance from stream species richness. Solid diamonds and solid fitted lines are 2002 catch, and open boxes with broken lines are 2003 catch. a) Total species richness among Ephemeroptera, Plecoptera, Trichoptera, and Simuliidae. Linear models were the best fit (2002 $R^2 = 0.95$; 2003 $R^2 = 0.89$). Intercepts are significantly different ($p = 0.004$); slopes are not ($p = 0.34$). b) Total richness minus Simuliidae. Inverse power functions were the best fit (2002 $R^2 = 0.84$; 2003 $R^2 = 0.94$). Neither slopes nor intercepts are different at $\alpha = 0.05$.

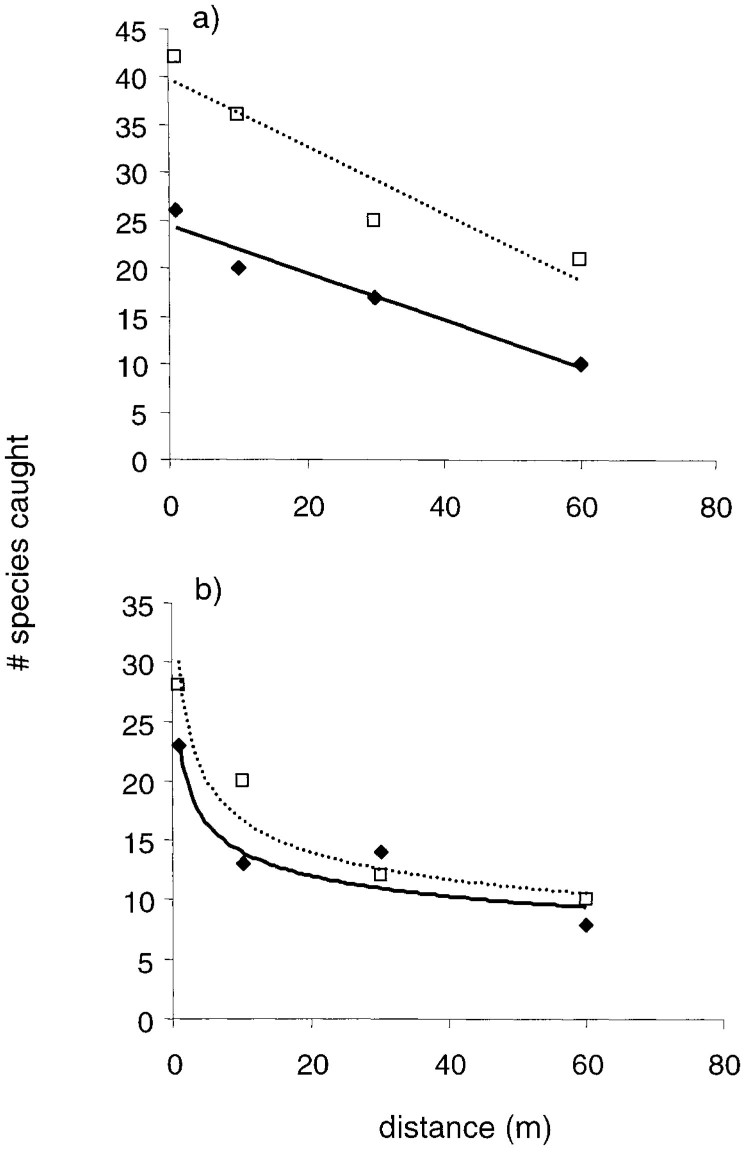


Figure 5.8. Sex ratios for a) *Asynarchus nigriculus*, b) *Ameletus celer*, c) *Cinygmula* spp., d) Simuliidae, e) *Alloperla pilosa*, and f) *Zapada haysi* with trap distance from the stream along the east side transect. Gray bars are male and white bars are female proportions. Data shown are from the year with the highest recorded abundance for each species. There were no significant relationships between distance and sex ratio.

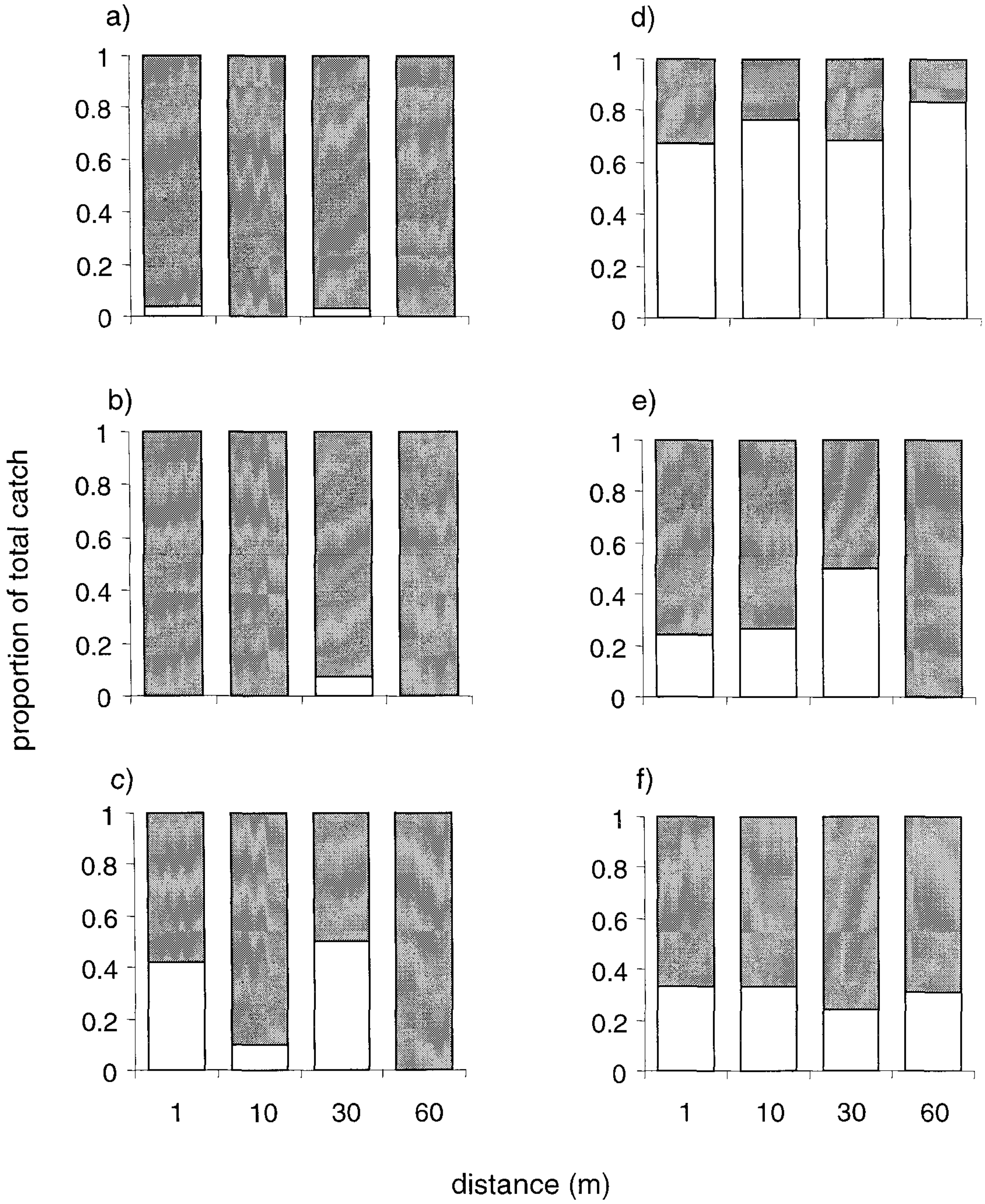


Figure 5.9. Trichoptera abundance with distance from stream along the east vs. west transects in 2003. a) all Trichoptera combined; b) *Asynarchus nigriculus*. Closed squares and open diamonds represent west and east transects, respectively. Lines are fitted negative exponential functions (see Table 5.2). Solid and broken lines represent west and east, respectively. Neither slopes nor intercepts are significantly different for either taxon.

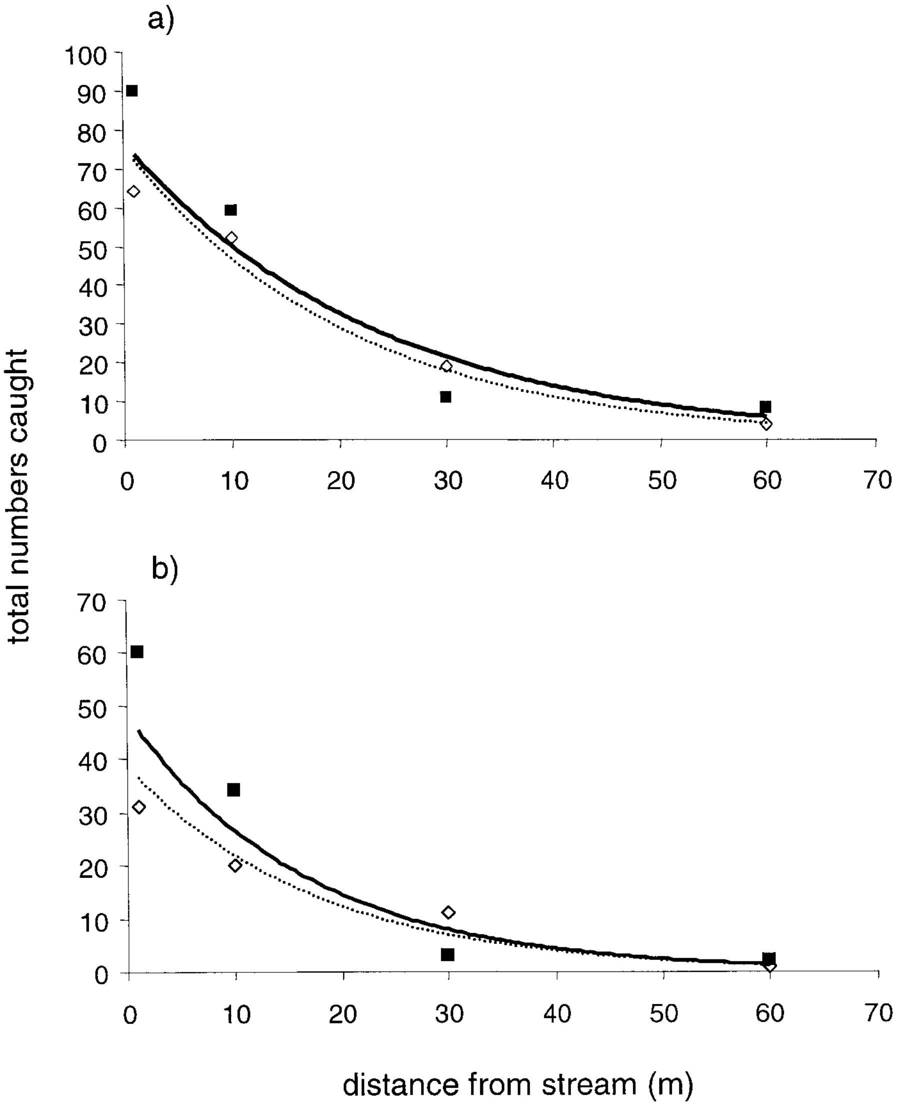


Figure 5.10. Plecoptera abundance with distance from stream along east vs. west transects, 2003. Symbols and lines are same as in Fig. 5.9 for a) all Plecoptera combined; b) *Zapada haysi*; c) *Alloperla pilosa*. Lines are fitted inverse power functions (see Table 5.2). For a and b, neither slopes nor intercepts are significantly different. For c, intercepts are different ($p = 0.045$), and slopes are nearly different ($p = 0.07$).

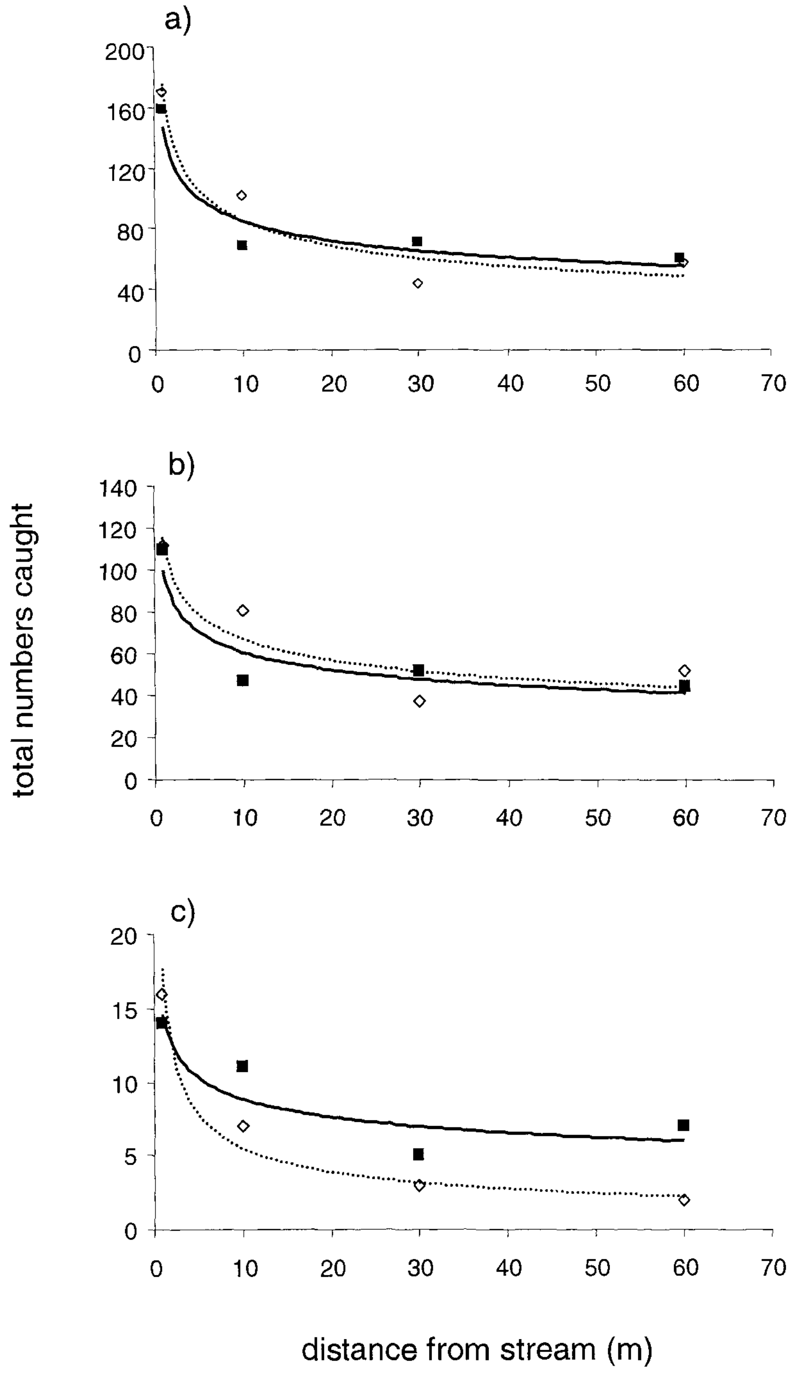


Figure 5.11. Ephemeroptera abundance with distance from stream along east vs. west transects, 2003. Symbols and lines are same as in Figs. 5.9 & 5.10 for a) all Ephemeroptera combined; b) *Cinygmula* spp.; c) *Ameletus celer*. Lines are fitted inverse power functions (see Table 5.2) for decreasing abundances only. For b, intercepts are significantly different ($p = 0.016$), as are slopes ($p = 0.04$).

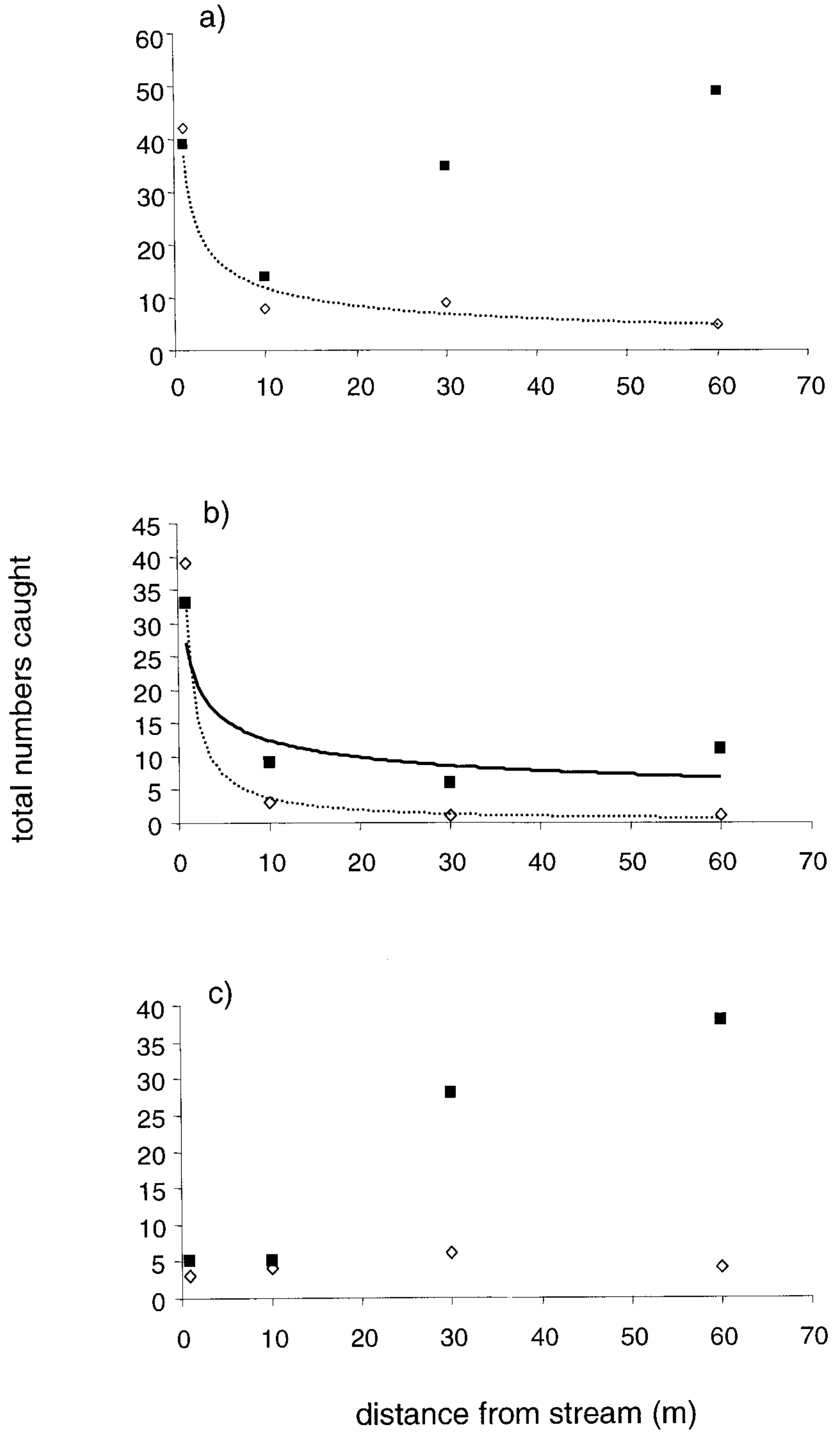


Figure 5.12. Simuliidae abundance with distance from stream along east vs. west transects, 2003. Symbols are same as in Figs. 5.9-5.11 for a) all Simuliidae combined, and b) *Stegopterna acra*. Abundance did not decrease with distance along either transect for either taxon (see Table 5.2).

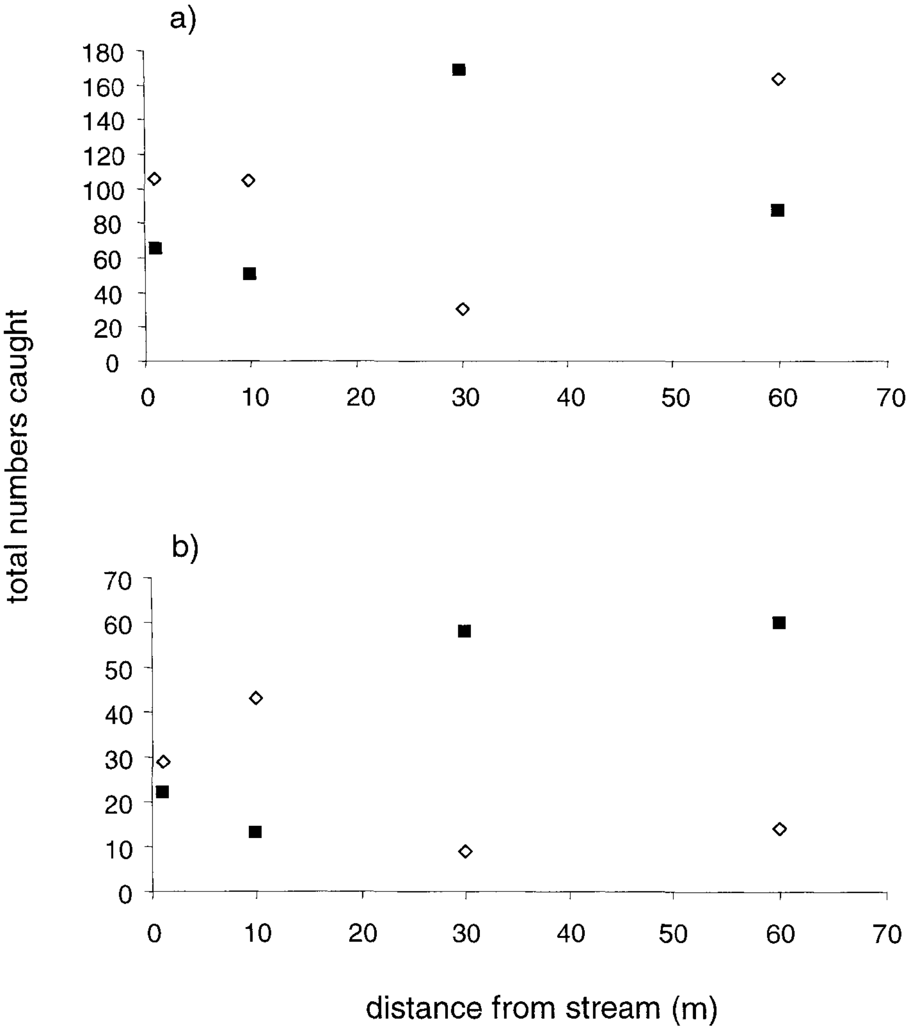
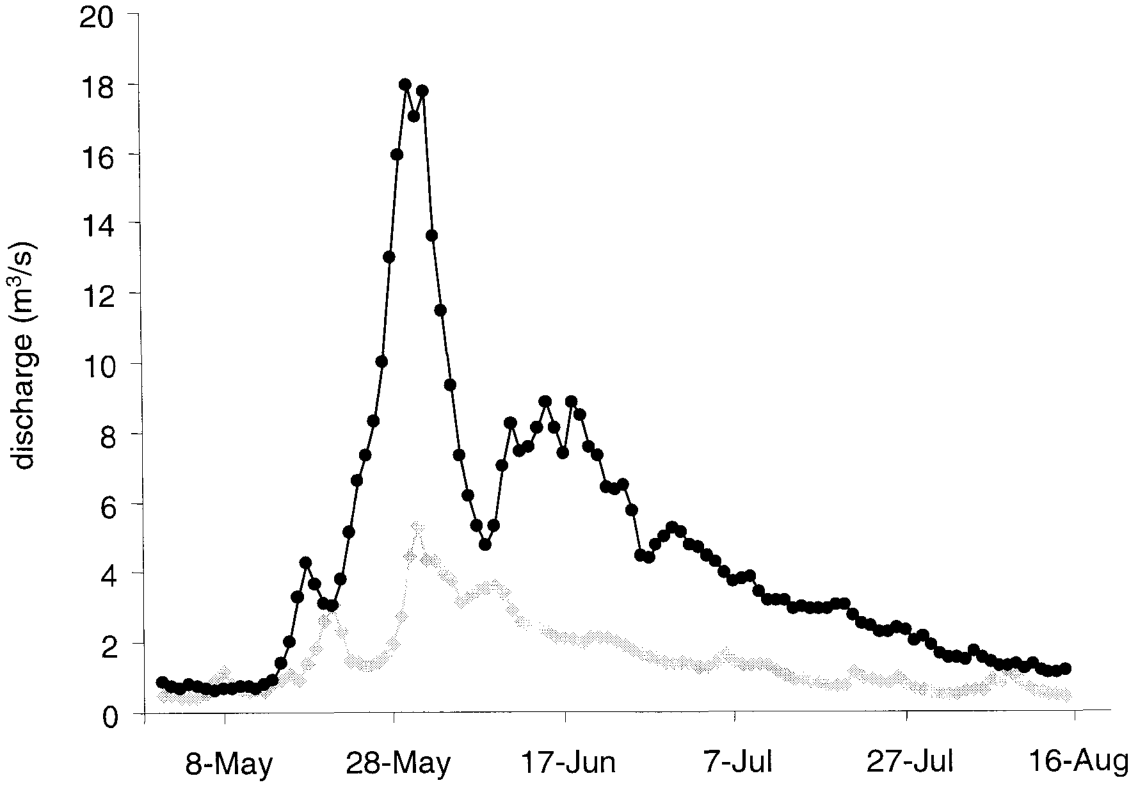


Figure 5.13. Hydrographs for 2002 (gray diamonds, lines) and 2003 (black circles, lines) during the peak runoff season on the Big Thompson River at 2400 m a.s.l. in RMNP. Data from USGS gauge #402114105350101 (http://water.usgs.gov).



Appendix 5.1. List of all species and total number caught of each in all Malaise traps. [1]CO state record; 3rd for US. [2]probably two species: *H. susanae* (Peterson) and *H. onychodactylus* (Coquillett). [3]includes *S. exulatum* (Adler, Currie & Wood) and/or *S. pilosum* (Knowlton & Rowe); the former is probably limited to alpine streams. 4*C. par* and *C. ramaleyi* lists males only; females are listed as *Cinygmula* spp.

	2002								2003															
	females				males				females								males							
	1E	10E	30E	60E	1E	10E	30E	60E	1E	10E	30E	60E	1W	10W	30W	60W	1E	10E	30E	60E	1W	10W	30W	60W
DIPTERA																								
Simuliidae																								
[1]*Greniera denaria* (Davies, Peterson & Wood)										2	1				3									
[2]*Helodon onychodactylus* complex		1		1					4	5	9	5	20	3	7	8	1				1		2	1
Prosimulium daviesi (Peterson & DeFoliart)		2				1																		
Prosimulium exigens (Dyar & Shannon)									2		1				1	1		2					1	
Prosimulium frohnei (Sommerman)												2			3			1					8	
Prosimulium shewelli (Peterson & DeFoliart)									2				1	2	4		2		1				4	3
Prosimulium travisi (Stone)						1			4	6		1	2	2	1	3	2	3				1		1
Prosimulium **new sp.**									1								1	1	1		1		1	
Prosimulium sp.			2						1															
Simulium arcticum complex	1								5	3		6	2	1	8	1	7	3	1	17	4	2	15	2
[3]*Simulium aureum* complex		2		1	1				6	6	2	23	1	1	7	1	2	1		3			3	1
Simulium carbunculum (Adler, Currie & Wood)									2	2	1	7	2	1	4	3								
Simulium craigi (Adler & Currie)										1	1			1	1									
Simulium curriei (Adler & Wood)									1	3	1			5	6	1								
Simulium pugetense (Dyar & Shannon)		1	1																					
Simulium silvestre (Rubtsov)									30	19	1	125	9	20	26			1		3				
Simulium vandalicum (Dyar & Shannon)										2		1					2			1				
Simulium venustum complex		1																						
Simulium vittatum (Zetterstedt)									2	4	2	18	1	1	16		10	5	1	10	2	4	12	1
Simulium (Nevermannia) sp.				1																				
Stegopterna acra (Currie, Adler & Wood)	5	14	9	5	6	9	5		18	34	3	9	14	8	25	32	11	9	6	5	8	5	33	28
PLECOPTERA																								
Chloroperlidae																								
Plumiperla diversa (Frison)	1	1				1								1		1								
Alloperla pilosa (Needham & Claassen)	5	4	1		16	11	1	1	2	5	2		7	2	1	5	14	2	1	2	7	9	4	2
Suwallia sp.										1														
Sweltsa borealis (Banks)	3				2		1		1						1							1		1
Sweltsa lamba (Needham & Claassen)	2		1		3		1		2				2	1			5	3			3	1		
Perlodidae																								
Isoperla sobria (Hagen)	3				4	2								1			2				2			
Megarcys signata (Hagen)													1			1								

Appendix 5.1 (cont.)

	2002								2003															
	females				males				females								males							
	1E	10E	30E	60E	1E	10E	30E	60E	1E	10E	30E	60E	1W	10W	30W	60W	1E	10E	30E	60E	1W	10W	30W	60W
PLECOPTERA (cont.)																								
Nemouridae																								
Zapada haysi (Ricker)	42	18	1	3	33	13	5	4	57	37	12	23	32	13	22	27	100	60	37	47	105	40	41	17
Zapada frigida (Claassen)													2			1	1	1			4		2	1
Amphinemura banksi (Baumann & Gaufin)													1				1							
Podmosta delicatula (Claassen)	3	4	1		5	1			4	1	1	1	4	2	3	1	8	1			2		2	
Podmosta decepta (Frison)					1				2		1		1	1	1			1	1				1	
Capniidae																								
Capnia gracilaria (Claassen)	9				6				11	3	3		11	1	1		6	2	1	1	4	1	1	
Capnia coloradensis (Claassen)									2				1	1										
Eucapnopsis brevicauda (Claassen)										1			2			1	1						1	
Leuctridae																								
Paraleuctra occidentalis (Banks)																				1		1		
Paraleuctra vershina (Gaufin & Ricker)									1														1	
TRICHOPTERA																								
Apataniidae																								
Allomyia chama (Denning)					2				8	3			10				5	7		1	17	3	3	1
Limnephilidae																								
Asynarchus nigriculus (Banks)	1		1		24	22	33	3	5	1	1			4			26	19	10	1	60	30	3	2
Chyranda centralis (Banks)					2																1			
Ecclisomyia maculosa (Banks)																								1
Hesperophylax designatus (Walker)											1												1	
Hesperophylax occidentalis (Banks)								1							1	1		1				1		
Limnephilus abbreviatus (Banks)	1	1	2	1		5	3	2	1	3				2				3			1	7		3
Limnephilus coloradensis (Banks)	1	1	1		1		3		4	4				1			5	6	5			5	1	
Limnephilus dispar (MacLachlan)									1															
Limnephilus diversus (Banks)	1				1				1	1		1			1			1		1		2		
Limnephilus janus (Ross)									1															
Limnephilus moestus (Banks)		2				1					1													
Limnephilus perpusillus (Walker)	1		1										1				1				1		1	
Limnephilus picturatus (MacLachlan)	3	8	1	1	5	8	1		1	2							3	2				2		
Psychoronia costalis (Banks)					7		2										2		1		1	2		
Psychoglypha ormiae (Ross)																	1							

Appendix 5.1 (cont.)

	2002								2003															
	females				males				females								males							
	1E	10E	30E	60E	1E	10E	30E	60E	1E	10E	30E	60E	1W	10W	30W	60W	1E	10E	30E	60E	1W	10W	30W	60W
TRICHOPTERA (cont.)																								
Rhyacophilidae																								
Rhyacophila harmstoni (Ross)					1					1			1								2			
Rhyacophila hyalinata (Banks)									1						1									
EPHEMEROPTERA																								
Heptageniidae																								
[4]*Cinygmula par* (Eaton)					8	3	1										25	2			6		1	2
[4]*Cinygmula ramaleyi* (Dodds)					24	6	1	1											2		14	7	1	
[4]*Cinygmula* spp.	23	1	2						14	1	1	1	13			9								
Ameletidae																								
Ameletus celer (McDunnough)			1		7	17	13	10				1			1		3	4	6	3	5	5	27	38
Baetidae																								
Baetis bicaudatus (Dodds)	4				3					1					1									
Siphlonuridae																								
Siphlonurus occidentalis (Eaton)																					1			