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Population Structure of the Vernal Pool Fairy Shrimp (*Branchinecta lynchi*) at Two Spatial Scales

THESIS

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by

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Thesis Committee:
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DEDICATION

To my parents, Richard and Patrica McClintock, for all their love and support.

The thesis of Kelly McClintock is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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ABSTRACT OF THE THESIS

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By

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Master of Science in Quantitative and System Biology

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Patterns of dispersal and gene-flow in freshwater invertebrates have often been difficult to interpret. Despite the assumed high potential for dispersal, populations of freshwater invertebrates display high genetic differentiation over small distances. There have been several explanations posed for this gene flow dispersal paradox, including strong priority effects or low realized dispersal. This study explores the spatial genetic structure of the freshwater invertebrate *Branchinecta lynchi*, a threatened vernal pool inhabitant, at two scales with a goal to determine the scale at which gene flow is important in shaping these patterns. Vernal pools were sampled at two different localities the San Luis National Wildlife Refuge Complex and a preserve adjacent to the University of California, Merced. Individuals were genotyped using both the mitochondrial cytochrome oxidase I subunit (COI) and nuclear amplified fragment length polymorphisms (AFLPs). Pairwise F_{st} values showed that genetic structure for this species was high, however, the only geographic pattern that emerged was isolation by distance at the local scale for the COI marker. Discrepancies between mitochondrial and AFLP markers may be explained several ways, including genotyping error, sex-biased dispersal and/or the longer time

to equilibrium of the nuclear genome. These results suggest that gene flow is important at the local scale, at least for mitochondrial DNA, while historical colonization patterns are likely maintained at the regional scale by priority effects. I infer that maintaining connectivity among vernal pool complexes through local dispersal vectors should be a management priority.

INTRODUCTION

The extent of dispersal and gene flow occurring between populations are important factors in understanding evolutionary differentiation, variation, and genetic structure across space and time (Slatkin 1987, Dieckman 1999, Bohonak and Roderkick 2001). Dispersal can be defined as the movement of individuals or propagules between populations that may or may not result in gene flow (Bilton et al. 2001) and is the constraining force on a species' ability to colonize new habitat patches or replace extinct populations (Jenkins and Buikema 1998, Shurin 2000). Gene flow, on the other hand, is an important evolutionary force that can either homogenize populations into one single panmictic population, counteract local adaption (Lenormand 2002), or be a source of novel genetic material and therefore variation within a population (Slatkin 1985, Slatkin 1987). While dispersal is required for gene flow, gene flow is not always the result of dispersal. Instead, dispersal into established populations leads to gene flow only if the individual contributes genetic material to the next generation. There are several ways to measure dispersal and gene flow including direct and indirect methods. Direct measures make use of physical marking of individuals to directly observe dispersal.. Indirect measures, on the other hand, rely on patterns of allele frequencies or sequence differences to predict gene flow between populations, but may not accurately depict the amount of dispersal occurring (Slatkin 1985).

Many freshwater invertebrates that live in temporally varying habitats use diapausing strategies to maintain populations through periods of drought (Caceres 1997). It is generally these dormant stages that are believed to be subject to passive dispersal while adults are usually confined to a single pool or pond during their lifetime (Bilton et al. 2001). Often this provides a challenge to direct measurements of dispersal as these resting stages are small making physical

marking and tracking almost impossible. Instead gene flow between populations of freshwater invertebrates is estimated with indirect measures which make use of genetics. The most common of these methods is use of the fixation index (F_{ST}) to estimate gene flow using the infinite island model of Wright (1943). While this indirect measure is useful in that it accounts for long-term averages of successful gene flow over time, there are often many of assumptions of the island model used in this calculation that are, and can be, violated in natural systems causing estimates of gene flow to be inaccurate. These include that populations are of equal size, exchange an equal amount of migrants, that gene flow and genetic drift have equilibrated, and that migration far exceeds mutation (Whitlock and McCauley 1999, Bohonak and Roderick 2001). Despite these limitations, indirect measures are still useful in that they can provide a picture of dispersal ability (Bohonak 1999).

In the past it has generally been accepted that passive movement of resting stages, and therefore dispersal of freshwater invertebrates, is high and uninhibited due to their many potential vectors for dispersal as well as their observed cosmopolitan distributions (De Meester et al. 2002, Bohonak and Jenkins 2003). Resting propagules are suspected to disperse in several different ways, including through animal vectors, wind, and water overflow (anemochory, zoochory and hydrochory). Numerous studies have shown that viable propagules can survive passage through the digestive tract of waterfowl, as well as attach to feathers or feet of wading birds, promoting dispersal between pools (Proctor et al. 1967, Figuerola and Green 2002, Figuerola et al. 2005, Brochet et al. 2010). Resting propagules of freshwater invertebrates also have been found to be distributed by other vertebrates that visit ponds or pools, such as salamanders (Bohonak and Whitman 1999), and terrestrial mammals such as the wild boar

(Vanschoenwinkel et al. 2008). Wind mediated dispersal of freshwater invertebrate resting stages has also been shown to occur through the use of sticky traps (Brendonck and Riddoch 1999) and windsocks (Jenkins and Underwood 1998). Despite the many ways in which these resting stages are hypothesized to move around a landscape, often indirect genetic estimates of gene flow between populations of freshwater invertebrates are found to be low and genetic differentiation high even between geographically proximate pools. This discrepancy led to proposal of a dispersal-gene flow paradox in which high dispersal does not translate into high gene flow (reviewed in De Meester et al. 2002). This would result in the aforementioned pattern of high genetic divergence between nearby populations and low gene flow despite the commonly presumed uninhibited ability to disperse. High genetic divergence over short geographic distance has been found in many freshwater invertebrates including Anostraca (Davies et al. 1997, Brendonck et al. 2000), Cladocera (Vanoverbeke and De Meester 1997, Allen et al. 2010), Copepoda (Bolieau and Hebert 1991) and Ostracoda (Chaplin and Ayre 1997).

Several hypotheses have been invoked to explain the apparent paradox between dispersal and gene flow in freshwater invertebrates. Bolieu et al. (1992) accounted for this paradox through the idea of a persistent founder effect. If the number of initial colonizers in the habitat is low, and the size of the initial source population large, then measures of genetic differentiation (such as F_{ST}) will initially be high (Bolieau et al. 1992). These high values of differentiation established by the potential of freshwater invertebrates to rapidly expand post colonization to fill an open habitat, are maintained over time by large population sizes and establishmentment of a large resting cyst bank. Large numbers of founding genotypes dilute future genetic contributions of immigrants by decreasing the probability of a small number of genotypes from increasing due

to drift. As a consequence populations will take much longer to reach migration-drift equilibrium and population genetic structure should be more dependent on how pools were colonization then contemporary patterns of gene flow (Bolieau 1992, De Meester et al. 2002). Genetic variation will be low depending on how many colonists successfully colonize a habitat, a trait which can be influenced by the rate at which colonization occurs (De Meester et al. 2002). In this case divergence between populations can be directly related to the number of founding colonizers and/or the way in which pools were initially colonized and not number of migrants currently being exchanged between populations (Bolieau 1992).

De Meester et al. (2002) modified the persistent found effect hypothesis by emphasizing the importance of local adaption in maintaining the resistance of initial genetic differentiation to change. Statistically, immigrant individuals are less likely then residents to have alleles adapted to the local environment giving those individuals that are already established a higher chance to produce offspring that will contribute to the gene pool of the next generation (De Meester et al. 2002). Hatching is also affected by different environmental conditions such as pH, temperature or low osmotic pressure (Brendonck 1996), thus differences in pool environments also could affect reproductive success of immigrants. If this is the case pools or ponds with similar environmental conditions may experience higher gene flow relative to other dissimilar pools. This would create an isolation by environment pattern in which ponds or pools with similar environmental conditions would be genetically more similar. This hypothesis stresses the role of local factors (e.g. resident species or hydrological conditions) in structuring communities of freshwater zooplankton and not dispersal limitation. These two hypotheses, the persistent

founder effect hypothesis and the monopolization hypothesis will both generate a pattern of strong priority effects.

Two studies have supported the idea that dispersal limitation is not a major factor in structuring freshwater invertebrate communities and that instead local factors, such as the established community in a pool, contribute to success of migrants. Shurin (2000), through experimental inoculation of ponds containing both intact zooplankton communities and reduced native communities with new species, showed that dispersal limitation was weak in comparison with the role of interactions with local residents. Furthermore Cohen and Shurin (2003) found that new artificial habitats were rapidly colonized by zooplankton implying that colonization is not dispersal limited and the possibility that local factors play a more important role in structuring communities.

The above theories depend on understanding of the actual frequency of dispersal occurring in freshwater invertebrates. The monopolization hypothesis and persistent founder effect hypothesis assume that dispersal is high and therefore other factors are influencing high structuring of freshwater invertebrate populations (De Meester et al. 2002). While the above two studies support the limited role dispersal might play in structuring freshwater invertebrate communities, there have been several other studies that have drawn contrasting conclusions about how frequently dispersal may occur in freshwater invertebrates. Bohonak and Jenkins (2003) argue that while there are many possible vectors for distribution of resting stages, they represent only the potential for dispersal. They advise caution in generalizing the role dispersal plays in shaping freshwater invertebrate communities. Jenkins and Underwood (1998) investigated the dispersal ability of freshwater invertebrates by incubating particulates from duck

feces and windsocks and found that only propagules from a subset of species in nearby communities were dispersed large distances through wind and rain. Likewise, environmentally similar experimental ponds are colonized stochastically, providing evidence against dispersal of all freshwater invertebrates being frequent and widespread (Jenkins and Buikema 1998). These studies instead advocate the importance of considering dispersal limitation in understanding patterns of genetic structuring between freshwater invertebrate populations and communities.

Anostracans are an order of freshwater invertebrates commonly known as fairy shrimp, or brine shrimp, that live in temporary waters or hypersaline lakes and produce resting cysts (Belk and Brtek 1995). Anostracans, like other freshwater invertebrates, have high population genetic structure (Davies et al. 1997, Bohonak 1998, Hulsman 2007). Studies done across species ranges, usually greater then 1 km, have explained this high population structure as a persistent founder effect maintaining historical patterns of colonization (Ketmaier et al. 2008, Muñoz et al 2008, McCafferty et al. 2010), yet isolation by distance, commonly found at distances under 1 km, suggests the importance of geographically limited gene flow at smaller scales (Hulsman 2007, Davies et al. 1997, Bohonak 1998). These varying conclusions reflect the evolutionary processes shaping population structure which may vary by scale.

In *Branchinecta sandiegonensis*, Davies et al. (1997) found a majority of F_{ST} values above 0.25 and a moderate pattern of isolation by distance (IBD) in pools sampled between 1 and 50 kilometers (km) apart. These *B. sandiegonensis* populations also showed low genetic diversity compared to other anostracans due to high fragmentation of the habitat and fewer dispersal vectors (Davies et al. 1997). Bohonak (1998) found highly structured populations of *B. coloradensis* among valleys separated by 5-10 km while populations were more similar within

valleys suggesting gene flow is greater at shorter distances. Estimates of dispersal corresponded well with ecological estimates of dispersal by salamanders (Bohonak and Whiteman 1999) suggesting a drift-gene flow equilibrium at least on the local scale in this species. Studies of *Branchipodopsis wolfi* have shown that dispersal may be limited to 2 km or less (Brendonck 2000). Another study in *B.wolfi* (Hulsman et al. 2007) found dispersal be limited by as little as 50 m indicating the importance of scale when studying patterns of dispersal limitation. This study had a rare opportunity to observe direct dispersal and also found that estimates of dispersal also correlated well with indirect estimates (Hulsman et al. 2007).

Several other studies have found high population genetic differentiation but no correlation between this structure and geographic distance. Generally these studies have been done at spatial scales larger then 1 km and have indicated rare long distance dispersal events. Many of these studies have employed mitochondrial markers rather than alloyzmes as did Davies et al. 1997, Bohonak et al. (1997), Brendonk et al. (2000) and Hulsman et al. (2007). McCafferty et al. (2010) found high structuring among populations of *Eubranchipus vernalis,* sampled across the state of Massachusetts, that did not correspond to geographic distance leading to the conclusion that dispersal limitation was not structuring these populations. Mediterranean *Artemia salina,* using COI, explained high pairwise Φ_{st} values (many greater then 0.5) between populations along with evidence of long distance dispersal events as priority effects (Muñoz et al. 2008). While this study found a pattern of isolation by distance ($R^2 = 0.246$) this was explained by sequential colonization and not current patterns of gene flow. In populations of *Branchipodopsis cf. wolfi* found on top of highly isolated inselbergs, Vanschoenwinkel et al. (2011) described a high partitioning of genetic variation within individual populations of *Branchipodopsis cf. wolfi*

along with a lack of isolation by distance pattern as evidence for the dispersal gene flow paradox. A recent study on *Branchinecta lynchi* across its range found that it showed weak geographic structuring as well as evidence for long distance dispersal events (Aguilar 2011).

The vernal pool fairy shrimp (*Branchinecta lynchi*) is a federally threatened endemic freshwater anostracan found in vernal pools in California and parts of Oregon. It occupies a wide swath of California including the Central Valley, Santa Rosa Plateau, Santa Barbara County, Ventura County, the Coast Ranges of Monterey County, as well as the Agate Desert of southwestern Oregon (Eng et al. 1990). *B. lynchi* is mostly associated with vernal pools, though it is also found in a variety of seasonal wetland habitats (Helm 1998, Eng et al. 1990). Its life cycle is fast, with reproduction taking place usually within 40 days, which allows it to complete its reproductive cycle in the small highly ephemeral habitats with which it is associated (Helm 1998). *B. lynchi*, like many freshwater invertebrates, produces resting cysts to survive periods of desiccation within their vernal pool habitat.

Vernal pools are ephemeral wetlands that provide habitat to many freshwater invertebrates including *B. lynchi* and other species of anostracans. Temporary wetlands, such as vernal pools, account for a high amount of biodiversity and endemism due to the unique flora and fauna that are highly adapted to the spatial and temporal variability of this ecosystem (King et al. 1996, Simovich 1998). Loss of these habitats can contribute substantially to losses of biodiversity due to the high number of different species per area (King et al. 1996). Fairy shrimp (Anostraca) are one of the more obvious occupants of vernal pools and their diversity is especially high in California. More then 40% of all anostracan described from North America are situated in

California (Eng et al. 1990). The genus *Branchinecta* shows high endemism and diversity and many species are considered endangered, within the Central Valley region.

The Central Valley region of California represents an area in which vernal pool habitat is prevalent, however, this habitat is being lost at an alarming rate due to human impacts such as agricultural and urbanization leading to destruction of current habitat and fragmentation of the remaining pools (Belk 1998). This region alone has already seen losses of up to 13% of vernal pool habitat between 1995 and 2005 (Holland 2005) and possibly up to 50-85% from pristine times (King et al. 1996). These losses have the potential to be compounded by changing global climate conditions as the seasonal cycles of inundation and desiccation of vernal pools are highly tied to temperature and rainfall (Pyke 2005a and b).

Understanding genetic connectivity between populations can have applications in the management of species. A better understanding of the scale at which dispersal is important in *B. lynchi* can help in defining the areas, or clusters of vernal pools, that should be managed for conservation purposes. Units for management are usually defined as units in which local processes such as birth and death are more important than immigration (Palsbøll et al. 2006). Understanding the extent of connectivity between populations of *B. lynchi* can define areas, whether local or regional, that need to be conserved to maintain current population dynamics. If gene flow is high at regional scales this would suggest that units of management for this species would be large, however, if gene flow is only important at local scales complexes should be considered independently.

Restoration of vernal pool habitat would also benefit from more detailed understanding of the extent of gene flow in *B. lynchi*. If local gene flow is important, then restoration projects

concerning this species would benefit from being in close proximity to an established pool complex preventing a loss of biotic connectivity among restored pools. Understanding the extent of vernal pool connectivity can help elucidate how increased habitat fragmentation may affect anostracan species as well as their ability to colonize new or restored habitats. Information on pool diversity could also shed light on reintroduction of individuals to newly established habitats. For example, if diversity within pools is high, a restored pool may benefit from several reintroductions of several different individuals to maintain a healthy per pool diversity level.

I studied population genetic structure of *B. lynchi* at two spatial scales using two different types of marker, nuclear amplified fragment length polymorphisms (AFLPs) and mitochondrial cytochrome oxidase subunit I (COI) sequences, to better understand the roles that dispersal, priority effects and local adaptation might play in shaping this structure. Two different areas from a portion of the range of *B. lynchi* in Merced County were sampled with distances between pools ranging from 42 m to 50 km apart. Based on what has been found in other anostracans, I expect to observe strong priority effects over my entire study area, a signature of which is a lack of isolation by distance. If limited dispersal is an important factor in genetically structuring populations of *B. lynchi* at local scales, I predict that genetic distance between pools will exhibit a patterns of isolation by distance indicative of a gene flow drift equilibrium caused by contemporary geographic restriction of gene flow. If there is restricted gene flow I hope to capture the spatial scale of this limitation within my sampling scheme. If local adaptation to soil conditions and how they interact with pool hydrology contributes to patterns of gene flow, I expect that soils more similar to each other will show lower genetic differentiation, creating an isolation by environment pattern in these populations. Finally, if persistent founder effects and

local adaptation are structuring populations of *B. lynchi* and not limited dispersal, I expect that populations will show low genetic diversity within a pool due to the genetic contribution of a small number of colonizing individuals predicted by this hypothesis.

METHODS

Sample Collection

Samples of *B. lynchi* were collected from two different localities, the San Luis National Wildlife Refuge Complex (SLNWRC) and a 6,000 acre preserve adjacent to the University of California, Merced, hereafter referred to as the Merced Grassland Reserve (MGR; Figure 1). Eight Pools on the SLNWRC were sampled from several different units (Kesterson, Arena Plains, West Bear Creek and Snobird) during the winter of 2011. On the MGR eighteen pools were sampled during the 2009 and 2010 field season. Individuals were collected using dips nets, identified in the field, and preserved in 95% ethanol for transportation back to the lab at the University of California, Merced. Distances between pools ranged from 42 meters within the MGR and up to 50 km between the MGR and the SLNWRC.

DNA extraction

DNA used in the amplification of COI was extracted using a Chelex method which uses a chelating resin to protect DNA and heat to destroy proteins and other cellular debris. A small portion, between 3 and 5 mm, of each individual was cut off and macerated in 100 μL of a 5% Chelex resin solution containing 2 μL of 20 mg/mL of Proteinase K. Samples were incubated for a minimum of 3 hours at 56°C with light agitation. After digestion samples were held at 97°C for 7 minutes and then spun down separating the Chelex resin and cellular debris from the supernatant. The supernatant containing the DNA was then removed and stored at -20°C until further analysis.

DNA used in the amplification of AFLP fragments were extracted using a 96-well plate high throughput method (Ivanova et al. 2006). A small portion of each individual, 3 to 5 mm,

was macerated in the presence of 200 µL of 'Insect lysis buffer' containing 1 mg/mL Proteinase K, and then incubated at 56°C overnight with light agitation. Following digestion, 200 µL of Binding Buffer was added to the samples and the mixture was run through a 96-well 0.3 µM glass fiber plate (PALL2 plate). Bound DNA was then washed once with 180 µL of 'Protein Wash Buffer' and once with 750 µL of 'Wash Buffer', then allowed to air dry for 30 minutes. Finally DNA was eluted with 100 µL of double distilled H_2O pre-warmed at 56°C, quantified using a nanodrop-1000 spectrophotometer, and stored at -20°C.

Amplification and sequencing of mitochondrial COI

A 710 base pair fragment of the COI gene was amplified using the standard primers LCO1490 (5' – GGTCAACAAATCATAAAGATATTG - 3') and HCO2198 (5' – TAAACTTCAGGGTGACCAAAAAATCA – 3'; Folmer et al. 1994). Each reaction contained 2 µL of 1:10 diluted Chelex extracted template DNA, 3 µL of 10x PCR buffer from Applied Biosystems Inc. (ABI, Foster City CA), 2.1 mM of MgCl2, 0.4 µM of dNTPs, 0.4 µM of each primer, 5 ng of BSA and 0.4 units of Taq polymerase in a 30 µL reaction. Reactions were run on an ABI 2720 thermocycler under the following conditions: an initial denaturation of 3 minutes at 94°C, 35 cycles of 94°C for 30 seconds, 48°C for 30 seconds and 72°C for 45 seconds followed by a final extension for 5 minutes at 72°C. PCR products were sent to the University of California, Berkeley Sequencing Facility for clean-up and sequencing in both directions.

Mitochondrial COI Data

Raw sequence data were uploaded into Sequencher (Genecodes, Inc.), trimmed, aligned and then checked by eye for errors. Sequences were then aligned in MEGA version 4.1 (Tamura et al. 2007) and trimmed to a final length of 651 base pairs. Pairwise F_{ST} values between

populations (Hudson et al. 1992), nucleotide diversity, and haplotype diversity were obtained using DNASP version 4 (Rozas 2003).

Amplification and genotyping of AFLP

AFLPs were amplified using slight modifications to established protocols (Vos et al. 1995) based on suggestions for optimization found in Trybush et al. (2006). All reactions were performed on an ABI 2720 thermocycler. Fifty to five-hundred nanograms of genomic DNA were first subjected to simultaneous restriction digest and adaptor ligation. This 16 μL reaction contained 5 units of EcoRI, 1 unit of MseI, and 63 units of T4 DNA ligase as well as 0.32 μM EcoRI adaptor (Vos et al. 1995), 3.2 μM of MseI adaptor (Vos et al. 1995), 1.6 μL of 10x T4 DNA ligase buffer, 8 ng of BSA and 50 μM of NaCl. This digestion-ligation (dig-lig) mix was incubated at 37^{o}C for 2 hours and then diluted with 84 μL of 1x TLE. The first selective amplification was done using 3 μL of the diluted dig-lig mix, the primers EcoRI+A (5' – GACTGCGTACCAATTA- 3') and MseI+C (5' - GATGAGTCCTGAGTAAC - 3') in a 15 μL reaction containing 1.5 μL of 10x PCR buffer from ABI, 0.7 μM of dNTPs, 0.13 μM of MgCl2, 0.4 μM of each primer and 0.4 units of Taq polymerase. The reaction was performed under the following conditions: an initial denaturation of 5 minutes at 65^{o}C, 30 cycles of 94^{o}C for 30 seconds, 56^{o}C for 30 seconds and 72^{o}C for 1 minute followed by a final extension of 30 minutes at 60^{o}C. Samples were checked for amplification by running out a subset of samples on a 2% agarose gel and looking for a smear below 500 base pairs. One microliter of a 1:20 dilution of the first amplification was used in the second selective amplification. The multiplex reaction was carried out using the Qiagen (Valencia, CA) multiplex kit. Five micro-liters of the Qiagen Master mix along with 0.7 μM of the primer MseI+CCA (5' - GATGAGTCCTGAGTAACCA - 3') and

0.7 μM of three dye-labeled primers EcoRI+AAG (5' -FAM-GACTGCGTACCAATTAAG - 3'), EcoRI+ACA (5' – NED-GACTGCGTACCAATTCACA – 3') & EcoRI+ACT (5' – VIC-GACTGCGTACCAATTCACT - 3'). The multiplex reaction was carried out under the following PCR conditions: an initial activation of the HotStarTaq (Qiagen Inc. Valencia, CA) at 95°C for 15 minutes, 13 cycles of 94°C for 30 seconds, 65°C for 1 minute with a 0.7°C step down per cycle followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 second and 72°C for 1 minute and a final extension of 72°C for 10 minutes. Final products were diluted 1:40 and sent for genotyping at the University of California, Los Angeles Gonda Genomics Facility.

AFLP Data

Raw AFLP data were uploaded and analyzed in Peak Scanner (ABI, Foster City CA). Parameters for calling true peaks from background noise were first assessed in OPTIFLP version 1.41 (Arthofer et al. 2011) for each dye-primer combination separately. One-hundred of the 395 samples were chosen for parameter optimization due to computational limitations of OPTIFLP which searches a parameter space indicated by the user. I selected the parameter space recommended by the program authors due to computational limitations and time (Table 1) (Arthofer et al. 2011). The resulting parameters (Table 1) were then used to call peaks for all samples in TINYFLP version 1.2 (Arthofer 2010) for each primer-dye combination separately. Automated peak calling is essential for producing consistent peak profiles and alleviates peak calling by eye that can be subjective (Whitlock et al. 2008, Arthofer et al. 2011). The files for each primer/dye combination were then concatenated using TINYCAT which is provided with TINYFLP. Based on peak statistics calculated by TINYFLP, samples were filtered for quality. Any individual which contained a valid peak number in the lower 10th percentile of all samples for

more then two primer combinations was removed. A peak was considered valid if it was called within the specified parameter space, therefore a low valid peak number could represent poor amplification. Loci which occurred at a frequency of less then 10% among all individuals were also removed. Filtered and scored AFLP peak data was then analyzed using AFLPSURV version 1.0 (Vekemans et al. 2002) to calculate pairwise F_{ST} values between populations, expected heterozygosity, and the percentage of polymorphic loci using a Bayesian method with non-uniform prior distributions assuming Hardy-Weinberg genotypic proportions (Zhivotovsky 1999).

Isolation by Distance

Analyses were done at two levels, the local level, within either the MGR or the SLNWC, or regional level encompassing both locations. Isolation by distance was assessed using the F_{ST} matrix output from AFLPSURV for AFLP data, the F_{ST} matrix output from DNASP for mitochondrial data and a distance matrix obtained by plotting GPS points of pools in DIVA-GIS (Hijmans et al. 2004). The significance of the correlations were assessed with 100,000 iterations of a simple Mantel's test in the program ZT (Bonnet and Van de Peer 2002).

Isolation by Environment

Data were gathered online for several soil properties from the USDA online soil survey data through SoilWeb (http://casoilresource.lawr.ucdavis.edu/drupal/node/902). Based on the soil series on which each pool was located the properties obtained from the database for the first soil horizon were: percent clay, percent sand, pH, Saturated Hydraulic Conductivity (mm/hr) and the K-factor, which describes erosion by rainwater. Using these five properties pairwise Mahalanobis distances were calculated between the different soil types using R (R Development Core Team

2008) and pairwise distances were taken between each pool. For COI data, three different soil types were sampled within the MGR (only two were compared for nuclear AFLP data) and eight different soil types were sampled within the SLNWRC. The pairwise soil distances were then plotted against the pairwise genetic distances (F_{ST}) and the significance of each correlation tested using a partial Mantel's test, controlling for geographic distance in the program ZT (Bonnet and Van de Peer 2002) with 100,00 iterations. The level of significance was determined using a sequential Bonferonni correction.

AFLP Genetic Structure

AFLP data were analyzed with STRUCTURE version 2.3.3 (Pritchard et al. 2000) using the admixture model with site of collection (pool) as prior information. The initial analysis used K values from 1 to 24 with one iteration at each K. The program was run for 250,000 steps including a burnin of 50,000. Data were then re-run for K=1 through K=10 for 4 iterations at each K. The Evanno method (Evanno et al. 2005) was used to determine the most appropriate K value for the data and multiple runs were combined in CLUMMP version 1.1.2 (Jakobsson and Rosenberg 2007).

Phylogenetic COI Network

A network was created using COI haplotypes in SPLITSTREE4 version 4.12.3 using the neighbor-net algorithm (Hudson and Bryant 2006) .

RESULTS

Mitochondrial Data

I sequenced a 651 base pair fragment of COI from 406 individuals sampled from 26 pools. The number of individuals sampled from each pool ranged from 7 (pool 17) to 31 (pool ARP102), with an average of 15 individuals sampled per pools. The number of haplotypes per pool ranged from one haplotype (pool 10) to 20 (pool ARP102), with an average of 7 haplotypes per pool. Of the 118 haplotypes found, only 6 were shared between the SLNWRC and the MGR. The percentage of private haplotypes found per pool ranged from 0% (pools 10, 23, 7, and 9) to a maximum of 83% private haplotypes (pool KST70). Haplotype diversity was also generally high and ranged from fixed in one population (pool 10) to 0.9700 (pool ARP103), with an average of 0.6600 (Table 2). Nucleotide diversity ranged from 0 (pool 10) to 0.0158 (pool ARP103) with an average of 0.0076 across all samples (Table 2). Pairwise F_{ST} values between pools ranged from 0 up to to 0.97 found between pool 11 and pool 10. Seventy-eight percent (256 of the 325) of comparisons had F_{st} values above 0.25 (Appendix 1). Additionally no significant correlation existed between number of haplotypes per pool ($r=0.1834$; $p=0.3911$) and haplotype diversity ($r=0.2464$; $p=0.2457$) and pool area in meters squared (Table 6).

AFLP Data

After data clean up 134 loci were obtained for 395 individuals from 24 pools. The number of individuals sampled per pool ranged from 6 (pool 10) to 24 (Pool 15; Table 3). The number of polymorphic loci per population ranged from 87 (pool 10) to 132 (pool ARP102) with an average of 116. Observed heterozygosity ranged from 0.22 (pool 8) to 0.39 (pool SB5), with an average of 0.31. Pairwise F_{ST} values between populations ranged from 0.007 to 0.26 with 72%

(198 of the 276) of comparisons being under 0.1 (Appendix 1). There was no correlation between haplotype diversity and number of polymorphic loci, or nucleotide diversity and number of polymorphic loci. Additionally no significant correlation existed between expected heterozygosity (r=0.0781; p=0.7169) or percent polymorphic loci (r=0.1636; p=0.4450) and pool area in meters squared (Table 6).

Isolation by Distance

A positive correlation between COI genetic distance and geographic distance was found only at a local scale, within the SLNWRC (r=0.4090; p=0.0195; Figure 2A) and the MGR (r=0.2246; p=0.0034; Figure 2B). No correlation was observed at the regional scale (r=-0.0386; p=0.2729; Figure 2C).

No significant correlation between genetic distance and geographic distance was found for AFLP data at any level: within the SLNWRC (r=0.0202; p=0.4472; Figure 4A) and the MGR (r=0.1136; p=0.1181; Figure 3B) or the region as a whole (both the MGR and the SLNWRC) (r=-0.0634; p=0.2555; Figure 3C) .

Isolation by Environment

After controlling for any correlation with geographic distance, COI distances were found to be significantly negatively correlated with Mahalanobis distances (r=-0.1608; p=0.0039) which incorporated all five soil properties, at the regional scale. All other correlations at the regional level were not significant (Table 4). No correlations locally within the MGR were found to be significant while pH within the SLNWRC was significantly correlated with genetic distance (p=0.6904; r=0.0001; Table 4).

At the regional scale AFLP genetic distances were significantly negatively correlated with Mahalanobis distance, as well as all individual pairwise soil property comparisons with the exception of pH (Table 5). Within the MGR and the SLNWRC all correlations between genetic distance and both Mahalanobis distances and individual soil property pairwise distances were not significant (Table 5).

AFLP Genetic Structure

The most appropriate K value for this data was found to be 4. There is no clear geographic pattern of assignment to one of the four clusters among the SLNWRC and the MGR. While there appears to be a large amount of assignment to the blue and purple clusters in the SLNWRC (Figure 4) and the MGR appears to have high amounts of ancestry in the green, purple and red clusters (Figure 5) exceptions exist. Pools 15 and 17 show large portions of blue and green ancestry but are located within the MGR (Figure 4 and 5).

Phylogenetic COI Network

The network shows that haplotypes form two clades, however there is no geographic pattern (Figure 6). While only a few haplotypes are found in both localities (Figure 6), the number of haplotypes from each location appear evenly distributed between clades.

Table 1: Parameters used for calling AFLP peaks in TINYFLP as well as the parameter space searched in OPTIFLP. These include the minimum called peak height, the minimum and maximum size of the peak in base pairs, and the minimum and maximum frequency in all sample for each peak. # of loci is the number of loci contributed from each primer pairing.

Primer	Dye	Min. Peak heigh	Min. Size (bp)	Max Size (bp)	Min. Frequency	Max Frequency	# Loci
EcoRI+AAG	FAM	50	90	290	14	95	47
EcoRI+ACT	VIC	80	60	270	14	86	35
EcoRI+ACA	NED	110	100	270	8	92	52
Parameter Space Searched	-	50-200	60-130	250-400	5 – 15	80-95	-

Table 2: Estimated basic diversity statistics for mitochondrial COI data - the number of individuals sampled (n), latitude and longitude for each sampled pool, number of segregating sites (#SS), number of haplotypes (#Hap.), haplotype diversity (Hap. Diversity) and nucleotide diversity (Pi).

Population	Latitude	Longitude	Pool Size	n	# Hap.	No. Private Hap	Hap. Diversity	Pi
Pool10	37.396440	-120.382670	344	10	1	0	0.0000	0.0000
Pool11	37.372510	-120.416520	169	22	3	1	0.1775	0.0013
Pool12a	37.384890	-120.375020	144	22	6	4	0.5325	0.0032
Pool15	37.381010	-120.362830	213	23	8	6	0.6324	0.0034
Pool16	37.379110	-120.362410	301	20	7	5	0.6895	0.0114
Pool17	37.377740	-120.364740	515	7	3	1	0.5238	0.0077
Pool18	37.378960	-120.363650	177	12	3	1	0.4394	0.0008
Pool21	37.383790	-120.403210	5933	18	10	6	0.9216	0.0052
Pool22	37.369920	-120.420220	221	14	6	3	0.6044	0.0120
Pool23	37.371670	-120.417790	179	9	3	0	0.5556	0.0034
Pool24	37.386440	-120.373420	275	12	4	1	0.7424	0.0124
Pool25	37.385930	-120.372830	214	18	4	2	0.4771	0.0051
Pool26	37.381390	-120.401410	7151	12	6	4	0.7576	0.0053
Pool6	37.408170	-120.368610	-	23	9	4	0.8696	0.0142
Pool7	37.407820	-120.368430	-	11	5	0	0.0818	0.0132
Pool8	37.411950	-120.364450	562	23	5	2	0.7352	0.0092
Pool9	37.406260	-120.374000	74	8	2	0	0.5357	0.0135
CP	37.362108	-120.394707	39148	12	5	2	0.6667	0.0030
ARP101	37.276880	-120.712480	795	19	12	5	0.9240	0.0093
ARP102	37.260260	-120.719640	1745	31	20	14	0.9570	0.0132
ARP103	37.263360	-120.719160	19135	19	16	11	0.9766	0.0158
KST70	37.274910	-120.906460	17394	9	7	5	0.9167	0.0090
KST203	37.275200	-120.893110	460	13	4	3	0.6795	0.0033
SB201	37.243620	-120.749960	474	7	6	3	0.9524	0.0101
SB5	37.250100	-120.743390	817	9	6	4	0.8889	0.0083
WBVP04	37.243847	-120.841300	707	23	9	5	0.8221	0.0048

Table 3: Basic diversity statistics calculated for AFLP data - the number of sample (n*), number of loci (#loc.), number of polymorphic loci (#loc_P), proportion of polymorphic loci at the 5% level (PLP) and expected heterozygosity (Hj) were calculated for AFLPs.

Population	n*	#loc.	#loc_P	PLP	Hj
Pool10	6	134	87	64.9	0.2491
Pool11	24	134	125	93.3	0.3450
Pool12a	23	134	119	88.8	0.3117
Pool15	24	134	102	76.1	0.2651
Pool16	21	134	105	78.4	0.2795
Pool17	13	134	108	80.6	0.2439
Pool18	19	134	118	88.1	0.3508
Pool21	22	134	130	97	0.3674
Pool22	16	134	103	76.9	0.2806
Pool23	13	134	114	85.1	0.2802
Pool24	13	134	130	97	0.3544
Pool25	20	134	124	92.5	0.3517
Pool26	14	134	116	86.6	0.2961
Pool6	-	-	-	-	-
Pool7	-	-	-	-	-
Pool8	8	134	90	67.2	0.2209
Pool9	17	134	130	97	0.3150
CP	13	134	129	96.3	0.3532
ARP101	21	134	121	90.3	0.3186
ARP102	24	134	132	98.5	0.3521
ARP103	20	134	105	78.4	0.2432
KST70	15	134	120	89.6	0.3024
KST203	9	134	104	77.6	0.2645
SB201	9	134	122	91	0.3308
SB5	8	134	129	96.3	0.3868
WBVP04	23	134	128	95.5	0.3339

Table 4: Correlation between Mahalanobis soil distances (M) and pairwise soil property distances with genetic distance for mitochondrial COI data. Values are reported with the r value followed by the significance (p-value). Asterisks indicate a significant relationship after a table wide sequential Bonferroni correction.

	Regional (r/p)	MGR (r/p)	SLNWRC (r/p)
M	-0.1608/0.0039*	-0.0167/0.4194	0.0707/0.3656
pH	-0.1232/0.0113	0.1250/0.0658	0.6904/0.0001*
Conductivity	-0.0241/0.3437	0.1250/0.0675	0.1632/0.2063
K-Factor	-0.0882/0.0936	0.0469/0.2870	0.0814/0.3451
Percent Sand	-0.0858/0.0788	-0.0632/0.2238	0.0649/0.3705
Percent Clay	0.0356/0.2897	0.1113/0.0906	0.0771/0.3496

Table 5: Correlation between Mahalanobis soil distances (M) and pairwise soil property distances with genetic distance for AFLP data. Values are reported with the r value followed by the significance (p-value). Asterisks indicate a significant relationship after a table wide sequential Bonferroni correction.

	Regional (r/p)	MGR (r/p)	SLNWRC (r/p)
M	-0.2279/0.0003*	-0.2017/0.0116	0.0204/0.4597
pH	-0.0411/0.2734	-0.2017/0.0120	-0.2495/0.1019
Conductivity	-0.1578/0.0090*	-0.2017/0.0115	-0.0544/0.4029
K-Factor	-0.2093/0.0004*	-0.2017/0.0116	0.0351/0.4301
Percent Sand	-0.1870/0.0025*	-0.2017/0.0123	0.1428/0.2370
Percent Clay	-0.1970/0.0006*	-0.2017/0.0123	0.4090/0.0237

Table 6: Significance of correlations between pool size and measures of diversity for both mitochondrial and nuclear markers. PLP is proportion of polymorphic loci at the 5% level Hj is expected heterozygosity, #Hap is the number of haplotypes per pool and HapDiv is the haplotype diversity per pool.

	r-value	p-value
PLP	0.1636	0.445
Hj	0.0781	0.7169
#Hap	0.1834	0.3911
HapDiv	0.2464	0.2457

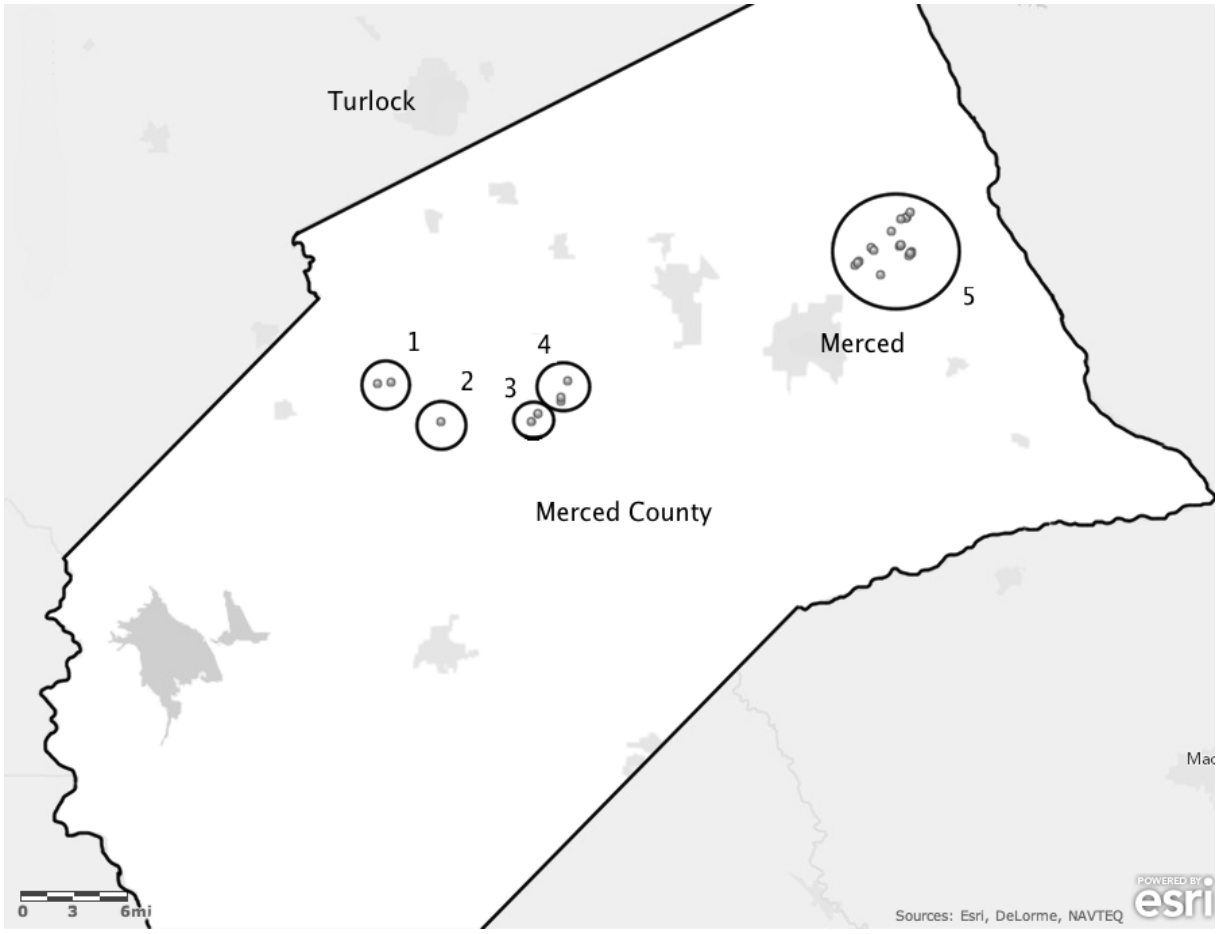

Figure 1: Map of Merced County and its location in California (inset) showing the locations and spatial distribution of the sampled vernal pools in relation to the city of Merced and Turlock. Circled locations indicate (1) the Kesterson Unit (KST) (2) the West Bear Creek Unit (WBVP) (3) the Snobird Unit (SB) (4) the Arena Plains Unit (ARP) and (5) the Merced Grassland Reserve.

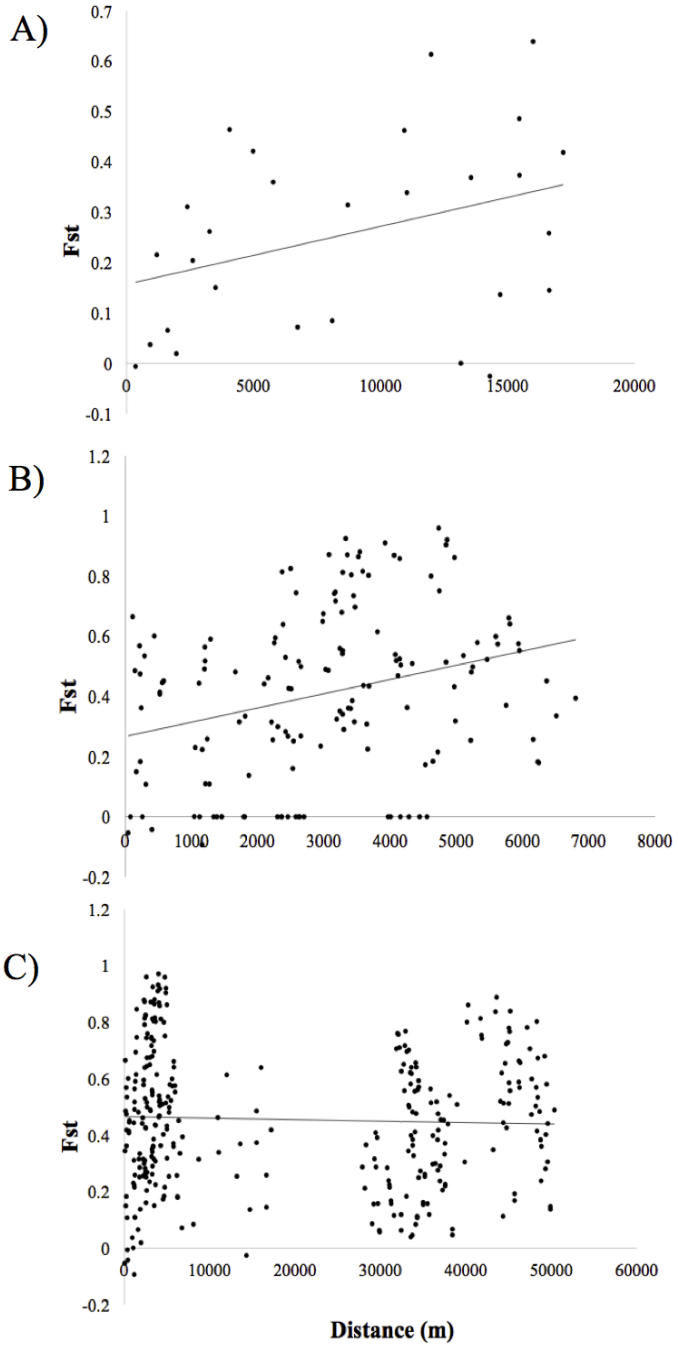

Figure 2: Pairwise F_{ST} values for COI data plotted against geographic distances between pools A) pairwise comparisons within SLNWRC; r=0.4090 p=0.0195.B) comparisons within Merced Grassland Reserve; r=0.2246 p=0.0034. C) all comparisons; r=-0.0386 p=0.2729. Note axis scale differences.

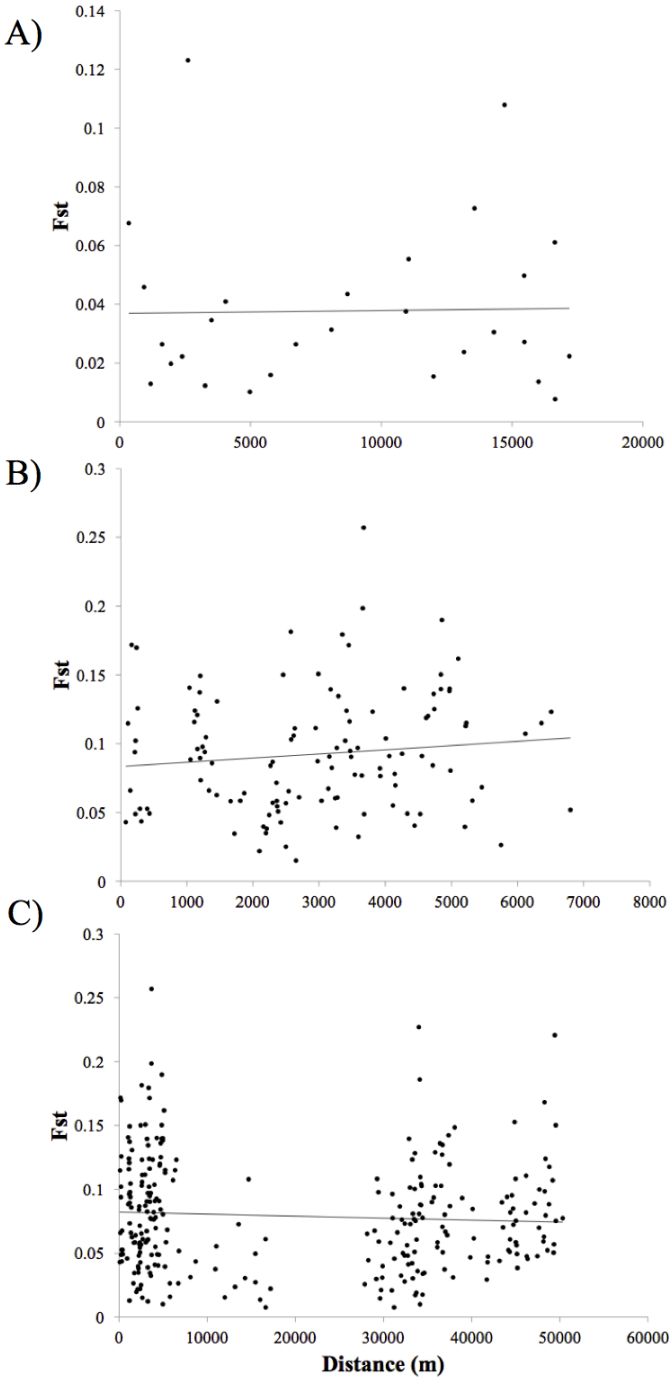

Figure 3: Pairwise F_{ST} values for AFLP data plotted against geographic distances between pools. A) pairwise comparisons comparisons within SLNWRC; r= r=0.0202, p=0.4472. B) comparisons within Merced Grassland Reserve; r=0.1136, p=0.1181. C) all comparisons; r=-0.0634, p=0.2555. Note axes scale differences.

Figure 4: Percentage of ancestry in a pool assigned to one of the 4 clusters determined by STRUCTURE for populations of *B.lynchi* in the SLNWRC based on AFLP data. (1) Kesterson Unit; (2) West Bear Creek Unit; (3) Snowbird Unit; (4) Arena Plains Unit.

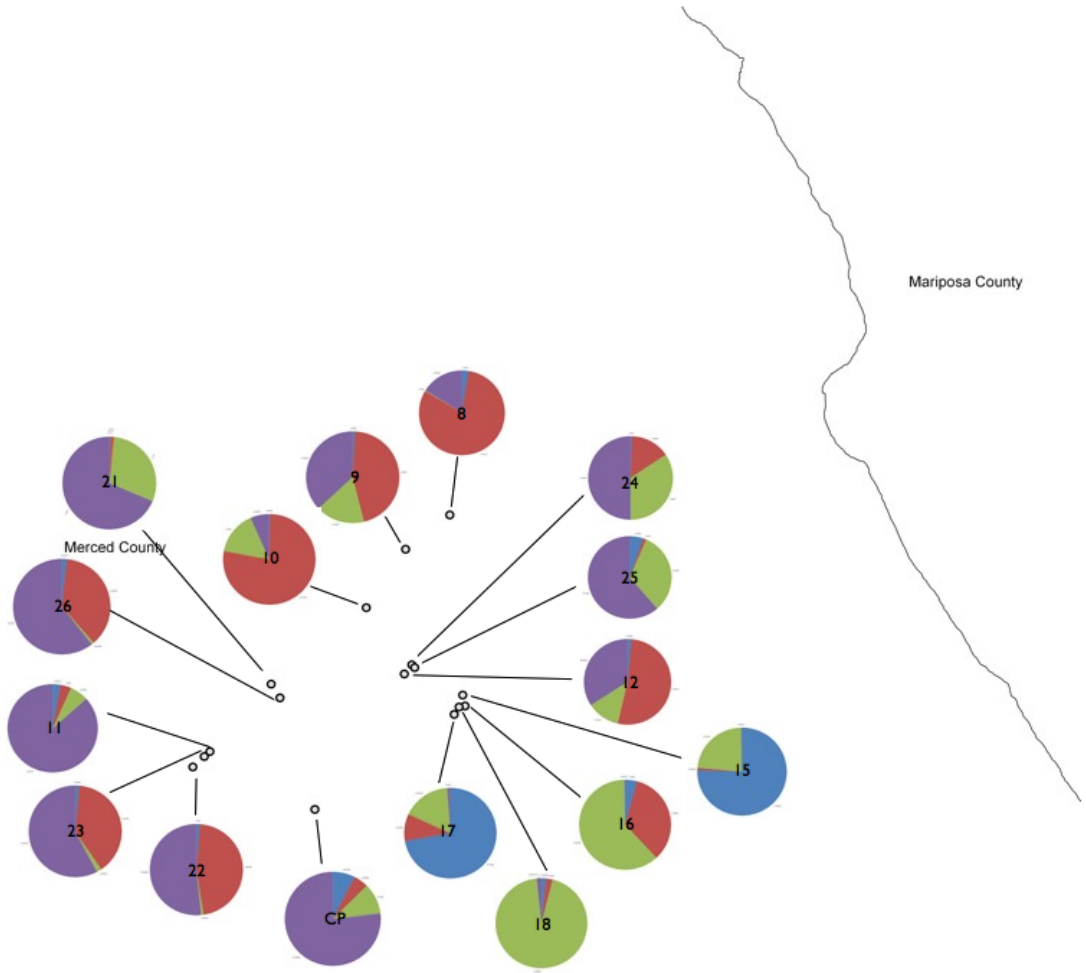

Figure 5: Proportion of assignment to one of the 4 clusters by STRUCTURE for populations of *B. lynchi* in the Merced Grassland Reserve based on AFLP data

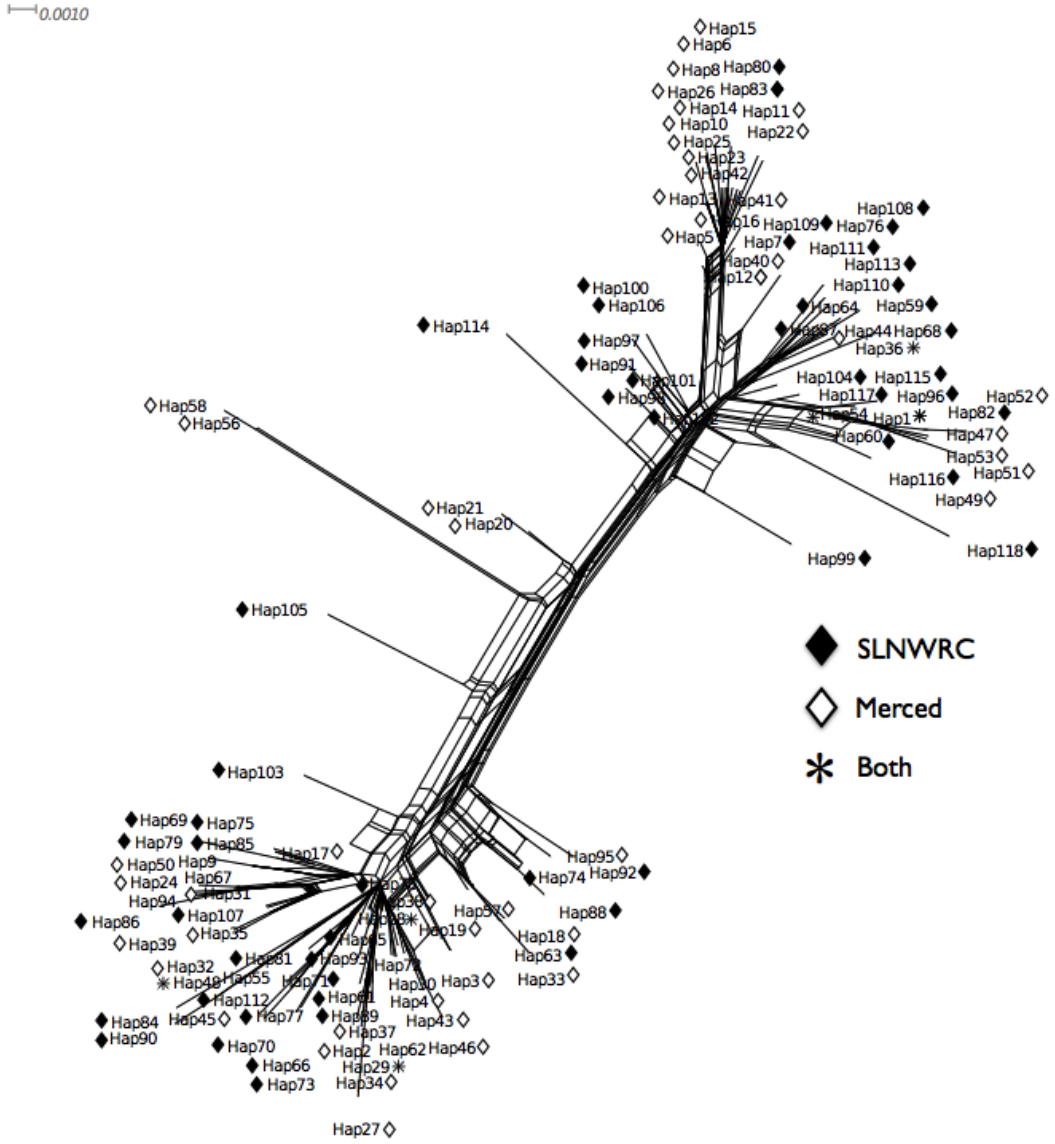

Figure 6: Neighbor-net network for 118 haplotypes found in *B. lynchi*

DISCUSSION

As is common for anostracans, (Davies et al. 1997, Bohonak 1998, Hulsman et al. 2007, Muñoz et al. 2008) populations of *Branchinecta lynchi* exhibit a high degree of genetic differentiation. A majority of COI F_{ST} values were found to be greater then 0.25 and isolation by distance (IBD) existed only at smaller spatial scales for the mitochondrial marker. These results suggest that these populations experience limited local gene flow and regional priority effects. Additionally, the influence that environment has on these patterns is difficult to interpret as both negative and positive correlations exist between environmental metrics and genetic distance.

Regional Scale

A persistent founder effect arises when a small number of initial colonizing genotypes rapidly monopolize an open habitat. Populations are expected to show high genetic differentiation lacking a geographic pattern as a consequence. The absence of isolation by distance (IBD) in both COI and AFLP data suggests of a lack of gene flow-drift equilibrium at the regional scale in *B. lynchi* (Slatkin 1993, Hutchinson and Templeton 1999) possibly resulting from a priority effect. Due to a lack of equilibrium, any conclusions about levels of gene flow at this scale need to be interpreted with caution as F_{ST} measures in a population not at drift-gene flow equilibrium may not accurately estimate measures of migrants exchanged (Wright 1943, Bossart and Prowell 1998, Bohonak and Roderik 2001). Despite the lack of drift-gene flow equilibrium, the wide variance in plotted pairwise F_{ST} values are suggestive of the small part gene flow may play in structuring populations at the regional level (Hutchinson and Templeton 1999). A low number of haplotypes shared between regions (6%) and a high number of private haplotypes per region is also consistent with decreased levels of gene flow between the MGR

and the SLNWRC. This conclusion of low gene flow is additionally supported by the STRUCTURE analysis of AFLP data which lacks any meaningful geographic pattern. If contemporary local gene flow was occurring this analysis should produce a pattern of clustering between nearby sites. At least for AFLPs, gene flow even between nearby pools appears to be low. As a caution, however, it is noted that there is the possibility of high noise or error within my AFLP data (see discussion below). The high degree of genetic differentiation observed here coupled with the high level of private haplotypes and lack of geographically meaningful patterns suggest a role for persistent founder effects in shaping population genetic structure of these populations.

Patterns of genetic structure are predicted by the persistent founder effect hypothesis to be related to past colonization events. The pattern of initial colonization by *B. lynchi* maintained across this spatial area appears to have been random. A COI network identified two clades in which haplotypes in both clades are from the MGR and the SLNWRC. The lack of reciprocally monophyletic clades between the two study areas suggests there is no deep historical isolation between the two sites. This might be expected since at an even spatial larger scale *B. lynchi* has shown only a weak signature of phylogeographic structure (Aguilar 2011). Additionally, although there are a few high pairwise F_{ST} greater then 0.1 values for AFLPs data, there is no clear subdivision by either localities or pools supporting a strong role for the random initial colonization of pools from a diverse set of source populations. If patterns of colonization had occurred in a non-random manner STRUCTURE would also be expected to separate populations in a geographically meaningful way. If pools had been colonized sequentially within each

locality, geographically proximate pools would share more similar ancestry, which is not in the case for these populations.

The monopolization hypothesis stresses the idea that local adaptation can prevent gene flow from occurring between pools (De Meester et al. 2002). While there exists a pattern of isolation by environment (IBE) at the regional level based on the Mahalanobius distances for AFLP data and COI data, the correlation is negative. This relationship would suggests that pools which are more dissimilar based on these environmental measurements experience higher levels of gene flow. This is opposite of what might be expected under the monopolization hypothesis, however, due to previous evidence which found patterns at the regional scale to be governed by priority effects, this negative relationship may also indicate how pools were initially colonized. For AFLP data only there also exists a significant negative relationships between individual environmental measurements and genetic distance (except for pH and conductivity).

There are several reasons why the relationships between these environmental measurements and genetic distance may not accurately explain the patterns found here. First, the sampling design that was executed, at least for the MGR, did not contain a wide array of different soil types required for a better picture of how genetic differentiation varies with distance between soil types. Additionally, the environmental measurements used here are a broad approximation of the soil type and water conditions found in each pool. Soil properties could vary even when pools occur on the same soil type therefore direct measurements of soil and water properties would need to be taken and analyzed on a per pool basis. Finally the soil properties used as explanatory variables here are only assumed to represent a biologically

relevant property, thus more testing would be needed to quantify what factors may affect success or failure of migrants versus residents.

Local Scale Patterns

Population structure among pools at the local scale based on the mitochondrial marker appears to reflect a pattern of limited local gene flow within localities. Within both the MGR and SLNWR a significant pattern of isolation by distance was found using the mitochondrial COI marker as would be expected if populations are experiencing geographically limited gene flow. This pattern is thought to be observed only if gene-flow drift equilibrium conditions have been reached in the system (Slatkin 1993). Although there is weak pattern of isolation by distance at a local scale it is possible that the gene flow pattern observed here is moving toward regional gene flow drift equilibrium. Isolation by distance is likely to form first over smaller spatial scales due to the increased chance of exchange between increasingly proximate populations (Crow and Aoki 1984). This is similar to case IV presented by Hutchinson and Templeton (1999) which shows isolation by distance patterns at smaller scales, but a random scatter of pairwise F_{ST} values at larger geographic distances. In this case gene flow is more important at smaller spatial scales, while for more distant populations drift is the dominant force in shaping structure (Hutchinson and Templeton 1999). Although it is impossible to predict whether the pattern is moving toward regional isolation by distance or becoming dominated by drift. This pattern could be an effect of the importance of persistent founder effects at larger geographic scales, while the influence of gene flow strengthens at smaller geographic scales. Such a pattern could be promoted by higher frequency of shorter distance dispersal events due to movement by more local vectors such as wind or large mammals that may reduce the length of a persistent founder effect while longer

distance dispersal such as movement by birds, are more rare and infrequent (Caceres and Soluk 2002). Additionally, Bolieau et al. (1992) showed that persistent founder effects could lead to a slower approach time to equilibrium even in the face of gene flow.

A pattern of isolation by distance at smaller scales is also consistent with several other studies that find gene flow is limited by geographic distance in anostracans. In populations of *Brachipodopsis wolfi,* a fairy shrimp species found in Africa, gene flow was found to be hindered by distances as small as 50 m (Hulsman et al. 2007). Another North American species, *Brachinecta coloradensis* from the Rocky mountains, displays genetic structuring that was found to be lower within a valley than between valleys, suggesting that gene flow was occurring at a smaller spatial scale (< 5 kilometers; Bohonak 1998). My comparisons within a region for the MGR are all below 7 km with an average distance of 3 km and for the SLNWRC the distances ranged from 0.9 km to 17 km with an average distance of 8 km between pools. Given the sampling design, and the weak signal of IBD, it is possible that gene flow is occurring at a smaller scale than explored in this study. Future studies should focus on smaller spatial sampling for this species to better understand the distance at which the pattern of IBD breaks down. For example, gene flow limitation in *Brachipodopsis wolfi* was originally found to be hindered by distances of 2 km or less (Brendonck et al. 2000), however another study of this species found the distance to be much lower at 50 meters (Hulsman et al. 2007).

In contrast to the mitochondrial marker, no IBD was detected for MGR or SLNWRC for nuclear AFLP markers. A lack of correlation between diversity measures using AFLPs and mtDNA suggests that there are additional factors leading to disagreement between these markers. These patterns suggest one of three possible scenarios: error or noise in my AFLP data that does

not capture the true pattern, female-biased dispersal in B. lynchi, *and/or a l*ag time to equilibri m of nuclear markers. Unfortunately with my data set it is impossible to rule out any of these pos ible scenarios nor are any of these mutually exclusive.

Locally, within the MGR for both COI and AFLP, there exists no significant patterns of isolation by environment (IBE). To reliably quantify this relationship sampling of multiple pools from multiple different soil types would provide a better picture of the relationship occurring at this geographic scale. On the other hand the SLNWRC shows no significant patterns of IBE for either marker type, with the exception of pH for COI . This comparison shows a significant positive correlation with genetic distance. The pH of a pool may be associated with different physiological requirements of vernal pool inhabitants such as hatching cues (Brendonck 1996). and this pattern might suggest that pH is a factor in preventing successful local gene flow within the SLNWRC at least for the mitochondrial genome. Considering the differences in what soil properties are significantly correlated to genetic distance, factors which are important in promoting or preventing local gene flow may be different by locality or pool complex.

Within pool variation

While some aspects of this system appear to fit characteristics of the monopolization hypothesis and persistent found effect hypothesis, others do not. If persistent founder effects and the monopolization hypothesis were occurring in these populations, then diversity within a pool it expected to be low due to the rapid colonization of a open habitat by a few individuals (Bolieau 1992 , De Meester et al. 2002), yet within pool diversity for samples of *B. lynchi* are generally high. With the exception of three pools, haplotype diversities on a per pool basis are all above 0.3. The number of haplotypes per pool also varies greatly. For example, all three of the

ARP pools have a large number of different haplotypes while Pool 10 contains only a single haplotype. In comparison, other studies on anostracans reporting a per pool diversity measure (generally heterozygosity) have found different degrees of variation (Davies et al. 1997, Bohonak 1998, Hulsman et al. 2007). When haplotype data is present, populations tend to generally have a high number of haplotypes per pool (McCafertey et al. 2010, Vanschoenwinkel et al. 2011). More and better measures of within population diversity should be examined to better understand the maintenance of diversity.

The divergence from the persistent founder effect hypothesis found in *B. lynchi* could be explained by colonization from highly diverse source populations or recurrent gene flow. If the source population is highly diverse, then the probability of several alleles establishing in a pool with fewer colonizing individuals is higher than if colonization occurred from a less diverse source. Elevated diversity within a pool could also be explained by colonization from multiple sources or by multiple colonization events. Multiple colonization events could occur if an open habitat is monopolized slowly, thereby increasing the probability of successful colonization by migrants for a longer period of time (Naihong et al. 2000, De Meester et al. 2002). In terms of time to monopolization larger habitats should take a longer time to colonize than smaller habitats due to increased space and resources. As is such higher diversity might be expected in larger pools (De Meester et al. 2002). This pattern was found in a Chinese *Artemia* (Anostraca) in which lake habitats with larger surface areas tended to show higher heterozygosities (Naihong et al. 2000). Although I found a positive relationship with genetic diversity measures and pool size these relationships were not statistically significant.

Modes of Dispersal

B. lynchi, like other freshwater invertebrates, has many possible vectors of passive dispersal. These include common suspected vectors such as birds (Figuerola and Green 2002, Figuerola et al. 2005, Brochet et al. 2010) and mammals (Vanschoenwinkel et al. 2008) in the MGR and the SLNWRC. The SLNWRC is a stopping point for many waterfowl and is located within the Pacific flyway, highlighting the possibility that dispersal has the potential to occur at the regional scale. Birds have the ability to disperse cysts much further than other vectors as they can fly large distances and may be most important for long distance dispersal events. Such events could account for the random distribution of haplotypes at the regional scale in this study.

Both the MGR and the SLNWRC are actively grazed by cattle. Studies of the impact of cattle grazing on vernal pools have found that grazing increases the occurrence of native flora and the diversity of pool invertebrates. (Marty 2004, Marty and Pyke 2004). In addition to these benefits, grazing cattle may also provide an important vector for dispersal of freshwater invertebrates. Generally cattle are grazed in one locality and therefore may represent an important local vector transporting cysts between pools on muddy fur or after ingestion and defecation. Wind may also be an important local dispersant for cysts of *B. lynchi* as wind appears to be less likely to produce dispersal over longer distances for many species (Jenkins and Underwood 1998, Brendonck and Riddoch 1999, Caceres and Soluk 2002). If local gene flow is important as was suggested here, then management of cattle grazing may provide an the additional benefit of promoting local gene flow.

AFLPs - Issues

There are several reasons that my AFLP data set may contain more noise than my mitochondrial data set. AFLPs are a commonly used dominant marker due to their ease of

obtaining many loci scattered randomly through the genome (Vos et al. 1995). AFLPs have been praised for their high reproducibility which allows for a high resolution fingerprinting of an individual (Mueller and Wolfenbarger 1999). However, AFLP markers can in some cases be considered less informative than other nuclear markers, such as microsatellites and single nucleotide polymorphisms (SNPs), as they are dominant and some information about an individual's genotype is lost when using them (Mueller and Wolfenbarger 1999). The power of this marker type lies instead in the large number or loci randomly obtained through the genome as well as the ease in which they are obtained (Mariette et al. 2002).

While AFLPs have been employed in other population genetic studies with success (e.g. Alacs et al. 2011, Crawford et al. 2011), there are still several pitfalls to remain cautious about when interpreting AFLP data. These include band homoplasy, subjective peak scoring and sampling effort (reviewed in Bronin et al. 2007). Homoplasy occurs when bands that are not homologous are represented by the same peak. A more common source of error in intraspecies comparisons that can occur is subjective peak scoring (Bronin et al. 2007, Meudt and Clark 2007). I have attempted to control for this common through the use of automatic peak calling software (Whitlock et al. 2008, Arthofer et al. 2011). To reliably quantify error in my data would require replicated profiles of individuals (Zang and Hare 2012) which I do not have. As a consequence the amount of error or noise and the success of my filtering processes for this dataset are unknown. It is also possible that my sampling effort of populations was too low. It has been suggested the sampling effort for RAPD data, a similar technique, should be 2-10 times that of microsatellites (Lynch & Milligan 1994). Krauss (2000) found that for genetic diversity results using AFLPs, samples of 30 individuals per population were needed for accurate results. My

samples size was on average 15 individuals per pool. Future work should take advantage of both more informative markers such co-dominant microsatellites or SNP markers, as well as focus on increasing sampling sizes when possible. The ability to quantify error through replicate profiles of individuals should also be taken into account especially if markers such as AFLP are to be used in the future.

The lack of concordant patterns of nuclear AFLP data and mtDNA could also represent a case of sex-biased dispersal. In *B. lynchi* evidence for gene flow-drift equilibrium in maternally inherited mitochondrial COI marker but not nuclear AFLPs suggests the possibility that female gene flow has occurred long enough and is frequent enough for equilibrium conditions to be reached, while male movement is not. The presence of sex-biased dispersal in *B. lynchi,* however, seems highly unlikely because dispersal in *B. lynchi* is passive and therefore should occur equally for males and females. Such a pattern could only arise if female *B. lynchi* received some benefit in a new environment over males including but not limited to increased frequency of hatching, survival to reproductive age or mating success. Despite the intuitive unlikelihood of this, such a pattern could be ruled out or tested in the future through other methods that have been used to observe sex-biased dispersal (Prugnolle and Meesus 2002) or more adequate sampling of males and females within a pool (Goudet et al. 2002).

Finally the discrepancies between genetic patterns of these two markers could be due to a slower approach to equilibrium conditions of nuclear markers than mitochondrial markers due to divergent mutation rates and/or effective population sizes (Birkey et al. 1983, Chesser and Baker, 1996). As is such, mitochondrial markers are expected to reach drift-migration equilibrium conditions faster than the diploid nuclear genome (Birkey et al. 1989, Friesen et al. 1996). Future

work could explore this possible scenario further through simulations of how effective populations sizes of nuclear and mitochondrial genomes in this system vary under the conditions of persistent founder effects.

Conservation

Habitat loss is the most detrimental force affecting populations of *B. lynchi* and other obligate vernal pool inhabitants (Belk 1998, Fugate 1998). Vernal pool restoration has occurred in California already including projects in San Diego County (Black and Zedler 1998), Santa Barbara (Ferren and Hubbard 1998) and Central California (Sutter and Francisco 1998, Ramp et al. 2002) with varying degrees of success. While restored pools tend to move toward resemblance to natural pools they often remain distinguishable even years after creation (Black and Zedler 1998, Ferren and Hubbard 1998, Sutter and Francisco 1998). Sutter and Fransico (1998) suggest a lack of habitat variability leading to lower biodiversity is one factors that can contribute to restoration success or failure.

Maintaining historical conditions of vernal pools, including historical hydrological conditions and biological connectivity, is another important factor for conservation of freshwater invertebrates (Amezaga et al. 2002). In terms of pool restoration for anostracans, and possibly other freshwater invertebrates, if gene flow is locally limited as was found here, then efforts should be made to restore pools near or within established pool complexes to restore connectivity. Likewise, due to the high genetic diversity and importance of local gene flow of this species a pool may not need to be restored from only its original cyst bank or a single cyst bank. It may be viable for a successful restoration to include parent material from multiple local sources.

Given that gene flow is important at the local scale for mtDNA, management of vernal pools may benefit from a focus on local scales rather than large regional areas. Along these same lines, protection of remaining vernal pool habitat should consider higher priority for remaining large intact vernal pool complexes as opposed to more fragmented habitats to preserve local connectivity. Management practices may want to consider maintaining local vectors that contribute to gene flow (e.g. cattle) to maintain connectivity in natural and restored vernal pool habitats. My results suggest that local factors are important in maintaining healthy vernal pool habitats in Central Valley of California.

Conclusion

In general for *B. lynchi* the patterns maintaining population structure for this species seem more complex than being explained by either monopolization hypothesis or dispersal limitation. Scale appears to be an important factor in determining population genetic structure in this species. Dispersal limitation and local gene flow appear to be important in structuring genetic patterns at a more local scale, while persistent founder effects maintain high genetic distances at regional scales. Although I was unable to determine the exact geographic distance at which dispersal is limited, future studies may look into a different sampling scale that can capture this distance. In conjunction with this it may also be of interest to look into a better measures of isolation by environment. Although the sampling scheme was not appropriate here there were several significant correlations, such as pH for the SLNWRC, that might suggest similarity of environment may facilitate some local gene flow.

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APPENDIX

Appendix 1: Pairwise F_{ST} Matrix calculated for the mitochondrial COI, shown on the top half of the matrix, and for AFLPs ,shown on the bottom half of the matrix.

	CP	**Pool10**	**Pool11**	**Pool12a**	**Pool15**	**Pool16**	**Pool17**	**Pool18**	**Pool21**
CP	0	0.0765	0.0482	0.0585	0.0904	0.1021	0.0673	0.1345	0.0251
Pool10	0.93211	0	0.1037	0.0627	0.1501	0.1112	0.1058	0.1814	0.0867
Pool11	0.5781	0.97112	0	0.0821	0.1396	0.1503	0.1187	0.1361	0.0346
Pool12a	0.8716	0.84594	0.9104	0	0.0962	0.1046	0.0734	0.1493	0.0567
Pool15	0.8644	0.82433	0.9039	0.2232	0	0.0939	0.0526	0.1697	0.0970
Pool16	0.3859	0.67409	0.5143	0.5906	0.5683	0	0.1257	0.1148	0.0769
Pool17	0.7471	0.64444	0.7999	0.1095	0.0000	0.41836	0	0.1717	0.0948
Pool18	0.9252	0.96031	0.9596	0.5178	0.3620	0.6649	0.1497	0	0.0775
Pool21	0.1604	0.87901	0.3154	0.8252	0.8161	0.3080	0.6971	0.8806	0
Pool22	0.5300	0.50980	0.6007	0.5094	0.4810	0.2541	0.3183	0.5360	0.4616
Pool23	0.2995	0.91892	0.4856	0.8692	0.8617	0.4324	0.7512	0.9208	0.1376
Pool24	0.2903	0.61502	0.4689	0.4750	0.4438	0.1086	0.2586	0.5640	0.2685
Pool25	0.8125	0.74656	0.8583	0.1831	0.0007	0.4912	0.0000	0.31110	0.75931
Pool26	0.2560	0.87224	0.4814	0.8142	0.8043	0.3155	0.6795	0.8709	0.1077
Pool6	0.5742	0.28543	0.6407	0.4993	0.4869	0.3408	0.3596	0.5522	0.5190
Pool7	0.5993	0.25172	0.6611	0.5162	0.4900	0.3514	0.3611	0.5596	0.5393
Pool8	0.2572	0.79139	0.4516	0.7422	0.7348	0.2247	0.6150	0.8024	0.1841
Pool9	0.4984	0.69388	0.5792	0.6391	0.6496	0.3243	0.5422	0.7173	0.4365
ARP101	0.1578	0.75678	0.3656	0.7101	0.6963	0.1834	0.5575	0.7680	0.0626
ARP102	0.2392	0.62139	0.3914	0.5814	0.5609	0.1075	0.4114	0.6409	0.1679
ARP103	0.2845	0.50614	0.4089	0.4997	0.4768	0.0840	0.3273	0.5554	0.2243
KST70	0.6573	0.41463	0.7233	0.4837	0.4412	0.3057	0.2811	0.5805	0.5883
KST203	0.8393	0.78161	0.8885	0.7060	0.6729	0.5339	0.5031	0.8025	0.7796
SB201	0.6564	0.27586	0.7172	0.4181	0.3711	0.3324	0.2055	0.4533	0.5929
SB5	0.7015	0.56373	0.7604	0.5150	0.4760	0.3837	0.2997	0.5184	0.6400

Appendix 1 continued: Pairwise F_{ST} Matrix calculated for the mitochondrial COI, shown on the top half of the matrix, and for AFLPs ,shown on the bottom half of the matrix.

	Pool22	**Pool23**	**Pool24**	**Pool25**	**Pool26**	**Pool6**	**Pool7**	**Pool8**	**Pool9**
CP	0.0428	0.0570	0.0389	0.0604	0.0350	-	-	0.1072	0.0395
Pool10	0.0404	0.0696	0.0858	0.1308	0.0715	-	-	0.0660	0.0660
Pool11	0.0493	0.0660	0.0550	0.0781	0.0582	-	-	0.0586	0.0586
Pool12a	0.0492	0.0910	0.0489	0.1021	0.0544	-	-	0.0507	0.0507
Pool15	0.1151	0.1399	0.1158	0.1406	0.1239	-	-	0.0872	0.0872
Pool16	0.1129	0.1382	0.0940	0.1372	0.1162	-	-	0.0825	0.0825
Pool17	0.0805	0.1251	0.0978	0.1208	0.0970	-	-	0.0609	0.0609
Pool18	0.1618	0.1898	0.0895	0.1240	0.1794	-	-	0.1394	0.1394
Pool21	0.0397	0.0641	0.0151	0.0611	0.0436	-	-	0.0324	0.0324
Pool22	0	0.0526	0.0489	0.0910	0.0219	-	-	0.0264	0.0264
Pool23	0.5347	0	0.0927	0.1401	0.0585	-	-	0.0683	0.0683
Pool24	0.1730	0.3630	0	0.0430	0.0655	-	-	0.0382	0.0382
Pool25	0.40259	0.81157	0.34444	0	0.1030	-	-	0.0840	0.0840
Pool26	0.4414	0.3345	0.2516	0.7445	0	-	-	0.0488	0.0488
Pool6	0.1796	0.5529	0.2677	0.4250	0.5043	0	-	-	-
Pool7	0.1825	0.5754	0.2827	0.4267	0.5249	0.0000	0	-	-
Pool8	0.3942	0.3356	0.2349	0.6753	0.2155	0.4460	0.4520	0	0.0884
Pool9	0.3704	0.5227	0.3149	0.5950	0.4348	0.4078	0.4146	0.2302	0
ARP101	0.2871	0.2122	0.1186	0.6263	0.0574	0.3639	0.3845	0.1127	0.3442
ARP102	0.1561	0.2872	0.0459	0.4821	0.1576	0.2538	0.2617	0.1189	0.2733
ARP103	0.0862	0.3159	0.0397	0.3987	0.2154	0.1544	0.1619	0.1575	0.2497
KST70	0.1129	0.6547	0.2381	0.3604	0.5686	0.1473	0.1376	0.4893	0.4021
KST203	0.3486	0.8372	0.4732	0.5988	0.7668	0.3831	0.3840	0.6797	0.5691
SB201	0.0626	0.6510	0.2376	0.2911	0.5705	0.0671	0.0467	0.5088	0.4394
SB5	0.1156	0.7058	0.2973	0.3982	0.6182	0.2259	0.2204	0.5404	0.4547

Appendix 1 continued: Pairwise F_{ST} Matrix calculated for the mitochondrial COI, shown on the top half of the matrix, and for AFLPs ,shown on the bottom half of the matrix.

	ARP101	**ARP102**	**ARP103**	**KST70**	**KST203**	**SB201**	**SB5**	**WBVP04**
CP	0.0213	0.0210	0.0582	0.0456	0.0385	0.0101	0.0303	0.0293
Pool10	0.0763	0.0768	0.1233	0.0984	0.0890	0.0706	0.1290	0.0941
Pool11	0.0445	0.0146	0.0977	0.0849	0.0702	0.0412	0.0325	0.0617
Pool12a	0.0501	0.0511	0.0809	0.0523	0.0478	0.0508	0.1029	0.0524
Pool15	0.0728	0.1027	0.0881	0.0754	0.0796	0.0869	0.1348	0.0755
Pool16	0.1014	0.1042	0.1860	0.1502	0.1239	0.1196	0.1271	0.1082
Pool17	0.0482	0.0808	0.0361	0.0505	0.0592	0.0642	0.1360	0.0723
Pool18	0.1395	0.1096	0.2270	0.2206	0.1681	0.1423	0.1028	0.1526
Pool21	0.0309	0.0077	0.0963	0.0818	0.0586	0.0176	0.0172	0.0429
Pool22	0.0257	0.0298	0.0676	0.0512	0.0440	0.0278	0.0663	0.0468
Pool23	0.0653	0.0591	0.1082	0.0953	0.0899	0.0562	0.0866	0.0846
Pool24	0.0483	0.0192	0.1003	0.0884	0.0699	0.0374	0.0547	0.0609
Pool25	0.0732	0.0607	0.1283	0.1177	0.0999	0.0802	0.0585	0.0819
Pool26	0.0399	0.0458	0.0776	0.0477	0.0562	0.0340	0.0754	0.0474
Pool6	-	-	-	-	-	-	-	-
Pool7	-	-	-	-	-	-	-	-
Pool8	0.0877	0.0936	0.0901	0.0775	0.1071	0.0932	0.0668	0.1106
Pool9	0.0421	0.0347	0.0778	0.0569	0.0630	0.0311	0.1485	0.0495
ARP101	0	0.0198	0.0264	0.0223	0.0136	0.0102	0.0409	0.0154
ARP102	0.0191	0	0.0676	0.0611	0.0497	0.0123	0.0222	0.0376
ARP103	0.0653	0.0000	0	0.0077	0.0272	0.0346	0.1231	0.0554
KST70	0.4189	0.2584	0.1448	0	0.0129	0.0305	0.1079	0.0264
KST203	0.6393	0.4859	0.3735	0.2155	0	0.0237	0.0727	0.0159
SB201	0.4214	0.2617	0.1503	0.0000	0.25430	0	0.0459	0.0313
SB5	0.4644	0.3108	0.2041	0.1364	0.3689	0.0370	0	0.0435

Appendix 2: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	CP	Pool10	Pool11	Pool12	Pool15	Pool16	Pool17	Pool18	Pool21	Pool22
Hap_1	-	10	-	-	-	-	-	-	-	-
Hap_2	-	-	20	-	-	-	-	-	-	1
Hap_3	-	-	1	-	-	-	-	-	-	1
Hap_4	-	-	1	-	-	-	-	-	-	-
Hap_5	-	-	-	1	-	-	-	-	-	-
Hap_6	-	-	-	15	-	-	-	-	-	-
Hap_7	-	-	-	3	14	3	5	2	-	-
Hap_8	-	-	-	1	-	-	-	-	-	-
Hap_9	7	-	-	1	-	-	-	-	-	-
Hap_10	-	-	-	1	-	-	-	-	-	-
Hap_11	-	-	-	-	2	-	-	-	-	-
Hap_12	-	-	-	-	1	-	-	-	-	-
Hap_13	-	-	-	-	1	-	-	-	-	-
Hap_14	-	-	-	-	1	-	-	-	-	-
Hap_15	-	-	-	-	2	-	-	-	-	-
Hap_16	-	-	-	-	1	-	-	-	-	-
Hap_17	-	-	-	-	1	-	-	-	-	-
Hap_18	-	-	-	-	-	11	-	-	-	-
Hap_19	-	-	-	-	-	2	-	-	-	-
Hap_20	-	-	-	-	-	1	-	-	-	-
Hap_21	-	-	-	-	-	1	-	-	1	-
Hap_22	-	-	-	-	-	1	-	-	-	-
Hap_23	-	-	-	-	-	1	-	-	-	-
Hap_24	-	-	-	-	-	-	1	-	-	-
Hap_25	-	-	-	-	-	-	1	9	-	-
Hap_26	-	-	-	-	-	-	-	1	-	-
Hap_27	-	-	-	-	-	-	-	-	2	-
Hap_28	-	-	-	-	-	-	-	-	4	-
Hap_29	2	-	-	-	-	-	-	-	3	-
Hap_30	-	-	-	-	-	-	-	-	2	-
Hap_31	-	-	-	-	-	-	-	-	1	-
Hap_32	-	-	-	-	-	-	-	-	1	-
Hap_33	-	-	-	-	-	-	-	-	1	-
Hap_34	-	-	-	-	-	-	-	-	2	-
Hap_35	-	-	-	-	-	-	-	-	1	-
Hap_36	-	-	-	-	-	-	-	-	-	9
Hap_37	-	-	-	-	-	-	-	-		1
Hap_38	-	-	-	-	-	-	-	-		1
Hap_39	-	-	-	-	-	-	-	-		1
Hap_40	-	-	-	-	-	-	-	-	-	-
Hap_41	-	-	-	-	-	-	-	-	-	-
Hap_42	-	-	-	-	-	-	-	-	-	-
Hap_43	-	-	-	-	-	-	-	-	-	-
Hap_44	-	-	-	-	-	-	-	-	-	-
Hap_45	-	-	-	-	-	-	-	-	-	-

Appendix 2 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	CP	Pool10	Pool11	Pool12	Pool15	Pool16	Pool17	Pool18	Pool21	Pool22
Hap_46	-	-	-	-	-	-	-	-	-	-
Hap_47	-	-	-	-	-	-	-	-	-	-
Hap_48	-	-	-	-	-	-	-	-	-	-
Hap_49	-	-	-	-	-	-	-	-	-	-
Hap_50	-	-	-	-	-	-	-	-	-	-
Hap_51	-	-	-	-	-	-	-	-	-	-
Hap_52	-	-	-	-	-	-	-	-	-	-
Hap_53	-	-	-	-	-	-	-	-	-	-
Hap_54	-	-	-	-	-	-	-	-	-	-
Hap_55	-	-	-	-	-	-	-	-	-	-
Hap_56	-	-	-	-	-	-	-	-	-	-
Hap_57	-	-	-	-	-	-	-	-	-	-
Hap_58	-	-	-	-	-	-	-	-	-	-
Hap_59	-	-	-	-	-	-	-	-	-	-
Hap_60	-	-	-	-	-	-	-	-	-	-
Hap_61	-	-	-	-	-	-	-	-	-	-
Hap_62	-	-	-	-	-	-	-	-	-	-
Hap_63	-	-	-	-	-	-	-	-	-	-
Hap_64	-	-	-	-	-	-	-	-	-	-
Hap_65	-	-	-	-	-	-	-	-	-	-
Hap_66	-	-	-	-	-	-	-	-	-	-
Hap_67	-	-	-	-	-	-	-	-	-	-
Hap_68	-	-	-	-	-	-	-	-	-	-
Hap_69	-	-	-	-	-	-	-	-	-	-
Hap_70	-	-	-	-	-	-	-	-	-	-
Hap_71	-	-	-	-	-	-	-	-	-	-
Hap_72	-	-	-	-	-	-	-	-	-	-
Hap_73	-	-	-	-	-	-	-	-	-	-
Hap_74	-	-	-	-	-	-	-	-	-	-
Hap_75	-	-	-	-	-	-	-	-	-	-
Hap_76	-	-	-	-	-	-	-	-	-	-
Hap_77	-	-	-	-	-	-	-	-	-	-
Hap_78	-	-	-	-	-	-	-	-	-	-
Hap_79	-	-	-	-	-	-	-	-	-	-
Hap_80	-	-	-	-	-	-	-	-	-	-
Hap_81	-	-	-	-	-	-	-	-	-	-
Hap_82	-	-	-	-	-	-	-	-	-	-
Hap_83	-	-	-	-	-	-	-	-	-	-
Hap_84	-	-	-	-	-	-	-	-	-	-
Hap_85	-	-	-	-	-	-	-	-	-	-
Hap_86	-	-	-	-	-	-	-	-	-	-
Hap_87	-	-	-	-	-	-	-	-	-	-
Hap_88	-	-	-	-	-	-	-	-	-	-
Hap_89	-	-	-	-	-	-	-	-	-	-
Hap_90	-	-	-	-	-	-	-	-	-	-

Appendix 2 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	CP	Pool10	Pool11	Pool12	Pool15	Pool16	Pool17	Pool18	Pool21	Pool22
Hap_91	-	-	-	-	-	-	-	-	-	-
Hap_92	-	-	-	-	-	-	-	-	-	-
Hap_93	-	-	-	-	-	-	-	-	-	-
Hap_94	1	-	-	-	-	-	-	-	-	-
Hap_95	1	-	-	-	-	-	-	-	-	-
Hap_96	-	-	-	-	-	-	-	-	-	-
Hap_97	-	-	-	-	-	-	-	-	-	-
Hap_98	-	-	-	-	-	-	-	-	-	-
Hap_99	-	-	-	-	-	-	-	-	-	-
Hap_100	-	-	-	-	-	-	-	-	-	-
Hap_101	-	-	-	-	-	-	-	-	-	-
Hap_102	-	-	-	-	-	-	-	-	-	-
Hap_103	-	-	-	-	-	-	-	-	-	-
Hap_104	-	-	-	-	-	-	-	-	-	-
Hap_105	-	-	-	-	-	-	-	-	-	-
Hap_106	-	-	-	-	-	-	-	-	-	-
Hap_107	-	-	-	-	-	-	-	-	-	-
Hap_108	-	-	-	-	-	-	-	-	-	-
Hap_109	-	-	-	-	-	-	-	-	-	-
Hap_110	-	-	-	-	-	-	-	-	-	-
Hap_111	-	-	-	-	-	-	-	-	-	-
Hap_112	-	-	-	-	-	-	-	-	-	-
Hap_113	-	-	-	-	-	-	-	-	-	-
Hap_114	-	-	-	-	-	-	-	-	-	-
Hap_115	-	-	-	-	-	-	-	-	-	-
Hap_116	-	-	-	-	-	-	-	-	-	-
Hap_117	-	-	-	-	-	-	-	-	-	-
Hap_118	-	-	-	-	-	-	-	-	-	-

Appendix 2 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	Pool23	Pool24	Pool25	Pool26	Pool6	Pool7	Pool8	Pool9
Hap_1	-	-	-	-	-	-	-	-
Hap_2	-	-	-	-	-	-	-	-
Hap_3	-	-	-	-	-	-	-	-
Hap_4	-	-	-	-	-	-	-	-
Hap_5	-	-	-	-	-	-	-	-
Hap_6	-	-	-	-	-	-	-	-
Hap_7	-	4	13	-	-	-	-	-
Hap_8	-	-	-	-	-	-	-	-
Hap_9	-	5	-	-	-	-	-	-
Hap_10	-	-	-	-	-	-	-	-
Hap_11	-	-	-	-	-	-	-	-
Hap_12	-	-	-	-	-	-	-	-
Hap_13	-	-	-	-	-	-	-	-
Hap_14	-	-	-	-	-	-	-	-
Hap_15	-	-	-	-	-	-	-	-
Hap_16	-	-	-	-	-	-	-	-
Hap_17	1	-	-	-	-	-	-	-
Hap_18	-	-	-	-	-	-	-	-
Hap_19	-	-	-	-	-	-	-	-
Hap_20	-	-	-	-	-	-	-	-
Hap_21	-	-	-	-	-	-	-	-
Hap_22	-	-	-	-	-	-	-	-
Hap_23	-	-	-	-	-	-	-	-
Hap_24	-	-	-	-	-	-	-	-
Hap_25	-	-	-	-	-	-	-	-
Hap_26	-	-	-	-	-	-	-	-
Hap_27	-	-	-	-	-	-	-	-
Hap_28	-	2	2	2	-	-	4	-
Hap_29	-	-	-	-	-	-	-	-
Hap_30	-	-	-	6	-	-	-	-
Hap_31	-	-	-	-	-	-	-	-
Hap_32	-	-	-	-	-	-	-	-
Hap_33	-	-	-	-	-	-	-	-
Hap_34	-	-	-	-	-	-	-	-
Hap_35	-	-	-	-	-	-	-	-
Hap_36	-	-	-	-	-	-	-	-
Hap_37	6	-	-	-	-	-	-	-
Hap_38	-	-	-	-	-	-	-	-
Hap_39	2	-	-	-	-	-	-	-
Hap_40	-	1	-	-	-	-	-	-
Hap_41	-	-	1	-	-	-	-	-
Hap_42	-	-	2	-	-	-	-	-
Hap_43	-	-	-	1	-	-	-	-
Hap_44	-	-	-	1	-	-	-	-
Hap_45	-	-	-	1	-	-	-	-

Appendix 2 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	Pool23	Pool24	Pool25	Pool26	Pool6	Pool7	Pool8	Pool9
Hap_46	-	-	-	1	-	-	-	-
Hap_47	-	-	-	-	3	-	-	-
Hap_48	-	-	-	-	4	1	-	-
Hap_49	-	-	-	-	7	3	-	-
Hap_50	-	-	-	-	2	-	-	-
Hap_51	-	-	-	-	2	1	-	-
Hap_52	-	-	-	-	1	-	-	-
Hap_53	-	-	-	-	1	-	-	-
Hap_54	-	-	-	-	2	4	-	-
Hap_55	-	-	-	-	1	2	10	3
Hap_56	-	-	-	-	-	-	1	5
Hap_57	-	-	-	-	-	-	6	-
Hap_58	-	-	-	-	-	-	2	-
Hap_59	-	-	-	-	-	-	-	-
Hap_60	-	-	-	-	-	-	-	-
Hap_61	-	-	-	-	-	-	-	-
Hap_62	-	-	-	-	-	-	-	-
Hap_63	-	-	-	-	-	-	-	-
Hap_64	-	-	-	-	-	-	-	-
Hap_65	-	-	-	-	-	-	-	-
Hap_66	-	-	-	-	-	-	-	-
Hap_67	-	-	-	-	-	-	-	-
Hap_68	-	-	-	-	-	-	-	-
Hap_69	-	-	-	-	-	-	-	-
Hap_70	-	-	-	-	-	-	-	-
Hap_71	-	-	-	-	-	-	-	-
Hap_72	-	-	-	-	-	-	-	-
Hap_73	-	-	-	-	-	-	-	-
Hap_74	-	-	-	-	-	-	-	-
Hap_75	-	-	-	-	-	-	-	-
Hap_76	-	-	-	-	-	-	-	-
Hap_77	-	-	-	-	-	-	-	-
Hap_78	-	-	-	-	-	-	-	-
Hap_79	-	-	-	-	-	-	-	-
Hap_80	-	-	-	-	-	-	-	-
Hap_81	-	-	-	-	-	-	-	-
Hap_82	-	-	-	-	-	-	-	-
Hap_83	-	-	-	-	-	-	-	-
Hap_84	-	-	-	-	-	-	-	-
Hap_85	-	-	-	-	-	-	-	-
Hap_86	-	-	-	-	-	-	-	-
Hap_87	-	-	-	-	-	-	-	-
Hap_88	-	-	-	-	-	-	-	-
Hap_89	-	-	-	-	-	-	-	-
Hap_90	-	-	-	-	-	-	-	-

Appendix 2 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the MGR

	Pool23	Pool24	Pool25	Pool26	Pool6	Pool7	Pool8	Pool9
Hap_91	-	-	-	-	-	-	-	-
Hap_92	-	-	-	-	-	-	-	-
Hap_93	-	-	-	-	-	-	-	-
Hap_94	-	-	-	-	-	-	-	-
Hap_95	-	-	-	-	-	-	-	-
Hap_96	-	-	-	-	-	-	-	-
Hap_97	-	-	-	-	-	-	-	-
Hap_98	-	-	-	-	-	-	-	-
Hap_99	-	-	-	-	-	-	-	-
Hap_100	-	-	-	-	-	-	-	-
Hap_101	-	-	-	-	-	-	-	-
Hap_102	-	-	-	-	-	-	-	-
Hap_103	-	-	-	-	-	-	-	-
Hap_104	-	-	-	-	-	-	-	-
Hap_105	-	-	-	-	-	-	-	-
Hap_106	-	-	-	-	-	-	-	-
Hap_107	-	-	-	-	-	-	-	-
Hap_108	-	-	-	-	-	-	-	-
Hap_109	-	-	-	-	-	-	-	-
Hap_110	-	-	-	-	-	-	-	-
Hap_111	-	-	-	-	-	-	-	-
Hap_112	-	-	-	-	-	-	-	-
Hap_113	-	-	-	-	-	-	-	-
Hap_114	-	-	-	-	-	-	-	-
Hap_115	-	-	-	-	-	-	-	-
Hap_116	-	-	-	-	-	-	-	-
Hap_117	-	-	-	-	-	-	-	-
Hap_118	-	-	-	-	-	-	-	-

Appendix 3: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the SLNWRC

	ARP101	ARP102	ARP103	KST70	KST203	SB201	SB5	WBVP04
Hap_1	-	1	-	-	-	1	-	-
Hap_2	-	-	-	-	-	-	-	-
Hap_3	-	-	-	-	-	-	-	-
Hap_4	-	-	-	-	-	-	-	-
Hap_5	-	-	-	-	-	-	-	-
Hap_6	-	-	-	-	-	-	-	-
Hap_7	-	-	-	-	-	-	1	-
Hap_8	-	-	-	-	-	-	-	-
Hap_9	-	-	-	-	-	-	-	-
Hap_10	-	-	-	-	-	-	-	-
Hap_11	-	-	-	-	-	-	-	-
Hap_12	-	-	-	-	-	-	-	-
Hap_13	-	-	-	-	-	-	-	-
Hap_14	-	-	-	-	-	-	-	-
Hap_15	-	-	-	-	-	-	-	-
Hap_16	-	-	-	-	-	-	-	-
Hap_17	-	-	-	-	-	-	-	-
Hap_18	-	-	-	-	-	-	-	-
Hap_19	-	-	-	-	-	-	-	-
Hap_20	-	-	-	-	-	-	-	-
Hap_21	-	-	-	-	-	-	-	-
Hap_22	-	-	-	-	-	-	-	-
Hap_23	-	-	-	-	-	-	-	-
Hap_24	-	-	-	-	-	-	-	-
Hap_25	-	-	-	-	-	-	-	-
Hap_26	-	-	-	-	-	-	-	-
Hap_27	-	-	-	-	-	-	-	-
Hap_28	5	5	-	-	-	-	-	-
Hap_29	1	-	1	-	-	-	-	-
Hap_30	2	-	-	-	-	-	-	-
Hap_31	-	-	-	-	-	-	-	-
Hap_32	-	-	-	-	-	-	-	-
Hap_33	-	-	-	-	-	-	-	-
Hap_34	-	-	-	-	-	-	-	-
Hap_35	-	-	-	-	-	-	-	-
Hap_36	-	1	-	-	-	-	-	-
Hap_37	-	-	-	-	-	-	-	-
Hap_38	-	-	-	-	-	-	-	-
Hap_39	-	-	-	-	-	-	-	-
Hap_40	-	-	-	-	-	-	-	-
Hap_41	-	-	-	-	-	-	-	-
Hap_42	-	-	-	-	-	-	-	-
Hap_43	-	-	-	-	-	-	-	-
Hap_44	-	-	-	-	-	-	-	-
Hap_45	-	-	-	-	-	-	-	-

Appendix 3 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the SLNWRC

	ARP101	ARP102	ARP103	KST70	KST203	SB201	SB5	WBVP04
Hap_46	-	-	-	-	-	-	-	-
Hap_47	-	-	-	-	-	-	-	-
Hap_48	2	-	-	-	-	-	-	-
Hap_49	-	-	-	-	-	-	-	-
Hap_50	-	-	-	-	-	-	-	-
Hap_51	-	-	-	-	-	-	-	-
Hap_52	-	-	-	-	-	-	-	-
Hap_53	-	-	-	-	-	-	-	-
Hap_54	-	-	1	-	-	1	-	-
Hap_55	-	-	-	-	-	-	-	-
Hap_56	-	-	-	-	-	-	-	-
Hap_57	-	-	-	-	-	-	-	-
Hap_58	-	-	-	-	-	-	-	-
Hap_59	1	3	3	-	-	2	-	-
Hap_60	1	-	-	-	-	-	-	8
Hap_61	1	-	-	-	-	-	-	-
Hap_62	2	2	-	-	-	-	-	-
Hap_63	1	-	-	-	-	-	-	-
Hap_64	1	-	-	-	-	-	-	-
Hap_65	1	-	-	-	-	-	-	-
Hap_66	1	-	-	-	-	-	-	-
Hap_67	-	1	-	-	-	-	-	-
Hap_68	-	3	-	-	-	-	-	-
Hap_69	-	1	-	-	-	-	-	-
Hap_70	-	1	-	-	-	-	-	-
Hap_71	-	1	-	-	-	-	-	-
Hap_72	-	1	-	-	-	-	-	-
Hap_73	-	1	-	-	-	-	-	-
Hap_74	-	3	2	-	-	-	-	-
Hap_75	-	1	-	-	-	-	-	-
Hap_76	-	1	-	-	-	-	-	-
Hap_77	-	1	-	-	-	-	-	-
Hap_78	-	1	-	-	-	-	-	-
Hap_79	-	1	-	-	-	-	-	-
Hap_80	-	1	-	-	-	-	-	-
Hap_81	-	1	-	-	-	-	-	-
Hap_82	-	-	1	-	-	-	-	-
Hap_83	-	-	1	-	-	-	-	-
Hap_84	-	-	1	-	-	-	-	-
Hap_85	-	-	1	-	-	-	-	-
Hap_86	-	-	1	-	-	-	-	-
Hap_87	-	-	1	-	-	-	3	-
Hap_88	-	-	1	-	-	-	-	-
Hap_89	-	-	1	-	-	-	-	-
Hap_90	-	-	1	-	-	-	-	-

Appendix 3 continued: Mitochondrial COI haplotypes found in *B. lynchi* sampled from the SLNWRC

	ARP101	ARP102	ARP103	KST70	KST203	SB201	SB5	WBVP04
Hap_91	-	-	1	-	-	-	-	-
Hap_92	-	-	1	-	-	-	-	-
Hap_93	-	-	1	-	-	-	-	-
Hap_94	-	-	-	-	-	-	-	-
Hap_95	-	-	-	-	-	-	-	-
Hap_96	-	-	-		-	-	-	-
Hap_97	-	-	-	-	3	-	-	-
Hap_98	-	-	-	-	7	-	-	2
Hap_99	-	-	-	-	2	-	-	-
Hap_100	-	-	-	-	1	-	-	-
Hap_101	-	-	-	1	-	-	-	6
Hap_102	-	-	-	3	-	-	-	1
Hap_103	-	-	-	1	-	-	-	-
Hap_104	-	-	-	1	-	-	-	-
Hap_105	-	-	-	1	-	-	-	-
Hap_106	-	-	-	1	-	-	-	-
Hap_107	-	-	-	-	-	1	-	-
Hap_108	-	-	-	-	-	1	-	-
Hap_109	-	-	-	-	-	1	-	-
Hap_110	-	-	-	-	-	-	1	-
Hap_111	-	-	-	-	-	-	1	-
Hap_112	-	-	-	-	-	-	1	-
Hap_113	-	-	-	-	-	-	2	-
Hap_114	-	-	-	-	-	-	-	1
Hap_115	-	-	-	-	-	-	-	2
Hap_116	-	-	-	-	-	-	-	1
Hap_117	-	-	-	-	-	-	-	1
Hap_118	-	-	-	-	-	-	-	1