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Regional Dispersal of Daphnia lumholtzi in North America Inferred from ISSR Genetic Markers

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Regional Dispersal of *Daphnia lumholtzi* in North America Inferred from ISSR Genetic Markers

By

G. Matthew Groves

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Abstract

The exotic zooplankter *Daphnia lumholtzi* is believed to have been introduced into Fairfield Lake, TX in 1983. Since introduction, *D. lumholtzi* has spread throughout the Midwest and Southeast United States. Due to its large head and tail spines, it may be less susceptible than native species to predation and therefore pose a threat to aquatic ecosystems. Development of management practices calls for study of the dispersal and potential impact of *D. lumholtzi* on invaded ecosystems.

Inter-simple sequence repeat (ISSR) markers were used in an effort to determine dispersal patterns of *D. lumholtzi*. Previous studies have shown that ISSRs possess sufficient levels of variability to reveal relationships at the population level. My objectives were to i) examine the utility of ISSRs in defining clonal groups within and across populations of *D. lumholtzi*, ii) look for genetic variation that may imply alternative sources of introduction of *D. lumholtzi* into North America and, iii) assess dispersal patterns of *D. lumholtzi* across parts of North America. Analysis included populations from Illinois, Kentucky, and Tennessee, as well as the population at Fairfield Lake, Texas.

Eighteen primers were screened and two were found to be variable with respect to the *D. lumholtzi* populations sampled. The primers **BECKY** (CA)7YC and **OMAR** (GAG)5RC produced a total of 812 ISSR fragments of which 677 (83%) were polymorphic. Overall similarity of individuals inferred from presence/absence of bands was used to draw conclusions about *D. lumholtzi* populations. Cluster analysis (UPGMA) of ISSR banding patterns revealed a potential presence of two clonal lineages within the Fairfield Lake population and a large amount of genetic variation within and

across all other populations sampled. Observed high levels of genetic variation in all populations except Fairfield Lake, suggests that Fairfield Lake is not the source population as is widely accepted. Furthermore, it appears that the genotypes present at Fairfield Lake are a subset of the numerous genotypes present in the other populations analyzed. If Fairfield Lake is in fact the source of introduction of *D. lumholtzi* into North America, my data suggest dispersal of *D. lumholtzi* across the U. S. resembles a "stepping stone" model of dispersal with one (or several) long distance dispersal event(s) originating at Fairfield Lake followed by several short distance dispersals.

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Introduction

Daphnia lumholtzi

The effect of exotic species on North American lakes and streams has become a concern in recent years. Exotic species can cause shifts in food webs, extinction of native species, and economic losses. Exotic species pose special problems because they often lack natural biological controls found in their native environments such as predators, competitors, pathogens, and parasites (Kolar 1997). Inability of management personnel to predict effects that introduced species may have on aquatic communities necessitates research focused on abiotic controls and biotic interaction to limit damage of exotics already present.

Daphnia lumholtzi (African water flea) is a cladoceran zooplankter native to Australia, Southwest Asia, and Africa (Work and Gophen 1995). *Daphnia lumholtzi* is an iteroparous species that most commonly reproduces via ameiotic parthenogenesis (King and Greenwood 1992). In large lakes where conditions are stable year round females dominate the population and reproduction may be entirely parthenogenic. In smaller bodies of water, where environmental conditions are not as stable, populations show annual cycles (Pennak 1989). A number of environmental factors can affect these cycles such as: overcrowding, poor water conditions, light intensity, or lack of food. Shifts in these conditions affects metabolism which appears to alter the chromosome mechanism in such a way that parthenogenic male eggs are produced rather than parthenogenic female eggs (Pennak 1989). The presence of males allows the population to survive unfavorable periods by sexually producing diapuasing ephippial eggs, which

remain viable even outside of water (Havel et. al. 2000). Such unfavorable periods vary geographically and can occur during hot or cold seasons.

Cladoceran zooplankters are predisposed to colonization of non-native habitats. They reproduce quickly and their ability to reproduce asexually allows an area to be colonized by the introduction of a single female. Colonization of new areas has been expedited by human action such as bait bucket release and intentional transfer of water or fish. Natural mechanisms such as passive transport by wind, birds, mammals and fish also contribute to dispersal of zooplankton. However, some research has suggested that these natural dispersal mechanisms may not have as great an effect on dispersal within the U.S. as previously thought. In eastern Kansas, Dzialowski et. al. (2000) conducted a survey of zooplankton in 40 small ponds. All ponds were within the watershed of an invaded reservoir but were inaccessible by recreational boat. All ponds lacked *D. lumholtzi*, suggesting that *D. lumholtzi* depend on human mediated dispersal for colonization. However, small size of the ponds may have precluded colonization due to physical factors such as surface area and depth or environmental factors such as nitrogen, phosphorous and chlorophyll *a* levels (Dzialowski *et al.* 2000).

Daphnia lumholtzi possesses additional characteristics that may have enhanced its ability to invade and colonize North America. *Daphnia lumholtzi* has adapted to withstand high summer water temperatures (up to 30° C) in the tropical/subtropical areas where it originated (Lennon 2001, Work and Gophen 1999). Ability to tolerate high temperatures may afford *D. lumholtzi* an advantage over native *Daphnia* of the Midwest and Southeast United States. In the late summer water temperatures become too warm for native *Daphnia* populations to persist so the population goes dormant via the

production of ephippial eggs (Work and Gophen 1999). During this time, *D. lumholtzi* can utilize food resources without competition from native species and flourish.

Displacement of native zooplankton during summer months was documented by Havens et. al. (2000) in a survey of zooplankton dynamics in a chain of subtropical lakes in Florida. It was found that the native *Daphnia ambigua* was dominant in fall and spring coinciding with lower water temperatures, higher transparency, and lower nutrient and chlorophyll *a* concentrations. In the summer months when water temperatures rose *D. lumholtzi* became dominant (Havens et. al. 2000). Another study showed *D. lumholtzi* to become abundant only in the late summer months, after the decline of much of the native zooplankton community. This difference in activity period suggests that *D. lumholtzi* may therefore not have a competitive impact on the native zooplankton assemblage (Work and Gophen 1999). The long term effects *D. lumholtzi* will have on invaded ecosystems remains uncertain.

While *D. lumholtzi* may not be directly competing with native daphnids, they may still have serious impact on aquatic ecosystems. Larger species of *Daphnia* such as *D. lumholtzi* filter feed on a wider size range of particles at a greater rate than smaller species common in the U.S.. This may result in lower algal concentrations and general reduction in biomass that may negatively affect lake ecosystems in which *D. lumholtzi* is present (Havens et. al. 2000).

Cyclomorphism

A number of *Daphnia* species are cyclomorphic, referring to their ability to change in morphology over time presumably to avoid predation (Brooks 1946). Factors such as temperature, food limitation, and presence of predators all may influence

expression of cyclomorphic characteristics (Sorenson and Sterner 1992). Chemical exudates of invertebrate predators, presence of soluble fish kairomones and changes in temperature all have been shown to elucidate a change in the morphology of cyclomorphic daphnia species (Swaffar and O'Brien 1996, Yurista 2000). Changes in helmet size, helmet shape, tail spine length, antennae length, and size of neck-teeth along dorsal midline may all manifest when individuals are exposed to certain environmental stimuli (Yurista 2000). The most notable example that *D. lumholtzi* exhibits, is variation in its long helmet and tail spines that each may exceed the carapace length.

The particularly long head and tail spines of *D. lumholtzi* make it less vulnerable than native cladocerans to predation by fish and invertebrates (Work and Gophen 1995). Many vertebrate predators are gape-limited, consuming only what they can swallow whole (Zaret 1980). The relatively long spines of *D. lumholtzi* defend against being consumed by gape-limited predators such as juvenile fish. Predators of this type traditionally feed on *Daphnia* species with the same body size as *D. lumholtzi,* not including spine length. *Daphnia lumholtzi* have been measured at up to 5mm in total length, which is larger than the size range of native North American species. In an aquatic community where *D. lumholtzi* is present, native species of zooplankton may be consumed by a certain size range of predators while *D. lumholtzi* may not. Antithetically, a recent study (Effert 1999) suggests that the spines of *D. lumholtzi* make it vulnerable to capture by the invertebrate predator, *Leptodora kindti,* an invertebrate predator that that may remove significant numbers of cladoceran prey.

Cyclomorphosis has been linked to clonal succession in some cladoceran species. Clonal succession occurs when there is a change in the relative frequencies of certain

genotypes in a population over time (Havel 1985). Clonal succession has been documented phenotypically and genetically in the cladoceran *Bosmina longrostris* and is believed to be the cause of cyclomorphosis in that species (Kerfoot 1975). Shifts in gene frequencies over time may also influence cyclomorphosis in *Daphnia* species. It is known that various clonal groups can exist within a population of *Daphnia pulex* (Hebert and Crease 1980). Furthermore, it has been documented that these clonal groups vary in abundance over time. Clonal groups in populations of *D. pulex* have been recognized from both life history and electrophoretic data (Lynch 1983, Hebert and Crease 1980). It is possible that similar clonal groups are present in *D. lumholtzi* populations.

Invasion history

Daphnia lumholtzi is known to thrive in disturbed habitats such as reservoirs, rivers, and ephemeral lakes in its native range. These habitats are similar to the habitats that *D. lumholtzi* has invaded in the U.S (Work and Gophen 1999). *Daphnia lumholtzi* was first detected in North America in Fairfield Lake in East Texas at (31.7°N, 96.0°W) in January, 1991 (Sorensen and Sterner 1992) and was later found in the fall of 1991 at Lake Stockton in Southwest Missouri (Havel and Hebert 1993). To date *D. lumholtzi* has been found in over 125 U.S. lakes and reservoirs (Lennon 2001), as far east as Florida and west to Arizona (Work and Gophen 1999). Recently *D. lumholtzi* has been found as far north as Lake Erie off the Ohio lake shore (Muzinic 2000).

Introduction of *D. lumholtzi* into the North America is thought to have occurred at Fairfield Lake in Texas, although the source or sources of introduction are uncertain. Fairfield Lake was created in the late 1960's and in 1983 was the first North American site stocked with Nile Perch *Lates niloticus* (Linnaeus). Nile Perch were imported from

Lake Victoria in Africa where *D. lumholtzi* are abundant. Adult *D. lumholtzi* may have been transferred with the water or ephippia may have been present in the digestive tracks of the fish themselves when imported. A cichlid (*Tilapia mossambica*) also was introduced into the Southern U.S. from Africa around that time and may have been the primary or a secondary source of introduction of *D. lumholtzi.* It has also been hypothesized that *D. lumholtzi* invaded the southern U.S. by way of the release of exotic fish or plants imported by the aquarium industry (Havel and Hebert 1993).

Previous genetic research

A study performed by Havel et. al. (2000) using allozyme and mitochondrial DNA showed high genetic similarity between U.S. populations and Afro-Asian populations of *D. lumholtzi.* A marked genetic difference between the Afro-Asian and Australian populations was also found. This supports the theory that *D. lumholtzi* currently found in the U. S. are from Africa or Asia as opposed to Australia. The application of a molecular clock to the mitochondrial 12s rDNA and cytochrome c oxidase subunit 1 (CO1) regions suggests the Australian population diverged from the Afro-Asian populations between 3.3 and 4 million years ago. This timeframe is based on a combination of Brower's (1994) estimate of nucleotide substitution in the arthropod mitochondrial genome of 2.3% per million years (12s RNA gene) and Knowlton's (1993) estimate of 2.2-2.6% nucleotide substitution per million years for the crustacean CO1 genome (Havel et. al. 2000). The different estimates of nucleotide substitution are necessary because the majority of the mitochondrial genome accrues nucleotide substitutions at a greater rate than the more conserved ribosomal RNA. In Havel's study, allozymes and mtDNA showed good utility for showing relationships between

populations of *D. lumholtzi* that diverged millions of years ago. In order to reveal relationships between populations of *D. lumholtzi* in North America that have diverged in the past 20 years a more variable genetic marker is necessary. High levels of genetic variation have been found in recently derived groups using techniques such as ISSRs, RAPDs, and microsatellites (Avise 1994). ISSRs particularly have shown to be highly variable and show great potential for population level studies (Robinson et al. 1997, Esselman et al.1999, Li and Ge 2001, Meng and Chen 2001). ISSRs have also been successful in identifying clonal diversity in population genetic studies (Zietkiewicz et al. 1994, Esselman et al. 1999, Li and Ge 2001) making them potentially well suited for this study, considering the potential clonal lineages present in U. S. populations. In the past ISSRs have primarily been used on plants. This is the first attempt to use ISSRs on a Cladoceran species.

ISSR markers

Inter-simple sequence repeat (ISSR) markers are the segments of a genome that lie between flanking regions of palindromic di-, tri-, tetra or pentanucleotide repeat motifs (Zietkiewicz *et al.* 1994). ISSR markers are generated using single PCR primers that anneal directly to these flanking regions of nucleotide repeats, also known as microsatellites. Since the primers anneal directly to the repeat regions, no prior knowledge of the target sequence is necessary (Li and Gi 2001, Godwin 1997). An anchoring sequence of one or more nucleotides is attached at either the 5′ or 3′ end of the primer. Anchoring sequences are intended to be complementary to the nucleotides directly adjacent to the repeating region to prevent strand slippage artifacts (Culley and Wolfe 2001). Slippage artifacts can cause the appearance of variation at a locus where

there is none and generate faulty data. Altering the anchoring sequence without altering the repeat motif can result in the amplification of different gene regions.

ISSR regions are abundant throughout the genome and evolve rapidly (Levinson and Gutman 1987). Regions that are of suitable length to be amplified via polymerase chain reaction (PCR) usually range from 0.3 to 2kb in size (Xu and Sun 2001). Regions that are too long will not have adequate extension time during PCR and will therefore not be amplified. ISSR's are inherited in a dominant or co-dominant Mendelian fashion and are scored diallelicly with bands present or absent (Wolfe *et al.* 1998, Gupta *et al.* 1994).

Objectives

The large size and unusual morphology of *D. lumholtzi* make it a potential threat to seasonal patterns of zooplankton abundance as well as algal-zooplankton interactions (Havens et. al. 2000). *D. lumholtzi* is not only a threat to plankton communities, higher trophic levels including commercial and sport fish could also be negatively effected (East et. al. 1999). Understanding the specific mechanisms and pathways that have led to *D. lumholtzi's* dispersal across North America will help to manage possible negative effects on ecosystems. The objectives of this study are: To examine the utility of ISSR genetic markers in defining clonal groups within populations of *D. lumholtzi*, to look for genetic variation that may imply multiple introductions of *D. lumholtzi* into North America, and to examine potential dispersal patterns of *D. lumholtzi* across the Midwest and Southeast U.S..

Methods

Sampling procedures

Zooplankton samples were taken from seven lakes across a geographical gradient from the suspected source population. Each lake was sampled at various locations both onshore and offshore via vertical tows with a Wisconsin Plankton Net (80-μm mesh). Lakes Taylorville, Decatur, Shelbyville, and, Springfield (Illinois) were sampled in early August 2001. Lake Barkley, Lake Kentucky (Kentucky) and Lake Douglas (Tennessee) were sampled in October 2001. Fairfield Reservoir (Texas) was sampled in May 2002. Live samples were kept on ice when necessary and returned to Eastern Illinois University to establish laboratory cultures.

Clonal cultures and DNA extraction

In order to have sufficient material for DNA extractions, clonal cultures were established for 30 individuals from each population. The individuals were labeled with their lake name of origin and an identification number corresponding to their place on a grid reading 1-6 on one axis and A-E on the other (ex. Shelbyville C3). Individuals were placed in 25 ml cups of synthetic hard water (Weber 1991) and kept at a constant temperature of 25° C in an incubator with a 12hr: 12hr light: dark cycle. Individuals were fed two drops each of *Selenastrum capricornutum* and YCT solution (Aquatic Biosystems) every 24 hours. Hard water was changed every 48 hours and neonates were separated into mass cultures in 500 ml beakers also kept at 25° C. Clonal cultures were kept until a minimum of 50 asexually produced clones per individual were available for DNA extraction. I found that using 50 clones per DNA extraction yielded sufficient

DNA for my analysis. It should be noted that water quality and overcrowding of mass cultures was monitored closely to prevent males being spawned as a response to environmental stress. Males can quickly reproduce sexually with the females present, forming ephippial eggs. Females carrying ephippia were excluded since ephippia are not pure clones of the parent female. DNA extractions were then performed using a Promega Wizard DNA Extraction kit (Promega Corporation, Madison, WI).

Eighteen ISSR primers were screened for their ability to produce informative banding patterns (Table 1). Primers screened were selected to incorporate a variety of nucleotide motifs. CA, AC, CAC, TC, CT, AG, GAG, GT, and GGGC motifs were represented. PCR amplifications were performed in 25 µl volumes using 2.5 µl Promega 10x buffer, 4.0 µl of 1.25 mM DNTPs, 0.5 µl of 50 µm primer, 0.5 µl TAQ Polymerase, 1 µl of DNA, and an optimized amount of 50 mM $MgCl_2$ ranging from 1.0 µl to 4.0 µl. ISSRs were amplified on a MJ Research PTC-100 temp cycler (MJ Research inc., Waltham, MA) using a program of 2 minutes at 94° C; 35 cycles of 50sec at 94° C, 50sec at 45° C (OMAR) or 47° C (BECKY), and 5min at 72° C.

ISSR analysis

PCR products were electrophoresed on horizontal 1.5% agarose gels for 210 minutes at 80 volts. This allowed the bromphenol blue marking dye to run out to 10cm. Gels were run with 5 µl ethidium bromide added before cooling and illuminated with UV light. Gel images were captured using a Kodak digital camera and scored using Kodak 1D Gel Electrophoresis Documentation Software (Eastman Kodak Corporation, Rochester). Replicate runs were performed to insure validity of banding patterns.

Table 1. Primers tested for population specific markers. Sequence motifs repeat the number of times shown between motif and anchor. In anchors Y= C or T, R= A or G.

Primer	Repeat Motif	Sequence	Annealing Temperature (Estimate)
BECKY	CA	(CA)7YC	47
CHRIS	CA	(CA)7YG	47
17899	CA	(CA)6RG	42
17898	CA	(CA)6RY	44
HANS	AC	(AC)7RG	47
MANNY	CAC	(CAC)4RC	45
AW-5	TC	(TC)6RG	45
844	CT	(CT)8RC	48
843	CT	(CT)8RA	48
MAO	CTC	(CTC)7RC	45
807-1	GA	(AG)8RG	48
SIBYL	AG	(AG)7RG	47
OMAR	GAG	(GAG)5RC	45
GOOFY	GT	(GT)7YG	47
17901	GT	(GT)6YR	45
AW-3	GT	(GT)6RG	45
AW-4	GT	(GT)6RC	45
AW-1	ATT	GGGC(ATT)5	48

Ultimately, two primers were chosen for inclusion in this study for their relatively informative banding patterns. These were BECKY (optimized at 1.5 mM $MgCl_2$) and Omar (optimized at 3.0 mM $MgCl_2$). BECKY and OMAR were scored and a binary data matrix based on presence or absence of bands was created.

Data analysis

A number of fundamental principles of ISSRs must be taken into account when analyzing data. Amplification products generated by simple sequence repeat primers represent only dominant and co-dominant alleles present at a given locus. The bands present represent either a dominant homozygote or a heterozygote and it is not possible to differentiate between the two genotypes. Therefore, genetic analyses where Hardy-Weinberg equilibrium is assumed are inappropriate due to the lack of differentiation between heterozygote and homozygote dominant markers (Culley and Wolfe 2001). Furthermore, the absence of a band does not necessarily indicate the presence of the homozygous recessive genotype. A number of factors can contribute to the absence of a band. Loss of a primer annealing site due to nucleotide sequence differences, insertions or deletions in the ISSR region between the two primer sites, and experimental error all can lead to loss of a band. It is because of this that the shared absence of a band in any two individuals cannot be assumed to be the result of a shared ancestor (Culley and Wolfe 2001). Due to these factors certain terms must be met in the analysis of ISSR data. Bands must be considered as purely presence or absence with no assumptions of allele frequencies and shared absence of bands of any individuals must not contribute to any measures of similarity.

The similarity between pairs of individuals was determined by Jacquard's coefficient (S_j) represented by the equation $S_j=a/(n-d)$ where a is the number of matched "1's", n is the total sample size, and d is the total number of matched "0's" (Zhu *et al.* 2001). This method meets the requirements of ISSR data by not making assumptions about allele frequencies and does not count shared absences of bands as a characteristic of similarity. A dendrogram was generated by cluster analysis of the similarity coefficients using UPGMA (unweighted pair-group method using arithmetic averages) in PCORD.

Results

Levels of success in creating clonal populations in the lab varied among populations. Therefore, the number of individuals included in the study from each body of water varies. Lakes Barkley and Douglas had the least with two surviving clonal populations each. Lakes Decatur and Springfield had 7 each. Lakes Taylorville and Shelbyville had 8 each. Fairfield Lake had the most successful clonal cultures with 11 (Table 2).

From the 18 primers screened, two primers, **BECKY** and **OMAR,** were found to have sufficient amplification products and variability to be informative. The primer **BECKY** produced a total of 344 bands with an average of 7.64 bands per individual. Of the 344 bands, 209 (61%) were polymorphic. **BECKY** produced 10 unique loci. Three of the unique bands were present across all individuals and all populations (Bands 6, 9, 10). The rest of the bands were variable across and within populations. All individuals possessed band 8 except for some individuals from the Lake Shelbyville population (D1, C3, E4, C1, A3, C2). The primer **OMAR** produced a total of 468 bands with an average of 10.40 bands per individual. **OMAR** generated 18 unique loci, all of which were polymorphic. There were no bands present across all individuals although band 20 was present in all individuals except one Springfield individual (B5). Band 11 was rare, being present in only two individuals (Shelbyvile D1, C3) as was band 12 which appeared only in Fairfield A3, Shelbyville D1, and Shelbyville C3.

Together the primers **BECKY** and **OMAR** generated 812 ISSR fragments (bands) with a combined average of 18.04 bands per individual. The length of fragments fell between 4,500 bp and 8,000 bp. Of all the bands produced, 677 (83%) were

Table 2. Number of successful clonal cultures from each population included in ISSR survey of *Daphnia lumholtzi* across Midwest and Southeast U.S.

Population	**Number of successful clonal cultures**
Lake Barkley, Kentucky	2
Lake Douglas, Tennessee	2
Lake Decatur, Illinois	7
Fairfield Lake, Texas	11
Lake Shelbyville, Illinois	8
Lake Taylorville, Illinois	8
Lake Springfield, Illinois	7

polymorphic. The combined primers generated 28 unique amplification products across all individuals sampled (10 from **BECKY** and 18 from **OMAR**) (Table 3).

The most notable banding pattern is the omission of 3 bands across all individuals from Fairfield Lake that are present in other individuals in other populations. Band 5 (**BECKY**) and bands 11 and 14 (**OMAR**) are not found in the Fairfield Lake population. However, band 5 is present in Decatur B2, C2, Shelbyville E4, A3, C2, E5, Taylorville E5, and Springfield B5, D3. Band 11 is found in Shelbyville D1, C3. Band 14 is found in DecaturA1, and Shelbyville D1, C3.

UPGMA analysis of all populations surveyed revealed a spurious distribution of most individuals regardless of population of origin (Figure 1). A number of individuals from Fairfield Lake grouped together with an individual from Lake Taylorville yet other individuals from Fairfield Lake grouped with individuals from Lakes Decatur, Douglas, Springfield, and Barkley. Resolution between individuals from other populations was generally low.

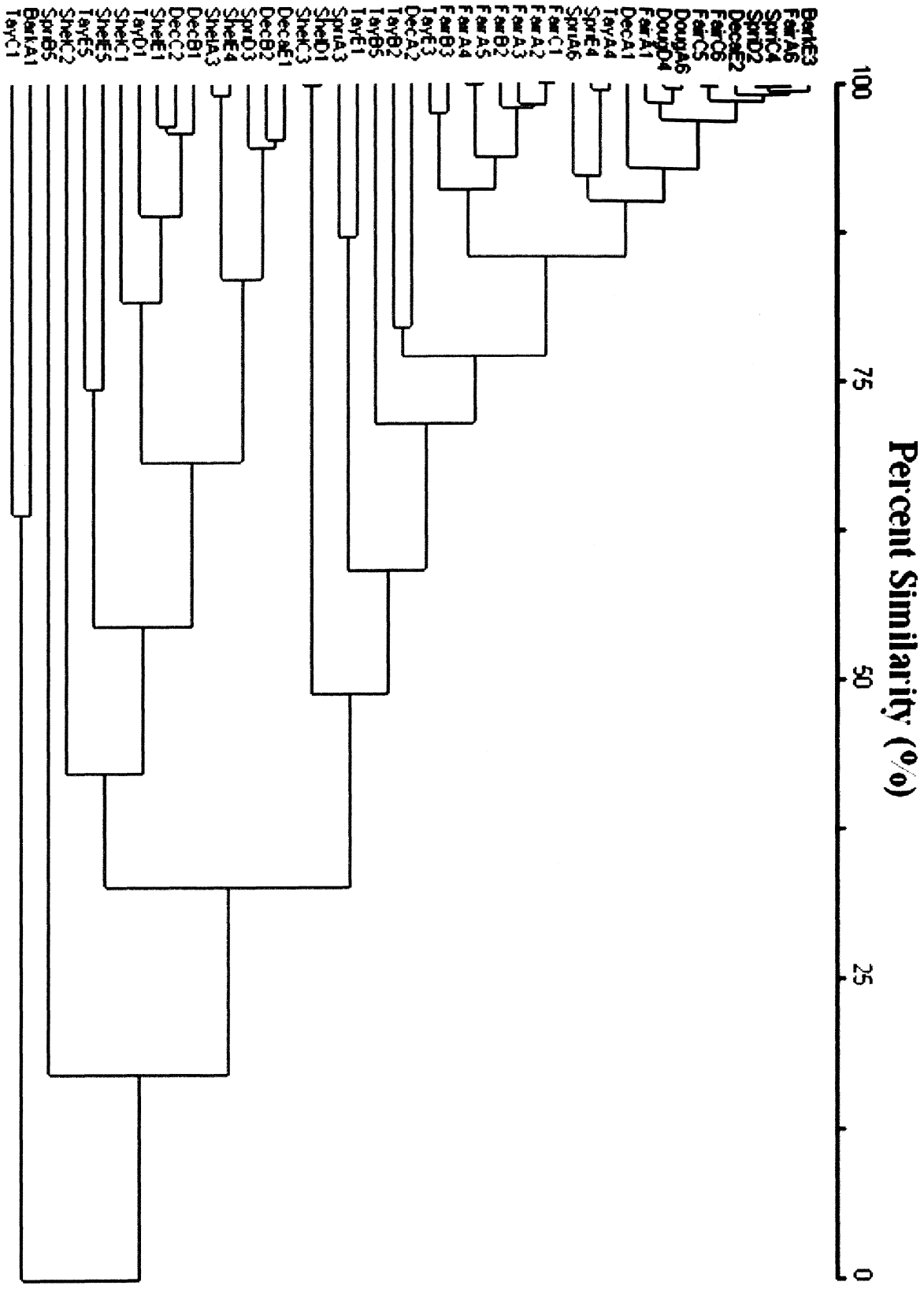
Table 3. Data matrix of ISSR band presence or absence in 7 populations of *Daphnia lumholtzi* based on the primers BECKY (B1-B10) and OMAR (B11-B28). A (1) denotes a band is present a (0) represents a bands absence.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15
Barkley E3	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0
Barkley A1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
Douglas A6	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0
Douglas D4	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0
Decatur E1	1	1	0	0	0	1	1	1	1	1	0	0	1	0	1
Decatur E2	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Decatur A2	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Decatur B1	1	1	1	0	0	1	1	1	1	1	0	0	1	0	1
Decatur A1	1	1	1	0	0	1	1	1	1	1	0	0	0	1	0
Decatur B2	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1
Decatur C2	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1
Fairfield C6	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0
Fairfield C5	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0
Fairfield C1	1	1	1	1	0	1	0	1	1	1	0	0	1	0	1
Fairfield B3	1	1	1	1	0	1	0	1	1	1	0	0	0	0	1
Fairfield B2	1	1	1	1	0	1	1	1	1	1	0	0	0	0	1
Fairfield A6	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Fairfield A5	1	1	1	0	0	1	0	1	1	1	0	0	1	0	1
Fairfield A4	1	1	1	0	0	1	1	1	1	1	0	0	1	0	1
Fairfield A3	1	1	1	1	0	1	1	1	1	1	0	1	1	0	1
Fairfiled A2	1	1	1	1	0	1	0	1	1	1	0	0	1	0	1
Fairfield A1	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Shelbyvile D1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0
Shelbyville C3	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0
Shelbyville E4	1	1	1	0	1	1	1	0	1	1	0	0	1	0	1
Shelbyville C1	1	1	1	1	0	1	1	0	1	1	0	0	1	0	1
Shelbyville A3	1	1	0	0	1	1	1	0	1	1	0	0	1	0	1
Shelbyville C2	1	1	1	0	1	1	0	0	1	1	0	0	1	0	1
Shelbyville E1	1	1	1	1	0	1	1	1	1	1	0	0	1	0	1
Shelbyville E5	1	1	0	1	1	1	0	1	1	1	0	0	1	0	1
Taylorville D1	1	1	1	0	0	1	0	1	1	1	0	0	1	0	1
Taylorville B2	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0
Taylorville C1	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0
Taylorville E5	1	1	0	1	1	1	0	1	1	1	0	0	1	0	1
Taylorville E1	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0
Taylorville E3	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0
Taylorville A4	1	1	0	1	0	1	1	1	1	1	0	0	0	0	0
Taylorville B5	1	1	0	1	0	1	0	1	1	1	0	0	0	0	0
Springfield B5	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0
Springfield A6	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Springfield C4	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Springfield E4	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0
Springfield D3	1	1	0	0	1	1	1	1	1	1	0	0	1	0	1
Springfield D2	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0
Springfield A3	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0

Table 3. (continued)

	B16	B17	B18	B19	B20	B21	B22	B23	B24	B25	B26	B27	B28
Barkley E3	1	0	1	1	1	1	1	1	1	1	1	1	1
Barkley A1	0	0	0	0	1	1	0	1	0	0	0	1	0
Douglas A6	1	0	1	1	1	1	1	1	1	1	1	1	1
Douglas D4	1	0	1	1	1	1	0	1	1	1	1	1	1
Decatur E1	0	0	0	0	1	1	1	1	1	0	1	0	0
Decatur E2	1	0	1	1	1	1	1	1	1	0	1	1	1
Decatur A2	1	0	1	1	1	1	1	1	1	0	0	1	0
Decatur B1	0	0	0	0	1	1	1	1	1	0	1	1	0
Decatur A1	1	1	1	1	1	1	1	1	1	1	1	1	1
Decatur B2	0	0	0	0	1	1	1	1	1	0	1	0	0
Decatur C2	0	0	0	1	1	1	1	1	1	0	1	1	0
Fairfield C6	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield C5	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield C1	1	1	1	1	1	1	1	1	1	1	1	1	1
Fairfield B3	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield B2	1	1	1	1	1	1	1	1	1	1	1	1	1
Fairfield A6	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield A5	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield A4	1	0	1	1	1	1	1	1	1	1	1	1	1
Fairfield A3	1	1	1	1	1	1	1	1	1	1	1	1	1
Fairfiled A2	1	1	1	1	1	1	1	1	1	1	1	1	1
Fairfield A1	1	0	1	1	1	1	0	1	1	1	1	1	1
Shelbyvile D1	1	1	1	1	1	1	1	1	0	1	1	1	0
Shelbyville C3	1	1	1	1	1	1	1	1	0	1	1	1	1
Shelbyville E4	0	0	0	0	1	1	1	0	1	0	1	0	0
Shelbyville C1	0	0	0	1	1	1	1	1	1	0	1	0	0
Shelbyville A3	0	0	0	0	1	1	1	0	1	0	1	0	0
Shelbyville C2	0	0	0	0	1	0	0	0	1	0	1	0	0
Shelbyville E1	0	0	0	1	1	1	1	1	1	0	1	1	0
Shelbyville E5	0	0	0	1	1	1	1	1	1	0	1	0	0
Taylorville D1	0	0	1	1	1	1	1	1	1	0	1	1	0
Taylorville B2	1	0	1	1	1	1	1	1	0	0	1	1	1
Taylorville C1	0	0	0	0	1	1	1	0	0	0	0	1	0
Taylorville E5	0	0	0	0	1	0	1	0	1	0	0	0	0
Taylorville E1	0	0	0	0	1	1	1	1	1	0	1	1	0
Taylorville E3	1	0	1	1	1	1	1	1	1	1	1	1	1
Taylorville A4	1	0	0	1	1	1	1	1	1	1	1	1	1
Taylorville B5	0	0	1	1	1	1	1	1	1	0	1	1	1
Springfield B5	0	0	0	0	0	0	1	0	1	0	0	0	0
Springfield A6	0	0	0	1	1	1	1	1	1	1	1	1	1
Springfield C4	1	0	1	1	1	1	1	1	1	1	1	1	1
Springfield E4	1	0	0	1	1	1	1	1	1	1	1	1	1
Springfield D3	0	0	0	0	1	1	1	1	1	0	0	0	0
Springfield D2	1	0	1	1	1	1	1	1	1	1	1	1	1
Springfield A3	1	0	0	1	1	1	1	1	1	1	1	1	0

Figure 1. Dendrogram generated by UPGMA cluster analysis of ISSR data from 7 populations of *Daphnia lumholtzi* based on Jaccard's coefficient of similarity. The scale represents relative percent similarity (0-100%).


BlankE3
FarA6
SpriC4
SpriD2
DecaE2
FarC6
FarC5
DougA6
DougD4
FarA1
DecA1
TayA4
SpriE4
SpriA6
FarC1
FarA2
FarA3
FarB2
FarA5
FarA4
FarB3
TayE3
DecA2
TayB2
TayB5
TayE1
SpriA3
SheD1
SheC3
DecaE1
DecB2
SpriD3
SheE4
SheA3
DecB1
DecC2
SheE1
TayD1
SheC1
SheE5
TayE5
SheC2
SpriB5
BlankA1
TayC1
100
75
50
25
0
Percent Similarity (%)

Discussion

Utility of ISSRs in defining clonal groups

The utility of ISSRs to define clonal groups within and between populations of *D. lumholtzi* in North America remains questionable. The UPGMA (relative percent similarity) analysis of all individuals sampled reveals that no population or clonal group was distinctly and consistently different from any other based on the ISSRs analyzed (Figure 1). Most individuals from Lakes Springfield, Shelbyville, Taylorville, Barkley, Douglas, and Decatur showed no sign of consistent grouping within or between populations, due to high levels of ISSR variability. It is likely that my ability to assess clonal groups within Lakes Douglas and Barkley was negatively affected by small sample size (two individuals from each). Further study with a larger sample size may be more successful at isolating clonal lineages.

There was overall less genetic variability shown in Fairfield Lake than in the other Lakes sampled. Fairfield C1 and A2 were 100% similar and grouped within a clade including seven (of 11) individuals from Fairfield Lake and one (of 8) individual from Lake Taylorville. This was the only instance where numerous individuals from one population showed high similarity to each other without equal levels of similarity to individuals from numerous other populations. This suggests that the population at Fairfield Lake is somewhat distinct and contains less genetic variation than the other populations sampled. Two clonal groups appear to be present within the Fairfield Lake population. One group being clearly defined, (Fairfield C1, A2, A3, B2, A5, A4, and B3) present at Fairfield Lake and Lake Taylorville. The other group (Fairfield A6, C6, C5, and A1) shows similarity to individuals from Lakes Barkley, Douglas, Springfield and

Decatur. This group includes one instance where identical clones are found across populations (Fairfield A6, Springfield C4, and Springfield D2). The more closely defined group seems to be somewhat unique to Fairfield Lake (and possibly Lake Taylorville). The other more loosely defined group appears to have dispersed more widely across other lakes sampled.

Multiple introductions of *Daphnia lumholtzi* into the U.S.

It is widely accepted, although not unequivocally proven that Fairfield Lake, TX was the source of introduction of *D. lumholtzi* into North America. To test this theory it was considered that differences in ISSR markers between the Fairfield Lake population and other populations would suggest that Fairfield Lake may not be the only source of introduction of *D. lumholtzi* into North America. Populations surveyed did in fact show variation from the Lake Fairfield population in at least one of two ways. Either by the presence of unique bands, absent in Fairfield Lake or by containing more ISSR variation than Fairfield Lake. The Fairfield Lake population is somewhat distinct in lacking 3 bands that appeared in other populations and contains less diversity than the other populations. The presence of bands in other populations not present in Fairfield Lake is a clear indicator that Fairfield Lake may not be the sole source of introduction.

Lack of genetic variation in what is believed to be the source population is contrary to basic tenants of gene flow to recently established populations. If Fairfield Lake is the source population for all others in the U. S., higher amounts of genetic variation should be present within Fairfield Lake rather than outside it. When founder events take place as *D. lumholtzi* disperses, each new population should have less genetic variation than the previous population. My results suggest the opposite with all

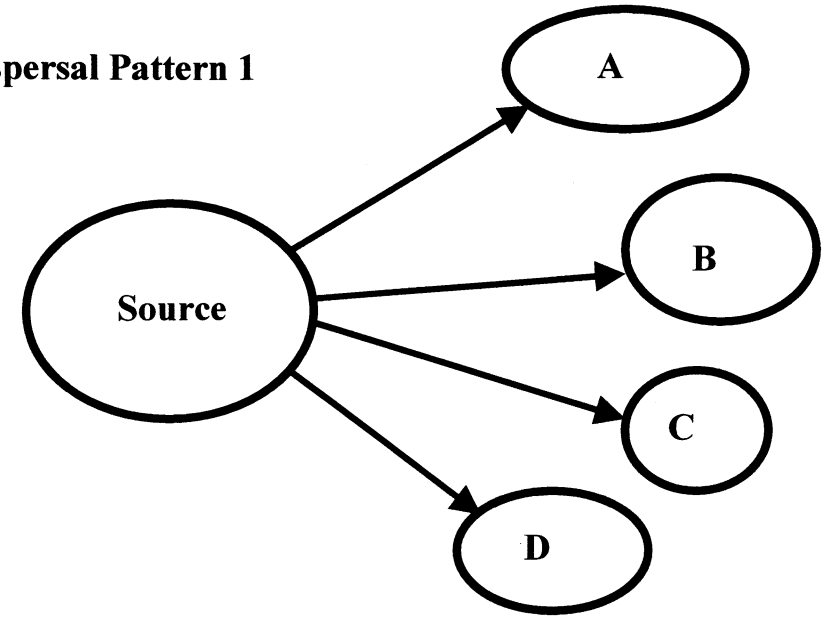
populations surveyed showing higher levels of genetic diversity than Fairfield Lake. These two factors suggest that Fairfield Lake may not be the only source of introduction of *D. lumholtzi* into the U. S..

Dispersal of *Daphnia lumholtzi* across the Midwest and Southeast U.S.

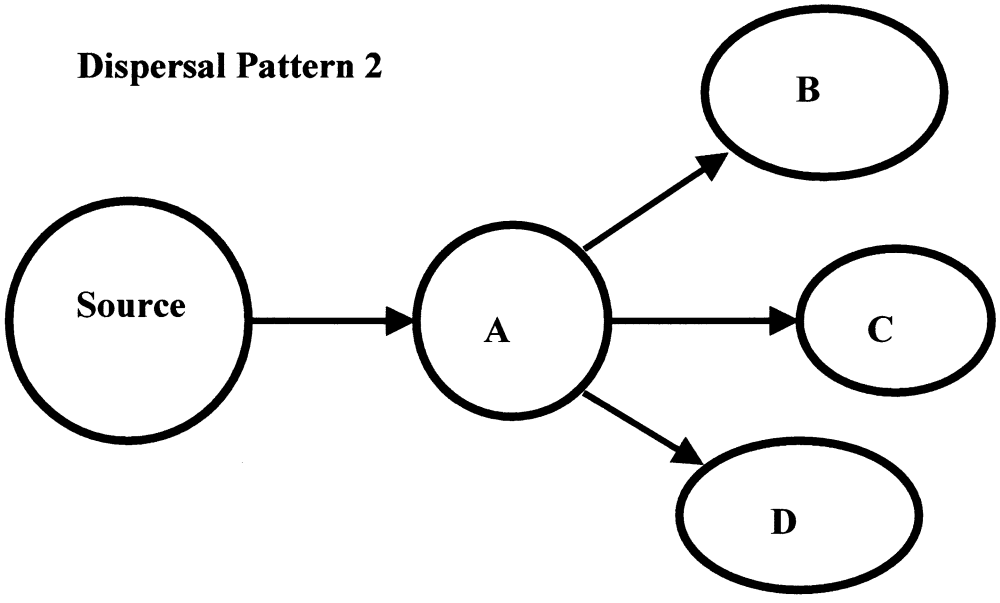
An effort was made to track the dispersal of *D. lumholtzi* across the Midwest and Southeast U.S based on the assumption that Fairfield Lake, TX is in fact the source of introduction into the U. S.. In judging the route of dispersal two basic radiation scenarios were hypothesized. Scenario 1 is composed of numerous long distance dispersal events all originating at the source of introduction and spreading directly to each new location. Scenario 2 is a "stepping stone" model involving a single long distance dispersal event from the source population to a given area followed by several short distance dispersal events (Figure 2). UPGMA cluster analysis of ISSR data supports the "stepping stone" model of scenario 2. It appears most likely a single long distance dispersal event from the Fairfield Lake source population to relatively few or a single location in the Midwest or Southeast U.S. may have occurred, followed by several short distance dispersal events. UPGMA cluster analysis of ISSR data shows most individuals from Fairfield Lake group together and most individuals from other populations widely distributed. Numerous dispersal events across populations and between populations may have contributed to the lack of resolution found between all other populations except for the Fairfield Lake population. If dispersal events were originating directly from Fairfield Lake (as in dispersal scenario 1), it is likely that all populations surveyed would more closely resemble both each other and the Fairfield Lake population.

Figure 2. Potential dispersal patterns of *Daphnia lumholtzi* across the Midwest and Southeast U.S.. Dispersal pattern 1 shows independent long range dispersal events from the source population. Dispersal pattern 2 shows one long range dispersal event followed by short range dispersal to nearby populations. If Fairfield Lake, TX is in fact the source of introduction, ISSR data collected suggests a radiation of *Daphnia lumholtzi* similar to dispersal pattern 2.

Dispersal Pattern 1



Dispersal Pattern 2



Sources of variation and stasis in cladoceran ISSRs

Overview

Inter-simple sequence repeat regions are assumed to lie in non-coding DNA regions and are therefore not subject to the genetic conservation instilled on the coding regions for life processes (Wolfe and Liston 1998, Camacho and Liston 2001). Because of this, any number of factors could alter the sequence of ISSR regions or their flanking repeat regions without negatively affecting the organism. The resulting hypervariability of ISSRs that makes them potentially so useful for investigating dispersal may have obscured patterns of dispersal which I was attempting to identify. To some extent, the results of this study were confounded by the large amount of genetic variation found within and among all populations surveyed.

Use of ISSRs in studies involving cladocerans has never been reported and the effect of this group's reproductive life history on ISSRs is unknown. Cladoceran populations reproduce asexually by parthenogenesis throughout much of the year, with limited bouts of sexual reproduction. Somatic mutations such as insertion or deletion events during parthenogenesis may alter ISSR regions. During sexual reproduction, crossing over or recombination events could result in genetic variation. Further sources of genetic variation could be the result of the hybridization of *D. lumholtzi* with local *Daphnia* species and/or the presence of multiple clonal groups within and throughout the populations sampled. Alternately, year round parthenogenesis and inter-dispersal between populations could have a stabilizing effect on genetic variation across and within populations.

Sources of genetic variation

Variation in ISSR regions could be the result of the presence of multiple clonal groups within and throughout the populations sampled. Hebert and Crease (1980) found 22 different clones of *Daphnia pulex* in 11 populations via electrophoresis. They also found as many as 7 clones coexisting in the same location. It has been hypothesized that *D. pulex* either is a highly variable species or an assortment of highly similar species (Dodson 1981). A similar situation could be occurring with *D. lumholtzi* populations in North America given my observation of the potential existence of two distinct clones in Fairfield Lake. Numerous clonal lineages could cause enough genetic variation so that populations are indistinguishable with the ISSRs chosen for this study. Also, larger sample sizes may have revealed additional distinct clones as could sampling at different times of the year. Furthermore, sampling at different times of the year may reveal patterns of clonal succession through time in relation to environmental conditions.

Genetic variation could also be the result of the hybridization of *D. lumholtzi* with native *Daphnia* species. Hybridization between various *Daphnia* species has been well documented (Hebert and Finston 1996, Hebert and Taylor 1993, Wolf and Mort 1996). Naturally occurring hybrids of *Daphnia hyaline, D. galeata,* and, *D. cuculatta* are known to coexist and have been identified with gel electrophoresis (Wolf 1987). *Daphnia lumholtzi* hybridizing with native *Daphnia* species at various locations could explain the wide range of genetic variation found within and between populations. Less genetic variation may exist in the *D. lumholtzi* population at Fairfield Lake because these individuals have had less opportunity to hybridize with congeners. In contrast, significant genetic variation may have been accumulated by populations that have spread

across multiple bodies of water. In the past ISSR markers have been used to successfully identify hybrids in the plant world (Wolfe et al. 1998). Identification of hybrids within the *Daphnia* species complex yet may be facilitated through the use of ISSRs. ISSRs could help resolve suspected natural hybrid lineages of *Daphnia* and identify possible hybrids of currently coexisting populations of native *Daphnia* species and *D. lumholtzi* if they exist.

Causes of genetic homogeneity

A constant year-round population lacking an ephippial life history stage could be responsible for the relatively small amount of genetic variation at Fairfield Lake. In warm climates and large lakes where water temperatures are constant *Daphnia* populations can be entirely parthenogenic year round (Pennak 1989). This has been particularly evident in *Daphnia rosea* (Pennak 1989) and could likely be the case for the population in Fairfield Lake due to *D. lumholtzi's* wide range of tolerances (Work and Gophen 1999, Lennon et al. 2001). Unfavorable conditions that may result during human mediated transport (bait bucket or ballast transport coupled with exposure to a new set of environmental conditions at new locations) likely would instigate the production of males and therefore sexual reproduction. As previously discussed, sexual reproduction may introduce ISSR variability via recombinant crossing over events. The source population at Fairfield Lake may be maintaining a constant asexual population and therefore have less ISSR variation than newly populated sites for this reason. Study of the population dynamics of Fairfield Lake *in situ* would be necessary to confirm this theory.

Repeated inter-dispersal between populations could be a source of genetic variation as previously discussed, or it could be a source of genetic homogeneity. Natural

and human mediated pathways may maintain genetic homogeneity across populations by creating a mixing affect. Zooplankton species are known to exhibit morphological stasis across even distant populations (Frey 1987). In light of this morphological stasis similar effects have been hypothesized regarding population inter-dispersal and gene flow (Mayer 1963). Constant inter-dispersal between sites could explain the lack of resolution between most populations in this study. Considering the speed with which *D. lumholtzi* has spread across the U. S., it is likely that many dispersal events occur between lakes already populated by *D. lumholtzi*. These events could spread population specific alleles that may develop, making them consistently present in all populations. Contrary to the idea that inter-dispersal would result in genetic homogenization, some allozyme and DNA studies have been able to show local population differentiation in various species of zooplankton that likely inter-disperse (DeMelo and Hebert 1994, Gómez et al. 1995). Morphological and genetic homogenization may not be a result of population inter-dispersal and gene flow (Colbourne et al. 1997). Further research is necessary to fully understand the specific factors involved in maintaining morphological and genetic stasis across populations of zooplankton.

Conclusions

Inter-simple sequence repeats proved to be only slightly successful at defining clonal groups of *D. lumholtzi*. Results suggest the presence of 2 potential clonal groups existing within Fairfield Lake, both of which were represented in other populations within the U.S.. My results also support the "stepping stone model" of dispersal from the source population at Fairfield Lake across the Midwest and Southeast U.S.. The large amount of variation present within and across all populations surveyed suggests that

Fairfield Lake may not be the only source of introduction of *D. lumholtzi* into North America.

The abundant genetic variation detected may have been present within the group of *D. lumholtzi* originally introduced into North America or may have resulted from the variety of mechanisms previously discussed. Alternatively, multiple introductions of *D. lumholtzi* into North America still cannot be ruled out as possible. Introduction and subsequent radiation of *D. lumholtzi* has occurred in just the last 25 years, a period of time which may be inadequate for establishment of population specific ISSRs. Recency of *D. lumholtzi* dispersal across the Midwest and Southeast U. S. may preclude evaluation of dispersal with ISSRs.

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