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Isolation Effects of Aquatic Habitat Fragmentation on Greenside (Etheostoma blennioides) and Fantail (Etheostoma flabellare) Darters

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Isolation Effects of Aquatic Habitat Fragmentation on Greenside (*Etheostoma blennioides*) and Fantail (*Etheostoma flabellare*) Darters

(TITLE)

BY

Rita M. Klein

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ABSTRACT

Aquatic habitat fragmentation is defined as the lack of connectivity between upstream and downstream populations and can occur along two gradients, the longitudinal gradient and the lateral gradient. The longitudinal gradient focuses on objects within the stream and is concerned with the disruption of stream flow, which is normally brought about by the presence of dams and bridges. Fragmentation along the lateral gradient, on the other hand, examines factors outside of the stream and is related to any loss or modification of the surrounding riparian zone.

In Illinois within the last 100 years, stream habitats have become increasingly fragmented by dams, pollution, and the loss of riparian zones, which have been brought about by an increase in channelization, wetland drainage, groundwater exploitation, and most importantly, agriculture. Organisms subjected to aquatic fragmentation may form isolated populations, with limited dispersal possibilities, leading to a reduction in gene flow and/or loss of genetic variation. Intolerant species, such as darters, may become isolated in distinct patches of good habitat leading to genetic differentiation between populations. To determine if aquatic habitat fragmentation has had an isolating effect on darters within a stream system, the following objectives were established: 1) estimate population size of three isolated populations of greenside and fantail darters, 2) determine the dispersal rate of individuals between isolated good patches, and 3) assess the degree of genetic isolation of darters between good patches.

Three hundred sixty-two greenside darters (*Etheostoma blennioides*) and 169 fantail darters (*Etheostoma flabellare*) from three good sites along Polecat Creek in Coles County, Illinois, were injected with an elastomer marking system and released back into

the stream. Ten recapture attempts yielded only one marked fantail, which precluded any other data being gathered for this species; however, overall greenside recapture rate was 23% (site 1 = 20%, site 2 = 6%, site 3 = 43%). Minimal greenside darter movement was found between riffles within sites, but no movement took place between sites. Allozyme analyses were performed on greenside darters to determine genetic differences between darter populations in distinct good patches. Mean heterozygosity was high for all three populations (0.21, 0.22, and 0.19, respectively) with no significant difference among sites. A modified Rogers' distance revealed that populations 1 and 2 were genetically similar, whereas population 3 was more genetically distinct. Thus, the isolating effects of habitat fragmentation have led to a decrease in overall population size and a lack of dispersal among sites. If population isolation is maintained and, at the same time, population size remains low and gene flow does not occur, we can predict that all three greenside darter populations will likely experience a loss of heterozygosity and/or rare alleles due to increased genetic drift and/or inbreeding, leading to possible extinction of the population.

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INTRODUCTION

In the United States and throughout the world, much of what was once single, large pieces of contiguous land has undergone habitat fragmentation, which is the breaking up of adjoining habitats into smaller, more isolated patches (Wilcove et al. 1986, Noss and Csuti 1997, Rosenblatt et al. 1999). Habitat fragmentation causes a reduction in total habitat area and redistributes the remaining area into patchy fragments (Wilcove et al. 1986, Rosenblatt et al. 1999), which may lead to a loss of various habitat types and changes in both abiotic and biotic factors of an area. For example, in Great Britain the fragmentation of habitats began over 5,000 years ago and today less than 5% of its original forests remain (Wilcove et al. 1986). Closer to home, Americans have been destroying the United States' habitats for over 300 years, which has led to widespread loss of native landscape due to farming and human expansion (Wilcove et al. 1986). A similar trend has been noted in Illinois, which has caused detrimental effects to organisms that depend on specific habitats for their existence (Page et al. 1997). To date, due to the destruction of native landscape by human disturbance (eg., agriculture), less than 1% of the original landscape in Illinois remains (Page et al. 1997).

When large-scale habitat fragmentation takes place, two potential changes to populations that may occur are reductions in both the effective population sizes and dispersal rates of individuals between patches (Wilcove et al. 1986). As an area becomes more fragmented, the effective population size, or the number of individuals in a population that are reproducing, and the actual total census count may be greatly reduced (Gilpin and Soulé 1986, Lacy 1987). In these fragmented habitats, migration is limited by an organism's ability to reach a patch of suitable habitat and as the landscape becomes

more fragmented the migration rate of an organism decreases markedly (Wilcove et al. 1986, Lehtinen et al. 1999, Collingham and Huntley 2000). Habitat fragmentation can create a landscape structure, which may limit how far organisms are able to migrate, and thus could result in limited dispersal rates of individuals between populations (Collingham and Huntley 2000), leading to a reduction in genetic diversity within populations due to the combined effects of random genetic drift and/or inbreeding (Meffe and Vrijenhoek 1988). Individuals reproducing in a population are responsible for population expansion and the maintenance of genetic variability; however, if only a few individuals produce the next generation, this may lead to future detrimental genetic effects.

Because of small effective population sizes and the limited dispersal ability of many organisms, isolated populations may experience genetic problems. Genetic drift and inbreeding are two of the genetic quandaries that researchers are alarmed about with populations that inhabit increasingly isolated patches (Sarre et al. 1990). The absence of migration between populations leads to a greater chance of inbreeding occurring within a population and genetic drift taking place between populations (Tibbets and Dowling 1996). In addition, if the good patches are geographically isolated from one another, dispersal between the populations may not take place, which could lead to a loss of allelic diversity (Schaal and Leverich 1996).

To date, there have been numerous studies that have investigated how habitat fragmentation of terrestrial ecosystems affects the survival, reproduction, and genetic composition of organisms living within those habitats. Findings have shown that, due to the increased isolation caused by habitat fragmentation, many terrestrial organisms such

as plants (Ouborg 1993, Schaal and Leverich 1996, Foré and Guttman 1999, Rosquist and Prentice 2000), birds (Verboom et al. 1991), lizards (Sarre et al. 1990), spiders (Ramirez and Haakonsen 1999), and insects (e.g. the golden sun moth; Clarke and O'Dwyer 2000), have experienced an overall decrease in genetic variability. A reduction in genetic variation will likely reduce an organism's ability to adapt to environmental changes and/or respond to environmental stressors, therefore leading to possible extinction of the population (Sarre et al. 1990).

Despite a wealth of information on fragmentation in terrestrial ecosystems, few studies have investigated habitat fragmentation in aquatic habitats. Aquatic habitat fragmentation, or the lack of connectivity between upstream and downstream populations, can occur along two gradients – a longitudinal or lateral gradient. The longitudinal gradient focuses on objects within the stream and is concerned with the disruption of stream flow, which is normally brought about by the presence of dams and bridges. Fragmentation along the lateral gradient, on the other hand, examines factors outside of the stream and is related to any loss or modification of the surrounding riparian zone.

In Illinois within the last 100 years, stream habitats have become increasingly fragmented by dams, pollution, and the loss of riparian zones, which have been brought about by an increase in channelization, wetland drainage, groundwater exploitation, and most importantly, agriculture (Karr et al. 1985, Page et al. 1997, Pringle 1997, Jones et al. 1999). As in fragments of terrestrial systems, organisms subjected to aquatic fragmentation (i.e. >70% loss of riparian zone; Page et al. 1997) may form isolated populations. If these populations are geographically separated, habitat fragmentation

could inhibit migration between populations and thus lead to a reduction in gene flow and/or a loss of genetic variation within populations (Tibbets and Dowling 1996, Page et al. 1997, Pringle 1997, Gilliam and Fraser 2001).

In general, the fragmentation of Illinois streams has led to the creation of small discrete good stream habitats that are isolated in a sea of poor habitat. This means that patches of good stream habitat (stretches that meander, have rocky substrate, good tree cover, and good riparian zone) are separated from one another by large patches of bad stream (channelized, silty substrate, poor tree cover, and little or no riparian zone). Given this scenario, the expected ramifications have previously been outlined by Meffe and Vrijenhoek (1988; Death Valley Model). These ramifications state that populations with small effective population sizes and reduced gene flow between populations will experience a loss of genetic variability *within* an isolated population due to the lack of gene flow between populations. It would also result in an increase in genetic divergence *between* isolated patches via genetic drift.

Genetic variability, both within and between populations, has been estimated for many organisms, including many of the terrestrial organisms mentioned previously. Allozyme analyses have been employed on fish species (Koehn 1970, Koehn et al. 1971, Merritt 1972, Farrington et al. 2000), including darters (Echelle et al. 1975, Echelle et al. 1976, Wiseman et al. 1978, Karlin and Rickett 1990, Wood and Mayden 1997, Turner and Trexler 1998). Echelle et al. (1976), Wiseman et al. (1978), and Karlin and Rickett (1990) studied populations of darters that were isolated to various degrees and found that genetic variation between populations was positively correlated to the extent of isolation

and that the increase in divergence can be attributed to a lack of gene flow/dispersal of individuals.

Darters are an ideal group of species for examining potential effects of aquatic fragmentation on dispersal rates and genetic variability. In part, this is due to the fact that darters are intolerant bottom-dwelling specialists that inhabit rocky or gravelly riffles with a swift current. Much of the darter's life is spent beneath or between rocks protected from the current and movement is accomplished by a series of short, quick dashes. Reproduction occurs in the spring and early summer, during which time many darters are brightly colored, and culminates with the attachment of eggs to various aspects of the bottom substrate. Thus, because these intolerant fish need a fast flowing current and gravel bottom substrate to survive and reproduce, darters may be unable to inhabit or transverse bad patches of stream, which are usually dominated by slow-moving or standing water.

Although aquatic systems have been shown to be fragmented (Page et al. 1997), it is unknown whether intolerant fish species, such as darters, found within these fragments are truly isolated from other such populations, or if there is some dispersal of organisms between patches. Thus, the overall goal of this study is to determine if aquatic habitat fragmentation has had an isolating effect on darter populations. The specific goals are to: 1) estimate population size of three populations inhabiting good patches, 2) determine the dispersal rate of individuals between these good patches, and 3) assess the degree of genetic isolation of darters between good patches.

MATERIALS AND METHODS

Study Site

Polecat Creek is located in Coles County, Illinois and is a 28.6-kilometer long, fourth-order stream that flows into the Embarras River near the city of Charleston in east-central Illinois (Fig. 1). Polecat Creek is ideal for a study dealing with population isolation questions using darters because it is highly fragmented and contains small distinct "good" habitat (sites) that are separated from one another by large areas of "bad" habitat. Three "good" sites were chosen along the length of Polecat Creek. A "good" site consists of a meandering stream with rocky substrate, good tree cover (≥70%), a riffle-pool-raceway sequence, and good riparian zone (Fig. 2). The good sites ranged from 63.1 meters to 271.2 meters in length and contained between two and six riffles (Table 1). Importantly, these good sites were separated from one another by stretches of stream that were entirely of poor quality (i.e. a channelized stream with no/poor riparian zone, no tree cover, and a sandy or silty substrate; Fig. 3). Each good site was designated by a number (1-3); site 1 was 1.0 kilometer downstream from site 2, which was 2.9 kilometers downstream from site 3.

Population Size Estimate and Dispersal Rates

Fantail darters, *Etheostoma flabellare,* and greenside darters, *Etheostoma blennioides*, were collected from riffles associated with the three good sites identified along Polecat Creek during the late spring/early summer of 2000. To estimate population size and potential dispersal between darter populations, 3-4 passes of a seine were made in each riffle of the good sites to collect individual darters of the two species. These fish

were marked using a subcutaneous injection of a marking compound (Northwest Marine Technology, Shaw Island,WA). To create the marking compound, 1 cc of a colored elastomer was mixed with 0.1 cc of curing agent (10:1 ratio) and inserted into injection syringes, following the procedure recommended by Northwest Marine Technology. The marking compound is a liquid when cold and solidifies as it warms, so it was prepared immediately before going out to the field and kept on ice when not in use.

Fish from each site were marked with a different color in order to identify which site they were originally marked during the study. For example, red, yellow, and orange were the colors assigned to sites 1, 2, and 3, respectively, for marking purposes (Table 1). Then, for each riffle within a site, a different anatomical region was marked on the fish (e.g., behind the eye, base of the dorsal fin, base of the pectoral fin, belly, side, or caudal peduncle). Using the marking system outlined above, any recaptured darter could be identified back to the site and riffle from which it had been originally marked. After injection of the marking compound into greenside and fantail darters at each riffle, the fish were placed in a bucket for 15-20 minutes to recover before being released back into the stream at the head of the individual riffles. Ten recapture attempts (approximately every two weeks) were made throughout the summer and fall to determine population size and dispersal of darters between good sites. Any unmarked darters caught during these recapture attempts were immediately marked using site and riffle specifications outlined above.

Sampling was performed with replacement of marked darters back into the stream; thus, population estimates were determined using the Schnabel population estimation formula (Van Den Avyle 1993):

$$\hat{N} = \frac{\sum_{t=1}^{n} C_t M_t}{\sum_{t=1}^{n} R_t},$$

where $\hat{N}$ is the estimated population size, n is the number of sample periods, t refers to the individual period, C is the number of fish collected (marked and unmarked) in a specific period, M is the total number of marked fish released prior to the sample period, and R refers to the number of marked fish found in C. The Schnabel population estimate is based on multiple sampling periods, during which the number of recaptures and the number of unmarked fish for each period are recorded. The Schnabel estimate assumes that no mortality takes place during the study, so in order for the population estimate to be valid, sampling takes place over a relatively short period of time. To determine confidence limits for the population estimate, the variance of $1/\hat{N}$ was first computed using the following equation:

$$V(1/\hat{N}) = \frac{\sum_{t=1}^{n} R_t}{\left(\sum_{t=1}^{n} C_t M_t\right)^2}$$

95% confidence intervals were determined by:

$$(1/\hat{N}) \pm \sqrt{\left(1.96 * V\left(1/\hat{N}\right)\right)},$$

from which the inverses of the limits were calculated to find the confidence interval of $\hat{N}$ itself.

Dispersal was determined from direct observations of marked individuals caught during the recapture periods. Captured darters were identified back to their site and riffle

of origin. Both within and among site dispersal was ascertained for the three populations of greenside and fantail darters.

Genetic Analyses

The same three good sites were kick-seined during the fall and winter of 2000 to collect greenside darters for enzyme analyses. Fantail darters were not included in the genetic analysis due to the fact that data could not be gathered for either population size or dispersal rates for this species. Approximately 30 greenside darters were captured from each good site along the stream. Captured fish were sacrificed in the field using MS222 (Sigma, A 5040), placed in labeled plastic bags and transported to the laboratory on ice, where they were frozen at -80°C. In preparation for electrophoretic analysis, one gram of skeletal muscle was dissected from each fish, placed in separate sterile microfuge tubes and then ground in 3-4 drops of 0.01 M tris-EDTA grinding buffer (pH 6.8). Filter paper wicks were used to soak up the extract from the tubes and then placed in labeled 96-well spot plates, which were then returned to -80°C for later use.

Subsequently, wicks were inserted into a 13% starch gel with either a lithium hydroxide or tris citrate buffer. Buffers and corresponding protein systems were chosen according to studies conducted by Karlin and Rickett (1990) and included: lithium hydroxide for leu-gly-gly (*PEP-B*; EC 3.4.11), leu-ala (*PEP-C*; EC 3.4.11), α-naphthyl-phosphate (*EST-1*; EC 3.1.1.1), β-naphthyl-phosphate (*EST-3*; EC 3.1.1.1), and α-naphthyl-butyrate (*EST-2*; EC 3.1.1.1); and tris citrate (pH 8.0) for lactate dehydrogenase (*LDH*; EC 1.1.1.27), malate dehydrogenase (*MDH*; EC 1.1.1.37), and glutamic oxalacetic transaminase (*GOT*; EC 2.6.1.1). Gels were refrigerated and electrophoresed at 111 volts

for the first two hours, increased to 138 volts for an additional 19 hours and then sliced and stained for the proteins.

All genetic analyses were performed using BIOSYS-1 (Swofford 1989). Allele frequencies, percent polymorphic loci (P), and mean heterozygosity (H) were calculated for each population. A chi-square test was then performed to determine deviation from Hardy-Weinberg expectations at each locus. Similarity between populations was estimated using Rogers' (1972) modified genetic distances. An analysis of variance was performed to determine if there were differences among sites in the number of rare alleles present and percent heterozygosity.

RESULTS

Population Size Estimate and Dispersal Rates

Three hundred sixty-two greenside darters were marked throughout the study period; 165 from site 1, 139 from site 2, and 58 from site 3. The recapture rates ranged from a low of 6% in site 2 to a high of 43% in site 3 (Table 2). A Schnabel population estimation formula yielded population estimates for greenside darters from all three sites using mark-recapture data. Using this approach, population estimates of 430, 876, and 136 were obtained for sites 1 through 3, respectively (Table 2). The population size estimate produced for site 2 is considered imprecise due to the low recapture rate. In addition to population estimates, 95% confidence intervals were calculated for each estimated population size (Table 2). There was a substantial confidence interval around the population size estimate at site 2 (586-1734), which supports the earlier statement that the calculated estimate for this population may be compromised due to a low recapture rate.

One hundred sixty-nine fantail darters were marked during this study; 138 at site 1, 29 at site 2, and 2 at site 3. There was a 0% recapture rate for sites 1 and 3, and only a 3% recapture rate (one individual) in site 2 for this species. Due to the single recapture at site 2, neither a population estimate nor dispersal assessment could be determined for any of the three fantail darter populations associated with the sites (Table 3). Thus, because of the low recapture rate of fantail darters, the remainder of my study was focused solely on greenside darter populations.

From direct observation of the kick-seining results during the five-month mark-recapture period (approximately every two weeks), there was no recorded movement of

greenside darters between sites. Furthermore, minimal movement occurred between riffles within a site; out of 369 greenside darters that were marked, only eight individuals (2.2%) moved within a site during this study. Seven of these eight darters (87.5%) moved upstream within a site with a mean migration distance of 47.8 m. The single individual (12.5%) that moved downstream migrated a total of 8.8 m.

Genetic Analyses

Ninety-seven greenside darters were used in the genetic analyses; 21 from population 1, 44 from population 2, and 32 from population 3. Eight enzymes were screened for variability; four of these enzymes yielded scoreable loci (malate dehydrogenase (MDH), leucyl alanine (PEP), α-naphthyl-phosphate (ANP), and α-naphthyl-butyrate (A-NB)). These four enzymes were coded for by 7 loci comprised of 14 alleles (Table 4) and were assayed to determine genetic variability, Hardy-Weinberg relationships, allele frequency, loss of rare alleles, heterozygosity, and genetic similarity and genetic distance within and between each of the three populations.

Seventy-one percent of the seven loci at site 1 were polymorphic, 57% were polymorphic at site 2, and 86% at site 3 (Table 5). Using a chi-square test, a significant difference ($p < 0.05$) was obtained between expected and observed allele frequency data for each population. The analysis revealed that 29%, 57%, and 71% of the 7 loci were out of Hardy-Weinberg equilibrium boundaries in populations 1, 2, and 3, respectively (Tables 6, 7, 8). In population 1, all alleles were within Hardy-Weinberg equilibrium except for PEP1 and A-NB; population 2 had MDH2, PEP3, and ANP2 in Hardy-Weinberg expectations; and population 3 had only MDH2 and ANP2 within Hardy-

Weinberg equilibrium, with MDH2 being a borderline case. The mean number of alleles per locus demonstrates that there was no significant loss of rare alleles in greenside darters within any of the populations; population 1 had a mean number of 1.9 alleles per locus, while populations 2 and 3 both had 2.0 alleles per locus (Table 5). Six alleles within these three populations have an allele frequency of less than ten percent (Table 4). For these alleles, mean persistence time was calculated and, as the allele frequency at a locus decreased, the number of generations until the allele would be purged from the population increased (Table 9). In addition, mean heterozygosity for the three populations was not significantly different and ranged from 0.19 in population 3 to 0.22 in population 2 (Figure 4).

Genetic divergence between the three populations was estimated using the Rogers' (1972) modified genetic distances. A dendogram (UPGMA) was constructed based upon our estimated distances (Fig. 5).

DISCUSSION

With habitat fragmentation becoming widespread throughout the world, many concerns are being raised regarding the potential effects of fragmentation on populations of both terrestrial and aquatic organisms. One potential outcome of habitat fragmentation is the formation of distinct isolated good patches in a sea of poor habitat. The formation of isolated patches may lead to a reduction in effective population size as well as preventing dispersal of individuals among patches of suitable habitat. The lack of connectivity between patches of suitable habitat may also create isolated populations that would be in danger of undergoing potentially detrimental genetic effects, such as genetic drift, inbreeding, loss or fixation of alleles, or reduced heterozygosity. Thus, the goal of the study was to determine if aquatic habitat fragmentation has occurred in Polecat Creek and, if so, what effects this fragmentation has had on the darter populations inhabiting the stream.

Population Size Estimate and Dispersal Rates

Based upon mark-recapture data, I estimated population size and calculated 95% confidence intervals for greenside darters from all three sample sites (Van den Avyle 1993; Table 2). Accurate population estimates were obtained for populations 1 and 3. The estimate for population 2, however, was imprecise and yielded an extremely large 95% confidence interval (a range of 1148) around the estimated population size. This imprecision was most likely due to the low number of recaptures at this site (Table 2). The inability to make accurate population estimates due to low recapture rates was reaffirmed by Van den Avyle (1993) who states that accurate population estimates and

confidence intervals cannot be made when the total number of recaptures in a study is small. In support of this conclusion, the accepted minimum recapture rate for obtaining accurate population size estimates for fish is 20%, determined from a mark-recapture study on stream fish populations in the Ouachita Mountains, west-central Arkansas (Warren and Pardew 1998). Greenside darters from sites 1 and 3 had recapture rates of 20% and 43%, respectively, and thus yielded reliable estimates of population size. However, the low recapture rate obtained at site 2 (6%) made it impossible to determine an accurate population size estimate.

One possible explanation for the low recapture rate at site 2 is flooding caused by extended periods of rainfall, which occurred throughout the study period. The reoccurring rainfall caused an increase in water level leading to difficulty in sampling the site as well as having a possible "washing-out" effect on the greenside darters from site 2. The possible washing out effect due to heavy rainfall has been seen in minnow and carp species that inhabit the Tachia River in Taiwan (Chang et al. 1999). The minnow and carp species (*Varicorhinus barbatulus, Leiocassis adiposalis, Hemimyzon formosanum,* and *Crossostoma lacustre*) of the Tachia River inhabit primarily upstream sections of the river; however, after an extended flooding period, they were observed and collected more frequently below Shikuan Dam in the lower portions of the river. Additionally, a 95% overall loss of aquatic organisms (including fish) has been reported after a flash flood in a 122 m reach of a montane desert stream (Lytle 2000). However, one problem with flooding as an explanation for the low recapture rate in site 2 is that sites 1 and 3 were both subject to the same flow conditions, yet recapture rates for these sites were sufficient

enough for accurate population estimates. Therefore, there may be another explanation for the low recapture rate in site 2.

A second possible explanation involves the loss of habitat heterogeneity due to flow regimes. As water level increases, riffles become raceways and the habitat of the stream becomes more homogeneous, leading to a lack of suitable habitat for darters (i.e. riffles). The substrate of the riffles in site 2 may have been unstable during high flow conditions and, if these conditions persisted, a habitat of poor quality may have been created for the darters, leading to these darters leaving the area. Holomuzki and Biggs (2000) reiterate this idea of flow creating poor habitats with their research on mayflies, caddisflies, and mudsnails. During high-flow disturbances, snails moved to more protected sublayers and were sheltered, while mayflies and caddisflies in unstable patches of substrate were dislodged. Since flooding and high flow events did occur during the study period, we can speculate that population 2 may have had a problem with unstable substrate, which led to greenside darters leaving the site and, therefore, inaccurate calculations of the population estimate and 95% confidence interval for site 2. Additional studies are required to assess the suitability of the substrate in site 2 for darter populations. Comparisons with the substrates from sites 1 and 3 may indicate that site 2 is a somewhat less stable habitat for greenside darters, which may have led to the low recapture rate found at the site.

Throughout the duration of the study period, greenside darters from sites 1 and 3 showed site fidelity not only to their initial capture site, but also to the riffle at which they were caught; only eight darters (2.2%) moved between riffles within a site during the mark-recapture period. Of the eight greenside darters that moved during this study, all

but a single individual (87.5%) moved in an upstream direction within the sites. The upstream movement observed in my study corresponds to results obtained by Mundahl and Ingersoll (1983) in which over 75% of all darter movements in the study occurred in an upstream direction. The site fidelity observed for greenside darters from sites 1 and 3 is similar to that seen for both johnny and fantail darters in Harker's Run, a southwestern Ohio stream. In this stream study conducted over a one-month period, it was found that over 96% of johnny darters and more than 87% of fantail darters moved little from their initial point of capture (Mundahl and Ingersoll 1983). In addition, a study conducted in an Oklahoma stream showed that orangebelly darters also exhibited site fidelity by remaining within a single riffle for an extended period of time (Scalet 1973).

A possible reason why greenside darters were able to exhibit high site fidelity during extended high flow conditions may be due to their large body size and increased muscle mass as compared to other darters. The increased body size may provide them with increased leverage to remain wedged between rocks in the substrate during adverse flow conditions. With their ability to anchor themselves between rocks, the darters put themselves in a strong position to withstand flooding events and high water disturbances as long as the substrate is stable.

Fantail darters seemed to show a lack of site fidelity and only a single fantail darter was recaptured during the entire study period. The lack of site fidelity in fantail darters seen in my study contradicts results from a study conducted in early autumn in an Ohio stream where 87% of fantail darters marked showed very little movement from their initial point of capture (Mundahl and Ingersoll 1983). A possible explanation for the lack of site fidelity in fantail darters during my study may be due to the extended spring high

flow conditions observed in all three sites. The increased water levels observed in these sites may have acted as a flushing agent and led to the removal of marked fantail darters from all sites. The lack of ability of fantails to maintain their position in the stream system during flooding conditions may be due to their small, sleek body morphology, which makes them more susceptible to be washed out of the site. The loss of small species from an entire area of stream during high flow conditions has also been observed by Chang et al. (1999) in a study where both minnow and carp species were swept downstream during flooding of the Tachia River. Due to a lack of information on population size or dispersal rate of fantail darters among sites, no conclusions could be made regarding the effects of habitat fragmentation as an isolating mechanism for this species.

Thus, using our estimates of population sizes for greenside darters (especially for sites 1 and 3), it is possible to make several predictions about the potential genetic effects of the observed habitat fragmentation. It has been suggested for stream-dwelling fish species that an N_e (effective population size) of at least 500 is needed to maintain sufficient levels of genetic variability in the absence of gene flow (Tringali and Bert 1998). The estimated population sizes of sites 1 and 3 are considerably less than 500 and it can be assumed that effective population size would likely be even smaller than the estimates. Thus, both of these sites appear to be prone to genetic changes common in small populations (i.e. inbreeding and genetic drift; Amos and Harwood 1998). Furthermore, no dispersal of darters between populations was observed. Thus, these populations seem to be effectively isolated from one another, which places them in

danger of genetic erosion taking place (i.e. loss of heterozygosity and rare alleles) and enhancing the population's chance of extinction (Ouborg 1993).

Genetic Analyses

Enzyme electrophoresis was used to test whether greenside darters from the three isolated sites in Polecat Creek (Fig. 1) are experiencing the predicted changes in genetic structure caused by fragmentation and the associated reduction in population sizes. The enzyme electrophoresis technique has been widely applied for estimating genetic diversity (Echelle et al. 1975, Randi et al. 1993, Clarke and O'Dwyer 2000, Farrington et al. 2000), testing for inbreeding (Rumball et al. 1994, Edmands and Potts 1997), and assessing the distribution of genetic diversity within and between populations (Tibbets and Dowling 1996, Díaz-Jaimes 1999, Foré and Guttman 1999, Rosquist and Prentice 2000), making it ideal for studying the various potential genetic effects to populations caused by isolation of suitable habitat.

When looking at percent polymorphic loci (P), site 2 has the lowest estimate (57.1%) while site 3 has the highest estimate (85.7%). The polymorphism observed in this study is similar to that seen for a study investigating the effects of ecology, life history, and water quality on genetic variation in greenside darters (polymorphic at 77.8% of the 14 scoreable loci; Heithaus and Laushman 1997). On the other hand, a study comparing gene flow estimates between greenside and fantail darters in different streams yielded a polymorphism range of 18.4 to 31.6% (Faber and White 2000), which is significantly lower than the values found in the current study. When comparing P estimated from other fish species (King and Pate 1992, P = 15.5%; Gajardo et al. 1998, P

= 21.8%; Farrington et al. 2000, P = 6.7% [black bream], P = 20% [yellowfin bream]) with those obtained for the greenside darters in Polecat Creek, we see that in a large number of cases our estimate of polymorphism is significantly higher. In addition, even genetic studies which utilized a large number of fish species did not yield percent polymorphisms in the range observed in this study (Selander 1976, P = 30.6% [14 fish species]; Nevo 1978, P = 15% [51 fish species]). The high genetic polymorphism observed in the study may be due to genetic drift, a lack of gene flow, a decrease in natural selection, and/or a strong selection pressure occurring within the sites.

The higher degree of polymorphism maintained in site 3 may indicate that this site either has some other source of gene flow or that the site has only recently become isolated. Gene flow can be ruled out because migration does not appear to occur. Site 3 may have been isolated for fewer generations, which may explain why a decrease in P was not observed.

In order to examine whether genetic changes were occurring within the populations, Hardy-Weinberg (H-W) expectations were studied. Assumptions for Hardy-Weinberg include a large population size, random mating, and a lack of mutation, migration, and natural selection. Only a small number of the seven studied loci are within H-W expectations (5 at site 1, 3 at site 2, and 2 at site 3); however, when the results of the tests are compared across all sites, a trend is noted for several loci. ANP2 and MDH2 are the only loci within H-W equilibrium across all sites. MDH1, PEP3, and ANP1 are within H-W expectations at some sites but not others. In contrast, both A-NB and PEP1 deviate from Hardy-Weinberg expectations across all sites sampled. These two loci (A-NB and PEP1) had reduced allele frequencies as compared to the other loci,

indicating a possible violation of the assumptions associated with the H-W theory. The violation of the assumptions of migration and mutation can probably be ruled out, the former because there was no noticeable dispersal taking place between greenside darter populations and the latter because there were no rare alleles noted during electrophoresis and the time available for mutations to occur within the isolated populations was limited.

It is possible that the Hardy-Weinberg assumptions of no genetic drift and/or inbreeding are being violated in these greenside darter populations. As noted above, estimates of population sizes for these sites were lower than 500, which is the estimate of the effective population size required to maintain genetic diversity (Tringali and Bert 1998). Thus, the reduced effective population size may produce matings that are likely not random in these greenside darter populations. Also, if populations are small and isolated from one another and inbreeding is taking place within each population, there is a greater chance that heterozygosity will decrease and rare alleles will become fixed within the populations. Genetic drift will also take place as more generations are produced with no gene flow occurring between populations, leading to an increase in genetic divergence among populations. Additionally, the natural selection assumption may have been violated. It is possible that selection may be acting to maintain heterozygosity at certain loci, thereby causing loci not to adhere to Hardy-Weinberg expectations. Therefore, due to the violation of the Hardy-Weinberg assumptions, the populations are not in genetic equilibrium and allelic frequencies are subject to a wide variety of changes.

Six alleles within the three populations had a frequency smaller than ten percent, which would make them susceptible to extinction in a small population (Tringali and Bert

1998). However, calculations determining time of allele persistence in the population indicate that mean persistence time of an allele increases as allele frequency decreases (Table 9). The increase in persistence time is due to the fact that the smaller the frequency of an allele, the more difficult it will be to completely purge the allele from the population (Hartl and Clark 1997). However, persistence is a function of population size (N), so as population size decreases, the time until purging will also decrease. Furthermore, with decreasing population size the potential for loss of rare alleles due to random genetic drift increases (Hartl and Clark 1997).

The mean numbers of alleles per locus (rare alleles) in the three populations of greenside darters were 1.9, 2.0, and 2.0, respectively (Table 5). These mean allele numbers are slightly higher than those observed for fish from other studies (Van Der Bank and Van Der Bank 1995, $\bar{A} = 1.44$; Bembo et al. 1996, $\bar{A} = 1.17$; Faber and White 2000, $\bar{A} = 1.18$ to 1.35). The higher mean number of alleles per loci observed in this study may have to do with the fact that loci were chosen specifically for their increased genetic variability and, possibly, that populations have not been isolated long enough for a noticeable decrease in population size to occur. At this time, it is impossible to predict whether the population size of darters in each site is stable or decreasing, though additional sampling in the future will shed insights into the demographics of these populations as well as the overall potential for loss of genetic diversity via purging of rare alleles.

Rogers' (1972) modified genetic distances were calculated to determine the degree of genetic divergence among populations. The modified genetic distance indicated that sites 1 and 2 had a small genetic divergence and were more closely

genetically related to one another than either was to site 3, which had a large amount of genetic divergence. One possible reason for the small amount of genetic divergence observed between sites 1 and 2 may be because these two sites were once connected and have only recently become isolated. A similar conclusion was reached in a study of the endangered golden sun moth (*Synemon plana*) in southeastern Australia (Clarke and O'Dwyer 2000). Even though a decrease in genetic diversity within golden sun moth populations was found during the study, there was a lack of genetic differentiation between populations, possibly indicating that these populations only recently became isolated through habitat fragmentation. Site 3, if at one time connected to the first two sites, has probably been isolated from them for a much longer time period because the genetic divergence is three times greater between site 3 and the remaining sites.

Another way to determine if a population is experiencing genetic drift and/or inbreeding is to compare the levels of heterozygosity (H) for the population with other populations of species known to exist in large populations (i.e. no drift) or with outcrossing species (i.e. low inbreeding). The expectations for either inbreeding or drift are an overall decrease in H. For the three greenside populations examined, the mean heterozygosity was around 0.20 for all three populations. The estimated heterozygosity for all three sites was similar (0.21, 0.22, 0.19, respectively) and high when compared with observed heterozygosity values for greenside darters from the Kokosing River, one of the cleanest rivers in Ohio (H_{obs} = 0.140; Heithaus and Laushman 1997). Comparison of allozyme variation and heterozygosity of 243 species of vertebrates (Nevo 1978) indicates that the 20% heterozygosity estimated for greenside darters in this study is higher than all other vertebrates studied, except for Branchiostoma. The high

heterozygosity found in my study is most likely due to deliberately choosing previously observed loci that displayed multiple alleles in order to detect the variability among geographically proximate populations.

As noted above, site 3 has the highest value for P and is more divergent than the other sites. However, the estimate of heterozygosity for site 3 is similar to sites 1 and 2. This is somewhat surprising because site 3 also appears to have the smallest overall population size (Table 2). The relatively high estimate of observed over expected heterozygosity may be the result of any one of several factors, or a combination thereof. For example, it is possible that site 3 may be experiencing gene flow from another population upstream. If even a single individual is able to migrate into a population and reproduce, then this can act to maintain heterozygosity at higher levels (Slatkin and Maruyama 1975, Lacy 1987). Therefore, even a rare migration into population 3 from a proximate upstream population may explain the observed results. The maintenance of high levels of heterozygosity observed in this study is similar to findings observed for island lizard populations (Sarre et al. 1990). Sarre et al. (1990) surveyed seven island populations that were isolated by rising sea levels 6000-8050 years ago and three adjacent mainland populations of the sleepy lizard, *Trachydosaurus rugosus*. Comparison of the genetic variability within and between these populations of lizards indicated differences in the allelic diversity between island and mainland populations, with a greater overall divergence in the former, but there was no observed decrease in heterozygosity in the insular populations. The lack of reduced heterozygosity in both the lizard study and mine may possibly be due to pressures such as selection and genetic drift occurring within the population.

Another explanation for the disparities observed in site 3 as compared to sites 1 and 2 may be that site 3 has been isolated for a longer period of time as compared with sites 1 and 2, resulting in a higher degree of genetic divergence, but has only recently experienced a reduction in population size and therefore has not yet lost much of its heterozygosity. Strong selection pressure at site 3 may be responsible for maintaining a high heterozygosity at certain loci in this population. Additional sampling is required to test these hypotheses.

Given the data at hand, I can conclude that habitat fragmentation has had an isolating effect on greenside darter populations. The isolating effects of habitat fragmentation have led to a decrease in overall population size and a lack of dispersal among sites. If population isolation is maintained and, at the same time, population size remains low and gene flow does not occur, we can predict that all three greenside darter populations will likely experience a loss of heterozygosity and/or rare alleles due to increased genetic drift and/or inbreeding, leading to possible extinction of the darter populations. Thus, the management decision to restore riparian zone along the highly fragmented banks of Polecat Creek may be instrumental in reestablishing connectivity among good sites. The increase in connectivity among sites should lead to migration and gene flow between populations and an overall decrease in the risk of extinction.

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Table 1. General details for the three isolated good sites sampled along Polecat Creek, Coles County, IL.

	Site 1	Site 2	Site 3
Length (meters)	271.2	207.6	63.1
Number of riffles	5	6	2
Elastomer color	red	yellow	orange

Table 2. Mark-recapture data for greenside darters from Polecat Creek in Coles County, IL.

	Site 1	Site 2	Site 3
Number greensides marked	165	139	58
Number gr. recaptured	33	8	25
% recapture rate	20	6	43
Schnabel population estimate	430	876	136
95% confidence interval	346-569	586-1734	106-189

Table 3. Mark-recapture data for fantail darters from Polecat Creek in Coles County, IL.

	Site 1	Site 2	Site 3
Number fantail marked	138	29	2
Number fan. recaptured	0	1	0
% recapture rate	0	3	0
Schnabel population estimate 95% confidence interval	N/A	N/A	N/A

Table 4. Allele frequencies in populations 1, 2, and 3 of greenside darters. N = number of individuals. A-F = various alleles.

Locus	Allele	Population 1 (N = 21)	2 (N = 44)	3 (N = 32)
MDH2				
	A	.976	.955	.516
	B	.024	.045	.484
MDH1				
	C	.810	.761	.516
	D	.190	.239	.484
PEP3				
	A	1.000	.989	.297
	B	.000	.011	.703
PEP1				
	E	.452	.352	.313
	F	.548	.648	.688
ANP1				
	A	.214	.068	.297
	B	.786	.932	.703
ANP2				
	C	.833	.955	.969
	D	.167	.045	.031
A-NB				
	A	.548	.477	.703
	B	.452	.523	.297

Table 5. Genetic variability at 7 loci in three greenside darter populations (one standard errors in parentheses) from Polecat Creek.

Population	Mean sample size per locus	Mean number of alleles per locus	Percent polymorphic loci *
1	21.0 (.0)	1.9 (.1)	71.4
2	44.0 (.0)	2.0 (.0)	57.1
3	32.0 (.0)	2.0 (.0)	85.7

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

Table 6. Chi-square test for deviation from Hardy-Weinberg equilibrium in population 1.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MDH2						
	A-A	20	20.000			
	A-B	1	1.000			
	B-B	0	.000			
				.000	1	1.000
MDH1						
	C-C	13	13.683			
	C-D	8	6.634			
	D-D	0	.683			
				.998	1	.318
PEP1						
	E-E	7	4.171			
	E-F	5	10.659			
	F-F	9	6.171			
				6.221	1	.013*
ANP1						
	A-A	2	.878			
	A-B	5	7.244			
	B-B	14	12.878			
				2.226	1	.136
ANP2						
	C-C	14	14.512			
	C-D	7	5.976			
	D-D	0	.512			
				.706	1	.401
A-NB						
	A-A	2	6.171			
	A-B	19	10.659			
	B-B	0	4.171			
				13.518	1	.000*

* out of Hardy-Weinberg equilibrium
$P < 0.05$

Table 7. Chi-square test for deviation from Hardy-Weinberg equilibrium in population 2.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MDH2						
	A-A	40	40.069			
	A-B	4	3.862			
	B-B	0	.069			
				.074	1	.786
MDH1						
	C-C	23	25.414			
	C-D	21	16.172			
	D-D	0	2.414			
				4.084	1	.043*
PEP3						
	A-A	43	43.000			
	A-B	1	1.000			
	B-B	0	.000			
				.000	1	1.000
PEP1						
	E-E	1	5.345			
	E-F	29	20.310			
	F-F	14	18.345			
				8.279	1	.004*
ANP1						
	A-A	2	.172			
	A-B	2	5.655			
	B-B	40	38.172			
				21.822	1	.000*
ANP2						
	C-C	40	40.069			
	C-D	4	3.862			
	D-D	0	.069			
				.074	1	.786
A-NB						
	A-A	3	9.897			
	A-B	36	22.207			
	B-B	5	11.897			
				17.371	1	.000*

* out of Hardy-Weinberg equilibrium
$P < 0.05$

Table 8. Chi-square test for deviation from Hardy-Weinberg equilibrium in population 3.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
MDH2						
	A-A	11	8.381			
	A-B	11	16.238			
	B-B	10	7.381			
				3.437	1	.064
MDH1						
	C-C	14	8.381			
	C-D	5	16.238			
	D-D	13	7.381			
				15.823	1	.000*
PEP3						
	A-A	9	2.714			
	A-B	1	13.571			
	B-B	22	15.714			
				28.716	1	.000*
PEP1						
	E-E	9	3.016			
	E-F	2	13.968			
	F-F	21	15.016			
				24.513	1	.000*
ANP1						
	A-A	0	2.714			
	A-B	19	13.571			
	B-B	13	15.714			
				5.355	1	.021*
ANP2						
	C-C	30	30.016			
	C-D	2	1.968			
	D-D	0	.016			
				.016	1	.898
A-NB						
	A-A	13	15.714			
	A-B	19	13.571			
	B-B	0	2.714			
				5.355	1	.021*

* out of Hardy-Weinberg equilibrium
$P < 0.05$

Table 9. Mean persistence time of alleles that were present at a frequency of less than ten percent. N = population size.

Population	Locus	Allele	Allele frequency	Number of generations until allele disappears
1	MDH2	B	0.024	6.42N
2	MDH2	B	0.045	5.28N
	PEP3	B	0.011	7.81N
	ANP1	A	0.068	4.51N
	ANP2	D	0.045	5.28N
3	ANP2	D	0.031	5.96N

Fig. 1. Location of the 3 sample sites along Polecat Creek, Coles County, Illinois.

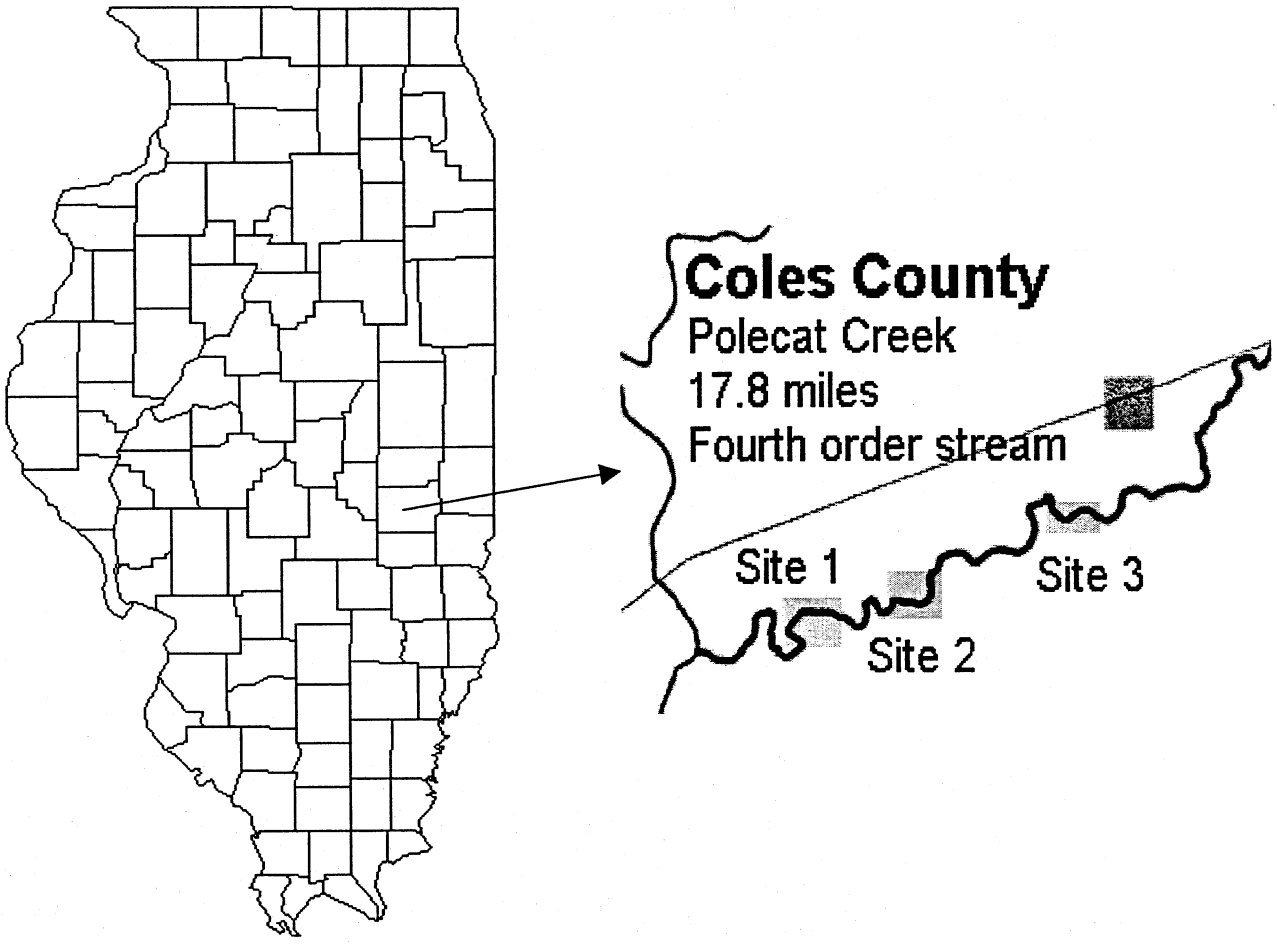

Coles County
Polecat Creek
17.8 miles
Fourth order stream
Site 1
Site 2
Site 3

Fig. 2. Example of a good site sampled in Polecat Creek. Good habitat is represented by a meandering stream, rocky substrate, good tree cover, and good riparian zone.

Fig. 3. Example of a poor habitat found in Polecat Creek. Poor sites are characterized by a channelized stream with silty substrate, poor tree cover, and little or no riparian zone.

Fig. 4. Mean heterozygosity and standard error of three greenside darter populations from Polecat Creek.

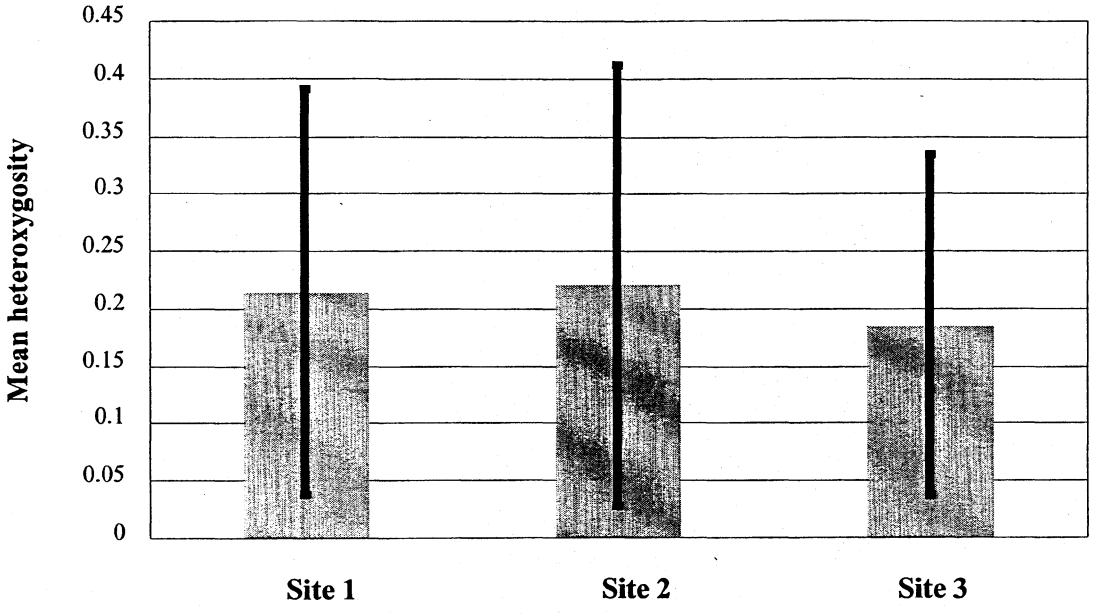
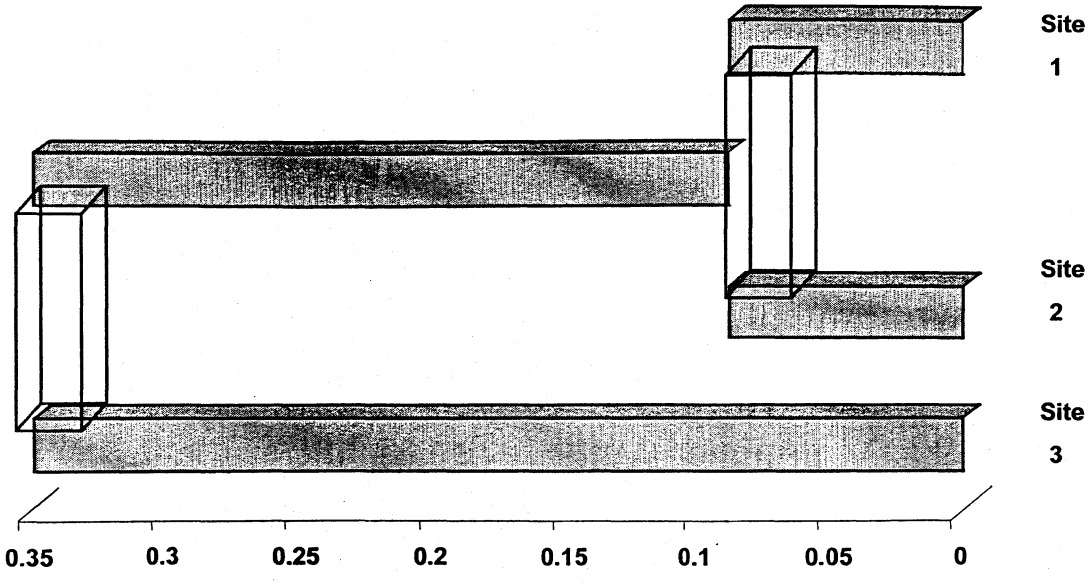

Mean heteroxygosity
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Site 1
Site 2
Site 3

Fig. 5. UPGMA dendogram based upon a modified Rogers distance. Branch lengths represent relative genetic distance.



Modified Rogers Distance