RH: Genetics of fragmentation in *Speyeria idalia*

Genetic Effects of Recent Habitat Fragmentation on the Wide Ranging, High Gene Flow Butterfly *Speyeria idalia* (Nymphalidae)

[1]Barry L Williams*, [2]Jeffrey D. Brawn, and [1]Ken N. Paige

[1]Department of Animal Biology , University of Illinois,Urbana, Illinois 61801

[2]Center for Wildlife Ecology, Illinois Natural History Survey, Champaign, Illinois 61820 and Department of Natural Resources and Environmental Sciences, University of Illinois,Urbana, IL 61801

*Current address:
Laboratory of Molecular Biology and Howard Hughes Medical Institute
University of Wisconsin
1525 Linden Drive
Madison , WI 53706
Fax 608-262-9343
bwillims@students.uiuc.edu

Date of receipt:

keywords: microsatellite, butterfly, habitat fragmentation, conservation, metapopulation, genetic.

Abstract

Detection of the genetic effects of recent habitat fragmentation in natural populations can be a difficult task, especially for high gene flow species. Previous analyses of mtDNA data from across the current range of *Speyeria idalia* suggested that the species exhibited high levels of gene flow among populations, with the exception of an isolated population in the eastern portion of its range. However, some populations are found on isolated habitat patches, separated from one another recently by large expanses of uninhabitable terrain, in the form of row crop agriculture. The goal of this study was to compare levels of genetic differentiation and diversity among populations found in relatively continuous habitat to populations in both recently and historically isolated habitat. Four microsatellite loci were used to genotype over 300 individuals from five populations in continuous habitat, five populations in recently fragmented habitat, and one historically isolated population. Results from the historically isolated population were concordant with previous analyses and suggest significant differentiation. Also, microsatellite data were consistent with the genetic effects of habitat fragmentation for the recently isolated populations, in the form of increased differentiation and decreased genetic diversity when compared to non-fragmented populations. This study is one of the first to identify the genetic effects of recent habitat fragmentation in a wide-ranging, high gene flow species.

Introduction

Anthropogenic habitat fragmentation of previously continuous habitats has been a topic of growing interest and concern in the fields of conservation biology, ecology, and evolutionary biology (Wilcox and Murphy 1985; Saunders *et al.* 1991; Frankham 1995; Young *et al.* 1996). Isolation of large populations into several smaller, isolated populations can alter both demographic and genetic factors, which leads to an increased risk of population extirpation (Goodman 1987; Lacy 1987; Lande and Barrowclough 1987; Lande 1988; Harrison and Hastings 1996). Several theoretical and experimental studies have determined the potential effects of isolation among populations, but inferring the effects of habitat fragmentation among natural populations can be a difficult task (Peacock and Smith 1997; Knutsen *et al.* 2000). Therefore, a first step in understanding habitat fragmentation in natural populations is to determine whether demographic or genetic data are consistent with the theoretically expected effects (Knutsen *et al.* 2000).

Conservation genetic studies have typically inferred the effects of habitat fragmentation by documenting patterns of genetic differentiation and levels of genetic diversity among fragmented populations (Harrison and Hastings 1996; Young *et al.* 1996). Ideally, such studies should take additional factors into account. First, studies on the genetic effects of recent habitat fragmentation should determine historical levels of isolation and differentiation among populations (Bermingham and Avise 1986; Cunningham and Moritz 1998). Historical population structure can have profound influences on the distribution of genetic variation among contemporary populations

such that any observed differentiation may be the result of long-term isolation, rather than recent, anthropogenic fragmentation (Cunningham and Moritz 1998). Alternatively, a lack of differentiation among populations based on similar allele frequencies could be the result of shared ancestry among populations, rather than ongoing gene flow among them (Avise *et al.* 1987).

Second, levels of differentiation and genetic diversity among fragmented populations should be compared to populations thought to be undisturbed (Jackson and Pounds 1979; Van Dongen *et al.* 1998; Brawn *et al.* 1996). Such comparisons have been effective at determining the effects of natural isolation among island versus mainland populations (Baker *et al.* 1990; Brawn *et al.* 1996; Bates 2000; Vucetich *et al.* 2001). However, finding both fragmented and non-fragmented populations can be difficult among naturally occurring populations, often because species aren't of conservation concern until only a few, isolated populations remain. Hence, efforts to conserve genetic diversity can benefit from early intervention, before all populations within a species' range have been influenced by anthropogenic change. Comparisons of fragmented and non-fragmented populations could be made among closely related species, but are best made at the intraspecific level because ecological or life history differences between species could also have profound influences on the distribution of genetic variation (Avise 1994).

The availability of "control" (= non-fragmented) populations is especially important for high gene flow species because levels of differentiation can be extremely low (Waples 1998). Such low levels of differentiation can be difficult to detect and

typically require the use of genetic markers with greater resolving power, e.g. microsatellites (Hughes and Quellar 1993; Waples 1998; Sunnucks 2000; Mossman and Waser 2001). The greater resolution provided by microsatellites theoretically allows for detection of even slight levels of differentiation in high gene flow species, but can also introduce several additional problems (Goldstein *et al.* 1995; Jarne and Lagoda 1996; Waples 1998; Hedrick 1999; Balloux *et al.* 2000). First, the increased resolution provided by hypervariable markers, like microsatellites, can yield statistically significant levels of differentiation among populations even when the biological relevance of such conclusions is questionable (Waples 1998; Hedrick 1999). For example, populations in fragmented habitat may reveal low but non-zero, statistically significant levels of differentiation that could be interpreted as biologically significant in terms of restricted gene flow due to habitat fragmentation (Hedrick 1999). However, if control populations also show similar absolute values and statistically significant levels of differentiation, the inference of restricted gene flow would be suspect. Alternatively, without data from control populations, low levels of differentiation in a high gene flow species could be interpreted as evidence of normal, high levels of ongoing gene flow when, in fact, habitat fragmentation may have altered patterns of genetic differentiation (Bossart and Prowell 1998; Waples 1998).

Second, the mutational processes responsible for the observed variation at microsatellite loci need to be properly incorporated into unbiased measures of genetic differentiation; hence, several alternative methods and measures for estimating levels of differentiation have been devised (Slatkin 1995; Goldstein *et al.* 1995; Bentzen *et al.*

1996; Valsecchi *et al.* 1997; Angers and Bernatchez 1998; Luikart and England 1999; Ellegren 2000). While some of these measures may identify significant differentiation among populations in a given scenario, alternative measures may not, leaving the investigator to infer which model of molecular evolution is best suited for a particular analysis. Hence, inferences concerning the effects of habitat fragmentation are more robust if they are based on samples from populations in both fragmented and non-fragmented habitat, multiple loci, and are concordant regardless of the genetic measure used to determine levels of differentiation.

This study examined the genetic effects of recent fragmentation on the butterfly *Speyeria idalia* (Lepidoptera: Nymphalidae) Drury using four microsatellite loci. The biogeographic distribution of this species is ideal for examining the effects of habitat fragmentation because some populations are found in relatively continuous habitat, some populations are found in habitat that has been highly fragmented within the last century, and one population has been historically isolated from all others (Williams 2001a,b). As a result, levels of genetic differentiation and diversity can be compared among fragmented, non-fragmented, and historically isolated populations. Finally, because *S. idalia* has been described as a high gene flow species (Hammond 1991; Williams 2001b), patterns of genetic differentiation may not be apparent unless they are examined at a large geographic scale (on the order of hundreds of kilometers). Enough populations of *S. idalia* are still remaining over a large enough area to make such large scale comparisons, both within and between regions, possible in this study.

Study species

Speyeria idalia is a univoltine species occurring in prairies, open range land, and marshes that contain its larval food sources of *Viola pedatifida, V. pedata, V. sagittata, V. papilionacea,* or *V. lanceolata* (Scudder 1889; Howe 1975; Opler and Krizek 1984; Scott 1986; Barton 1996). Previous studies of *S. idalia* described a biogeographic distribution whereby several populations occur in relatively non-fragmented habitat in the Great Plains of the U.S., from the Dakotas south to western Missouri and eastern Colorado (Hammond 1995; Swengel 1997; Debinski and Kelly 1998; Kelly and Debinski 1998; Williams 2001b)(Fig. 1). While pristine prairie habitats found in this region may be somewhat isolated from one another, populations are connected by habitats like grazed rangeland and riparian corridors that can accommodate *S. idalia* to some extent (Kelly and Debinski 1998; B. Williams personal observation). However, populations found in the Midwestern states of Wisconsin, Illinois, and Iowa are separated from one another by large expanses of uninhabitable terrain in the form of row crop agriculture. Hammond (1991) noted that *S. idalia* is a strong flyer, so this species may be able to maintain high levels of gene flow among populations in the face of increasing habitat fragmentation. Fragmentation of the Midwestern populations has only been present, at most, since the 1860's (Hammond 1995; Swengel 1997, Warner *et al.* 2000). Consequently, any genetic effects of habitat fragmentation are likely to be of recent origin. Finally, two extremely isolated populations are found in eastern Pennsylvania and western Virginia (Barton 1996; Williams 2001a,b). The Virginia population was found in 1997, and estimates based on

mark-recapture indicate a population size of less than 100 (Williams 2001a); hence, tissue from this population was not available for analysis. Conversely, the Pennsylvania population is estimated to number in the hundreds to thousands (Barton 1996).

Analyses of mitochondrial DNA (mtDNA) variation among populations suggest that while the Pennsylvania population was clearly morphologically and genetically differentiated from all other populations, little genetic structure existed among any of the Great Plains or Midwestern populations (Williams 2001a, b). These data suggest that *S. idalia* is a high gene flow species; therefore, an examination of recent changes in population structure will likely require microsatellite markers. Because much of *S. idalia*'s range occurs over land that was glaciated within the last 10,000 years (Pielou 1991), the lack of genealogical patterns among populations may be due to recent range expansion from a relatively small subset of refugia populations (Williams 2001b). Hence, there is no *a priori* reason, based on genealogical data, to suspect that populations in the Midwest versus Great Plains should exhibit substantially different patterns of genetic variation at microsatellite loci, with the exception of the potential effects of genetic isolation from recent habitat fragmentation. Alternatively, the Pennsylvania population should exhibit high levels of genetic differentiation when compared to all other populations, in accordance with the observed differentiation in mtDNA.

In summary, this study will address the following questions. First, can microsatellites be used to detect the genetic effects of habitat fragmentation, not evident from mtDNA analyses, among Midwestern populations? If so, we predict that

Midwestern populations should exhibit higher levels of genetic differentiation and lower genetic diversity among populations when compared to Great Plains populations. The biogeographic distribution of *S. idalia* means that comparisons among fragmented and non-fragmented populations can be made at a much larger geographic scale than is typically examined in natural populations. Second, is the differentiation of the Pennsylvania population observed from mtDNA consistent with patterns observed from nuclear microsatellite loci? Previously examined genealogical data provides information on the historical population structure across the range of this species (Williams 2001b). These data suggest, *a priori*, that the Pennsylvania population should exhibit significant differentiation from all populations due to historical isolation, whereas Midwestern and Great Plains populations only differ in the degree of habitat fragmentation. Hence, any differences observed in the level of differentiation and genetic diversity between Midwestern and Great Plains populations would be the result of recent habitat fragmentation, whereas differentiation of the Pennsylvania population would be the result of long term, evolutionary divergence.

Materials and Methods

Sample collection and DNA isolation

Samples of 25 to 30 individuals were collected from a total of 11 populations in the summers of 1997 and 1998, with the exception of the Nachusa population in northern Illinois, which was deemed sensitive and therefore only 15 individuals were sampled (Fig. 1). Five populations were sampled from both the Great Plains and Midwestern portions of the species' range, as well as the Pennsylvania population (Fig. 1). The geographic distance among populations was, on average, greater among populations in the Great Plains (470.8 ± 237.2 km) than the Midwest (248.7 ± 113.2 km), and the distance between the Pennsylvania population and all others was relatively much larger (1483.7 ± 371.1 km). Whole specimens were collected at most locations, with the exception of samples from Pennsylvania, Illinois, Iowa, and Wisconsin. All of those populations are either state protected or deemed sensitive by landowners. In those populations, the posterior leg on the right side was removed and then each specimen was released alive.

A sterile razor blade was used to homogenize either a single leg or section of the thorax into a "slurry" of tissue. Homogenized tissue was incubated at 65° C for 3-12 hours in digestion buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, 20 μL dithiothreitol, 0.4 mg Proteinase K), followed by standard organic extraction procedures (Sambrook *et al.* 1989).

PCR amplification of microsatellites

Microsatellite loci were identified in a previous study (Williams, unpublished data), which produced 4 loci with 46, 38, 76, and 60 alleles for loci 13, 17, 18 and 31, respectively. Each microsatellite locus was amplified individually in reactions containing 40 ng genomic DNA, 20 mM Tris-HCl, 50 mM KCl, 3 mM $MgCl_2$, 0.25 mM of each dNTP, 5 μM each primer, 0.5 U Ampli-*Taq* Gold DNA polymerase (Perkin-Elmer), and water to a final volume of 20 uL. Each PCR reaction was then subjected to an initial denaturation step at 94° C for 12 minutes, followed by 35 cycles of amplification at 94° C for 30 seconds, 57° C for 30 seconds, and 72° C for 1 minute. The annealing temperature for locus 18 was 55° C instead of 57° C. PCR products were amplified with one primer of each primer pair end-labelled with a fluorescent dye, either 6-FAM, HEX, or TAMRA, and then mixed with a size standard (Genescan-500 ROX) and run on an ABI 377 at the University of Illinois W.M. Keck Center for Comparative and Functional Genomics. Genotypes were determined with Genotyper software (Perkin-Elmer).

Data analyses

Allele frequencies were determined by direct counts and the number of alleles per population per locus (A), expected heterozygosity (He), and observed heterozygosity (Ho) were calculated according to Nei (1987) as implemented in GENEPOP (Raymond and Rousset 1995). Departures from random associations of allele frequencies between population pairs were tested with the exact test of Raymond and Rousset (1995), with 1000 iterations of the Markov chain method (Guo and

Thompson 1992). Critical values were adjusted for multiple statistical tests with the Bonferroni correction (Sokal and Rohlf 1995). Estimates of genetic variation can be influenced by assumptions concerning the model of evolution for a given molecular marker. Both an infinite allele model (IAM) and step-wise mutation model (SMM) have been applied to microsatellite data, and which model is appropriate for a given level of inquiry has been a topic of much debate (Goldstein *et al.* 1995b; Bentzen *et al.* 1996; Valsecchi *et al.* 1997). Differentiation among populations was determined using both global estimates and pairwise comparisons of θst and Rst values, estimated with FSTAT (Goudet 1995) and MICROSAT (Minch 1996) software packages respectively, where θst is consistent with an IAM (Weir and Cockerham 1984) and Rst is consistent with a SMM (Slatkin 1995). Finally, genetic distances among population pairs were estimated with the Cavalli-Sforza and Edwards' (1967) chord distance, which does not make underlying assumptions concerning the particular model of molecular evolution. Chord distances were estimated with the computer package PHYLIP (Felsenstein 1993). Hence, we have incorporated a variety of different measures in order to determine if the observed patterns of genetic differentiation are consistent across methodologies.

Results

Differentiation among populations

We detected several instances of non-random associations among alleles (Fig. 2). Allelic differentiation was significant for all populations, among Great Plains populations, and among Midwestern populations at each locus (N = 11, 5, and 5 respectively; $P < 0.001$ in each case). This fact is not surprising given the high allelic diversity and associated high statistical power at each of these four microsatellite loci. For the ten possible pairwise comparisons at each locus, significant allelic differentiation was more common among Midwestern than Great Plains populations (N = 40, $\mu \pm$ S.E. = 7.5 ± 1.73 and 3.00 ± 2.16, respectively, averaged across loci), and in almost all ten pairwise comparisons of each population with Pennsylvania (N = 40, $\mu \pm$ S.E. = 9.25 ± 1.50, averaged across loci; Fig. 2). Therefore, exact tests of allelic differentiation were consistent with greater differentiation among Midwestern populations and even greater differentiation for the Pennsylvania population.

Measures of genetic differentiation were also consistent with the effects of habitat fragmentation. All three multilocus measures, θst, Rst, and chord distances, revealed higher levels of differentiation among the fragmented Midwestern populations than non-fragmented Great Plains populations (Fig. 3). This pattern was consistent for each locus individually (data not shown) and for global values of θst (0.016 versus 0.049) and Rst (0.022 versus 0.107) among Great Plains and Midwestern populations respectively. Finally, pairwise comparisons of all populations with the Pennsylvania population were consistently the highest observed (Fig. 3).

For each measure of genetic differentiation, isolation by distance was examined by calculating correlations between geographic and genetic distances (Hutchinson and Templeton 1999). Correlations were calculated for all populations, only Great Plains populations, and only Midwestern populations using θst, Rst, and chord distances. When all populations were included in the analysis, the correlations were significant for all three measures (data not shown). However, the significance of this correlation was due entirely to the relatively large genetic and geographic distance separating the Pennsylvania population. No isolation by distance was found in either the Great Plains or Midwestern populations (data not shown). Hence, a true pattern of increasing genetic differentiation with increasing geographic distance was not apparent in these data.

Genetic Diversity

Levels of allelic variation were consistent with smaller population sizes for Midwestern populations when compared to Great Plains populations ($N = 20$, $\mu \pm$ S.E. = 16.15 ± 5.26 and 22.65 ± 5.54, respectively, averaged across loci)(Fig. 4). Allelic diversity was also lowest in the Pennsylvania population ($N = 4$, $\mu \pm$ S.E. = 9.0 ± 3.37, averaged across loci)(Fig. 4). Levels of expected heterozygosity were lower in Midwestern than in Great Plains populations and were lower again in the Pennsylvania population (Fig. 5). However, the observed levels of heterozygosity were not always consistent with patterns of expected heterozygosity and were typically lower than expected levels based on Hardy-Weinberg equilibrium (Fig. 6).

Discussion

The general patterns observed at all four microsatellite loci are consistent with the predicted genetic effects of recent habitat fragmentation. Both theoretical and experimental studies have outlined patterns of genetic differentiation expected from habitat fragmentation (Lande and Barrowclough 1987; Templeton *et al.* 1990; Harrison and Hastings 1996; Frankham 1995; Templeton 1998; Spencer *et al.* 2000). First, populations in fragmented habitat may experience restricted gene flow among populations, resulting in higher levels of genetic differentiation among populations (Harrison and Hastings 1996; Hutchinson and Templeton 1999). Second, isolated populations may be more likely to experience population bottlenecks, which in turn leads to reduced genetic variability (Wilcox and Murphy 1985; Saunders *et al.* 1991; Frankham 1995; Bouzat *et al.* 1998a, 1998b; Westemeier *et al.* 1998). Given that microsatellites exhibit several alleles per locus, a reduction in genetic variability is likely to be manifested as a reduction in allelic diversity (Spencer *et al.* 2000). Also, both expected and observed levels of heterozygosity should be lower in bottlenecked populations and both heterozygosity levels will vary depending on the severity and length of the bottleneck, as well as the mating system and life history characteristics of the species (i.e. naturally inbred or colonial species tend to have low levels of heterozygosity)(Charlesworth and Charlesworth 1987; Frankham 1995, 1996; Spencer 2000).

Previous studies of natural populations on a wide range of taxa have also examined, and found, genetic data consistent with habitat fragmentation. Some studies

examined the effects of natural, long-term fragmentation (Brawn *et al.* 1996; Cunningham and Moritz 1998; Barratt *et al.* 1999; Clark *et al.* 1999; Seppa and Laurila 1999; Bates 2000; Vucetich *et al.* 2001; Wolf *et al.* 2000) although more commonly, studies focused on recent, anthropogenic habitat fragmentation at relatively small geographic scales (e.g., Gaines *et al.* 1997; Peacock and Smith 1997; Aldrich *et al.* 1998; Gibbs 1998; Van Dongen 1998; Dayanandan *et al.* 1999; Gerlach and Musolf 2000; Knutsen *et al.* 2000; Mossman and Waser 2001). In some cases, habitat fragmentation can lead to an increase in gene flow among fragmented populations, contrary to the expected pattern, because gene flow among fragmented populations is enhanced in species that exhibit wind pollination (Foré *et al.* 1991; Young *et al.* 1993). The most commonly observed results from studies of habitat fragmentation reveal significant levels of differentiation among populations, and low levels of genetic variation within populations, relative to related taxa (e.g. Gaines *et al.* 1997; Young *et al.* 1999). However, these studies cannot adequately determine if the observed genetic patterns are the result of recent habitat fragmentation, population history, or are indicative of expected natural levels, because intraspecific control populations are lacking. One way to avoid this problem in long lived species is to examine genetic structure among adults present before habitat fragmentation took place, and compare those patterns to genetic variation among juveniles in the same fragmented habitat (Aldrich *et al.* 1998; Dayanandan *et al.* 1999). Fortunately, the number of studies that include control populations is growing (Young *et al.* 1993; Peacock and Smith 1997; Bouzat *et al.* 1998b; Gibbs 1998; Van Dongen 1998; Gerlach and Musolf 2000;

Knusten 2000; Mossman and Waser 2001). However, these studies typically focus on species thought to have relatively low levels of vagility, possibly because fragmentation is more likely to disrupt gene flow in those species. Alternatively, low gene flow species may also be more likely to experience local adaptation to a given area and, consequently, are less prone to changes resulting from habitat loss and fragmentation (Mopper and Strauss 1998).

High gene flow species, on the other hand, may require extensive gene flow among populations in order to remain evolutionarily dynamic and persistent (Waples 1998). Habitat fragmentation could therefore lead to an increased likelihood of population extirpation for high gene flow species. However, species with greater vagility present several logistical difficulties in determining the effects of habitat fragmentation, as discussed earlier. This study is the first to identify genetic patterns consistent with recent habitat fragmentation in a wide ranging, high gene flow species at a large geographic scale.

The pattern of increased genetic diversity among Midwestern populations, relative to Great Plains populations, was consistent for both IAM and SMM models of molecular evolution (Fig. 3). The pattern was also observed regardless of whether or not the method made assumptions concerning the underlying mutational process observed in microsatellite loci (Figs. 2 & 3). Clearly, habitat fragmentation has disrupted the level of gene flow observed among contemporary Midwestern populations of *S. idalia*. Note that the absolute value of differentiation was low; for example, among Midwestern populations θst was 0.049. If one was willing to accept the

assumptions associated with estimating migration rates from Fst (Wright 1943; Bossart and Prowell 1998; Templeton 1998; Waples 1998; Whitlock and McCauley 1999), the estimated Nm would be a relatively high value of 4.8 migrants per generation, This value could be misinterpreted as indicative of ongoing genetic exchange among populations rather than because of shared ancestry. Without the relative comparisons from control populations, such high estimates of gene flow could be considered indicative of continuous exchange of individuals among populations. Alternatively, all measures of genetic differentiation among Midwestern populations, derived from bootstrapping across loci, were statistically significant (data not shown). Again, without the relative comparisons from control populations, we might have incorrectly assumed that statistically significant levels of differentiation were equivalent to restricted gene flow among populations. Hence, this study provides another example on the importance of including control populations in the determination of the genetic effects of habitat fragmentation.

Levels of differentiation observed for the Pennsylvania population were consistent with previous results from analyses of mtDNA, which indicated a long history of isolation. One implication from the Williams (2001b) study was that the observed differentiation at a single locus (mtDNA) may be the result of stochastic lineage sorting from a polymorphic ancestral population. The relatively high level of differentiation observed at all four microsatellite loci support the hypothesis that the observed differentiation is the result of long term population isolation. A survey of 30 individuals for mtDNA variation resulted in a single shared haplotype in the population

(Williams 2001b) and the reduced allelic variation in Pennsylvania observed with microsatellites is also consistent with a population bottleneck. A second potential explanation for differentiation of the Pennsylvania population may still be fixation of unique alleles following a founder event for that population, although additional data will be required to resolve the issue (e.g., Glenn *et al.* 1999).

Allelic variation and expected heterozygosity among populations were also consistent with the effects of habitat fragmentation for the Midwestern populations, although observed heterozygosity was not. These results are in accordance with the patterns observed by Spencer *et al.* (2000). Their experimental study examined the effects of population bottlenecks on microsatellite loci in mesocosm populations of *Gambusia affinis* (Poeciliidae). Allelic richness was a more sensitive indicator of bottlenecks than was expected heterozygosity, while observed heterozygosity was not correlated with the number of founding individuals. Spencer *et al.* (2000) attribute this pattern to a number of potential explanations, including gametic sampling error with a small number of founding individuals, inbreeding depression, or selection at linked loci. However, in their study the observed levels of heterozygosity were often higher than those expected based on Hardy-Weinberg equilibrium. One troubling aspect of this study is that the observed heterozygosity was not consistent with the expected levels.

Null alleles

Given the reduction in heterozygosity across all populations, we must address the possibility that these loci exhibited null alleles. Null alleles result from a lack of PCR amplification, often due to nucleotide substitutions in the priming site for the

respective allele (Petkau and Strobeck 1995). As a result, observed heterozygosity may be low because the investigators incorrectly genotyped heterozygotes as homozygotes for every individual carrying null alleles. A null allele was also implicated in a similar study of microsatellite variation in a butterfly (Keyghobadi *et al.* 1999), in which they suggest that mutation rates at nucleotides adjacent to microsatellite repeats may be elevated relative to the remainder of the genome. One method for the detection of null alleles is through the observed peak intensity of genotypes observed on genotyping software (Petkau and Strobeck 1995; Keyghobadi *et al.* 1999). If PCR conditions are held constant, then peak intensities for homozygotes should be roughly twice as strong as for heterozygotes. If a null allele is present, heterozygous and homozygous individuals consistently have equivalent peak intensities. All peak intensities for homozygotes in this study were greater than those observed for heterzygotes, although some variation in peak intensity was observed (data not shown). Alternative explanations for the low levels of observed heterozygosity include selection at linked loci and inbreeding among individuals across most populations. Virtually nothing is known about inbreeding / outbreeding levels for *S. idalia*, so more data will be required to resolve the issue.

Conservation implications

The effect of fragmentation on populations of *S. idalia* has management implications as well. Previous studies on butterflies have documented their increased sensitivity to habitat fragmentation in terms of both levels of biodiversity and inbreeding depression (Saccheri *et al.* 1998; Zschokke *et al.* 2000; Nieminen *et al.*

2001), including studies documenting the effects of habitat fragmentation on the persistence of *S. idalia* populations (Hammond and McCorkle 1984; Nagel *et al.* 1991; Debinski and Kelly 1998; Kelly and Debinski 1998; Swengel 1997). *Speyeria idalia* is dependant on the several small patches of prairie habitat found in the Midwest for continued existence in that region (Panzer *et al.* 1995). However, while some population extirpation of *S. idalia* within the Midwest is clearly due to habitat loss, it is not clear why populations are absent in some of the remnant prairie patches and present in others. Moreover, studies of habitat management methods in the Midwest have reached conflicting conclusions concerning the effects of different habitat management regimes on *S. idalia* (Swengel 1996; Schwartz 1998; Huebschman 2000). Some data suggest that the commonly employed method of burning prairies may result in extirpation of *S. idalia* from those prairies, while other studies suggest that fire does not alter the ability of *S. idalia* to exist on prairie remnants (Swengel 1996; Schwartz 1998; Huebschman 2000). The results from this study indicate that Midwestern populations are experiencing the effects of habitat fragmentation and are therefore also more likely to experience the associated increase in extinction risk due to both genetic and demographic factors (Lande 1988; Frankham 1995; Westemeier *et al.* 1998). Conservation and management efforts will need to recognize that remnant prairie patches are required for the maintenance of *S. idalia* populations, and that more intermediate populations are required to maintain normal levels of genetic exchange among populations. Also, habitat managers will need to resolve the issue of which

method of prairie disturbance is most effective at maintaining population size for *S. idalia* in order to maintain the continued existence of this species in the Midwest.

Acknowledgements

This work was suported by a cooperative research agreement with the U.S. Fish and Wildlife Service, and grants from the University of Illinois Program in Ecology and Evolution, University of Illinois Graduate College, and Illinois Department of Natural Resources to BLW.

References

Aldrich PR, Hamrick JL, Chavarriage P, Kochert G (1998) Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology*,,**7**, 933-944.

Angers B, Bernatchez L (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution*, **15**, 143-159.

Avise JC (1994) *Molecular markers, natural history and evolution.* Chapman and Hall, New York.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Reviews in Ecology and Systematics*, **18**, 489-522.

Baker AJ, Dennison MD, Lynch A, LeGrand G (1990) Genetic divergence in peripherally isolated populations of chaffinches in the Atlantic Islands. *Evolution*, **44**, 981-999.

Balloux F, Brünner H, Lugon-Moulin N, Hausser J, Goudet J (2000) Microsatellites can be misleading: An empirical and simulation study. *Evolution*, **54**, 1414-1422.

Barratt EM, Gurnell J, Malarky G, Deaville R, Bruford MW (1999) Genetic structure of fragmented populations of red squirrel (*Sciurus vulgaris*) in the UK.

Molecular Ecology, **8**, S55-S63.

Barton B (1996) *Final report on the regal fritillary 1992-1995*. Report. U.S. Department of Defense. Annville, Pennsylvania.

Bates JM (2000) Allozymic genetic structure and natural habitat fragmentation: data for five species of amazonian forest birds. *Condor*, **102**, 770-783.

Bentzen P, Taggart CT, RuzzanteDE, Cook D (1996) Microsatellite polymorphism and the populations structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries Aquatic Science*, **53**, 2706-2721.

Bermingham E, Avise JC (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113**, 939-965.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons, and new directions. *Trends in Ecology and Evolution*, **15**, 538-543.

Bouzat JL, Cheng HH, Lewin HA, Westemeier RW, Brawn JR, Paige KP (1998a) Genetic evaluation of a demographic bottleneck in the greater prairie chicken. *Conservation Biology*, **12**, 836-843.

Bouzat JL, Lewin HA, Paige KN (1998b) The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. *American Naturalist*, **152**, 1-6.

Brawn JD, Collins TM, Medina M, Bermingham E (1996) Associations between physical isolation and geographical variation within three species of Neotropical birds. *Molecular Ecology*, **4**, 33-46.

1 Cavalli-Sforza, LL, and Edwards AWF (1967) Phylogenetic analysis: models and
2 estimation procedures. *Evolution*, **32**, 550-570.
3 Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary
4 consequences. *Annual Reviews in Ecology and Systematics*, **18**, 237-268.
5 Clark AM, Bowen BW, Branch LC (1999) Effects of natural habitat fragmentation on
6 an endemic scrub lizard (*Sceloporus woodi*): an historical perspective based on a
7 mitochondrial DNA gene genealogy. *Molecular Ecology*, **8**, 1093-1104.
8 Cunningham M, Moritz C (1998) Genetic effects of forest fragmentation on a
9 rainforest restricted lizard (Scincidae: *Gnypetoscincus queenslandiae*).
10 *Biological Conservation*, **83**, 19-30.
11 Dayanandan S, Dole J, Bawa K, Kesseli R (1999) Population structure delineated with
12 microsatellite markers in fragmented populations of a tropical tree, *Carapa*
13 *guianensis* (Meliaceae). *Molecular Ecology*, **8**, 1585-1592.
14 Debinski DM, Kelly L (1998) Decline of Iowa populations of the regal fritillary
15 (Speyeria idalia) Drury. *Journal of the Iowa Academy of Sciences*, **105**, 16-22.
16 Ellegren H (2000) Microsatellite mutations in the germline: implications for
17 evolutionary inference. *Trends in Genetics*, **16**, 551-558.
18 Felsenstein, J. 1993. PHYLIP (phylogeny inference package). Version 3.5c.
19 Distributed by the author, Department of Genetics, University of Washington,
20 Seattle.
21 Foré SA, Hickey RJ, Nankat JL, Guttman SI, Schaefer RL (1991) Genetic structure
22 after forest fragmentation: a landscape ecology perspective on *Acer saccharum*.

Canadian Journal of Botany, **70**, 1659-1668.

Frankham R (1995) Conservation genetics. *Annual Reviews in Genetics*, **29**, 305-327.

Gaines MS, Diffendorfer JE, Tamarin RH, Whittam TS (1997) The effects of habitat fragmentation on the genetic structure of small mammal populations. *Journal of Heredity*, **88**, 294-304.

Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology*, **14**, 1066-1074.

Gibbs JP (1998) Genetic structure of redback salamander Plethodon cinereus populations in continuous and fragmented forests. *Biological Conservation*, **86**, 77-81.

Glenn TC, Stephan W, Braun MJ (1999) Effects of a population bottleneck on whooping crane mitochondrial DNA variation. *Conservation Biology*, **13**, 1097-1107.

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. *Genetics*, **139**, 463-471.

Goodman D (1987) The demography of chance extinction. In *Viable populations for conservation*, ed, ME Soule pp. 11-43. Cambridge University Press, New York.

Goudet J (1995) FSTAT (Version 1.2); a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485-486.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **43**, 805-811.

Hammond PC (1991) Patterns of geographic variation and evolution in polytypic

butterflies. *Journal of Research on the Lepidoptera*, **29**, 54-76.

Hammond PC (1995) Conservation of biodiversity in native prairie communities in the United States. *Journal of the Kansas Entomological Society*, **68**, 1-6.

Hammond PC, McCorkle DV (1984) The decline and extinction of Speyeria populations resulting from human environmental disturbances (Nymphalidae: Argynninae). *Journal of Research on the Lepidoptera*, **22**, 217-224.

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution*, **11**, 180-183.

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313-318.

Howe WH (1975) *The butterflies of North America*. Doubleday, Garden City, New York.

Huebschman JJ, Bragg TB (2000) Response of regal fritillary (*Speyeria idalia*) to spring burning in an eastern Nebraska tallgrass prairie, USA. *Natural Areas Journal*, **20**, 386-388.

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology*, **2**, 131-137.

Hutchinson DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1989-1914.

Jackson JF, Pounds JA (1979) Comments on assessing the differentiating effect of gene flow. *Systematic Zoology*, **28**, 78-85.

1 Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back.
2 *Trends in Ecology and Evolution*, **11**, 424-429.
3 Keyghobadi N, Roland J, Strobeck C (1999) Influence of landscape on the population
4 genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae).
5 *Molecular Ecology*, **8**, 1481-1496.
6 Kelly, L., Debinski D (1998) Relationship of host plant density to size and abundance
7 of the regal fritillary *Speyeria idalia* Drury (Nymphalidae). *Journal of the*
8 *Lepidopterists Society,* **52**, 262-276.
9 Knutsen H, Rukke BA, Jorde PE, Ims RA (2000) Genetic differentiation among
10 populations of the beetle *Bolitophagus reticulatus* (Coleoptera: Tenebrionidae)
11 in a fragmented and a continuous landscape. *Heredity*, **84**, 667-676.
12 Lacy RC (1987) Loss of genetic diversity from managed populations: Interacting
13 effects of drift, mutation, immigration, selection and population subdivision.
14 *Conservation Biology*, **1**, 143-158.
15 Lande R (1988) Genetics and demography in biological conservation. *Science*, **241**,
16 1455-1460.
17 Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and
18 their use in population management. In *Viable populations for conservation*, ed,
19 ME Soule pp. 87-123. Cambridge University Press, New York.
20 Luikart G, England PE (1999) Statistical analysis of microsatellite data. *Trends in*
21 *Ecology and Evolution*, **14**, 253-256.

Minch, E. 1996. MICROSAT. Version 1.4. Stanford University Medical Center, Stanford.

Mopper S, Strauss SY (1998) *Genetic structure and local adaptation in natural insect populations: effects of ecology, life history, and behavior*. Chapman & Hall, New York.

Mossman CA, Waser PM (2001) Effects of habitat fragmentation on population genetic structure in the white-footed mouse (*Peromyscus leucopus*). *Canadian Journal of Zoology*, **79**, 285-295.

Nagel HG, Nightengale T, Dankert N (1991) Regal fritillary butterfly population estimation and natural history on Rowe sanctuary, Nebraska. *Prairie Naturalist*, **23**, 145-152.

Nei M (1987) *Molecular evolutionary genetics*. Columbia University Press, New York.

Nieminen M, Singer MC, Fortelius W, Schöps K, Hanski I (2001) Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *American Naturalist*, **157**, 237-244.

Opler PA, Krizek GO (1984) *Butterflies east of the Great Plains, an illustrated natural history*. Johns Hopkins University Press, Baltimore, Maryland.

Peacock MM, Smith AT (1997) The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (Ochotona princeps) metapopulation. *Oecologia*, **112**, 524-533.

Petkau D, Strobeck C (1995) The molecular basis and evolutionary history of a

microsatellite null allele in bears. *Molecular Ecology*, **4**, 519-520.

Pielou EC (1991) *After the ice age: the return of life to glaciated North America.* University of Chicago Press, Chicago.

Raymond M, Rousset RF (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491-494.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual.* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, **5**, 18-32.

Schwartz M (1998) Ecology forum: Effects of fire and hay management on butterflies. *Rx Fire Notes*, **7**, 7-13.

Scott JA (1986) *Butterflies of North America.* Stanford University Press, Stanford, California.

Scudder S (1889) *Butterflies of the eastern United States.* Cambridge University Press, Cambridge, Massachusetts.

Seppa P, Laurila A (1999) Genetic structure of island populations of the anurans *Rana temporaria* and *Bufo bufo*. *Heredity*, **82**, 309-317.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457-462.

Sokal RR, Rohlf F.J. (1995) *Biometry: the principles and practice of statistics in*

biological research. 3rd edition. W.H. Freeman and Company, New York.

Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology*, **9**, 1517-1528.

Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, **15**, 199-203.

Swengel AB (1996) Effects of fire and hay management on abundance of prairie butterflies. *Biological Conservation*, **76**, 73-85.

Swengel AB (1997) Habitat association of sympatric violet-feeding fritillaries (*Euptoieta, Speyeria, Boloria*)(Lepidoptera: Nymphalidae) in tallgrass prairie. *The Great Lakes Entomologist*, **3**, 1-18.

Swengel AB (1998) Effects of management on butterfly abundance in tallgrass prairie and pine barrens. *Biological Conservation*, **83**, 77-89.

Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology*, **7**, 381-397.

Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*, **77**, 13-27.

Valsecchi E, Palsboll P, Hale P, *et al.* (1997) Microsatellite genetic distance between oceanic populations of the humpback whale (*Megaptera movaeangliae*). *Molecular Biology Evolution*, **14**, 355-362.

Van Dongen S, Backeljau T, Matthysen E, Dhondt AA (1998) Genetic population

structure of the winter moth (*Operophtera brumata* L.)(Lepidoptera, Geometridae) in a fragmented landscape. *Heredity*, **80**, 92-100.

Vucetich LM, Vucetich JA, Joshi CP, Waite TA, Peterson RO (2001) Genetic (RAPD) diversity in *Peromyscus maniculatus* populations in a naturally fragmented landscape. *Molecular Ecology*, **10**, 35-40.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438-450.

Warner RE, Etter SL, David LM, Mankin PC (2000) Annual set-aside programs: a long-term perspective of habitat quality in Illinois and the Midwest. Wildlife Society Bulletin, **28**, 347-354.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.

Westemeier RW, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bousat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695-1698.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: Fst $\neq$ 1/(4Nm + 1). *Heredity*, **82**, 117-125.

Wilcox BA, Murphy DD (1985) Conservation strategy: the effects of fragmentation on extinction. *American Naturalist*, **125**, 879-887.

Williams BL (2001a) Patterns of morphological variation in *Speyeria idalia* (Lepidoptera: Nymphalidae) with implications for taxonomy and conservation. *Annals of the Entomological Society of America*, **94**, 239-243.

Williams BL (2001b) Conservation genetics, extinction, and taxonomic status: A case history of the regal fritillary. *Conservation Biology*, In Press.

Wolf AT, Harrison SP, Hamrick JL (2000) Influence of habitat patchiness on genetic diversity and spatial structure of a serpentine endemic plant. *Conservation Biology*, 14, 454-463.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114-138.

Young AG, Merriam HG, Warwick SI (1993) The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh. (sugar maple) populations. *Heredity*, **71**, 277-289.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413-419.

Young AG, Brown AHD, Zich FA (1999) Genetic structure of fragmented populations of the endangered daisy Rutidosis leptorrhynchoides. *Conservation Biology*, **13**, 256-265.

Zschokke S, Dolt C, Rusterholz H, Oggier P, Braschler B, Thommen GH, Ludin E, Erhardt A, Baur B (2000) Short-term responses of plants and invertebrates to experimental small-scale grassland fragmentation. *Oecologia*, **125**, 559-572.

Author Information Box

Barry Williams completed this work as a doctoral student at the University of Illinois. He is currently a postdoctoral researcher at the University of Wisconsin and is

interested in the evolution of adaptive phenotypes, and the population genetics and conservation of insects. Jeffery Brawn is an Associate Professor at the Illinois Natural History Survey and University of Illinois, and is studying life history evolution, ecology, and conservation of birds. Ken Paige is an Associate Professor at the University of Illinois, and is studying the evolutionary ecology of plant / animal interactions, the genetics of plant responses to herbivory, and the conservation genetics of natural populations.

Figure 1. Range of *Speyeria idalia* and sample locations for each of 11 populations, indicated with open circles. Grey areas represent the current distribution of *S. idalia*. The large grey section in the western portion of *S. idalia*'s range is not meant to indicate a single large population, only that several, uncharacterized populations reside in this region.

Figure 2. Results for exact tests of allelic differentiation among populations of *Speyeria idalia*. Bars indicate the number, out of 10 possible pairwise comparisons, that were significant ($P < 0.05$) in each region. Solid bars represent comparisons among Great Plains populations, hatched bars represent comparisons among Midwestern populations, and open bars represent comparisons of all populations with the Pennsylvania population. Each of the comparisons are grouped by locus.

Figure 3. Estimates of genetic differentiation among populations of *Speyeria idalia*. Each bar indicates the average of ten possible pairwise comparisons among populations within the corresponding region. Error bars indicate plus or minus one standard error. Solid bars represent comparisons among Great Plains populations, hatched bars represent comparisons among Midwestern populations, and open bars represent comparisons of all populations with the Pennsylvania population. Each of the comparisons are grouped by the measure of differentiation.

Figure 4. Observed number of alleles per locus among populations of *Speyeria idalia*. Each bar indicates the mean number of alleles observed among populations in the corresponding region. Error bars indicate plus or minus one standard error. Solid bars represent the average among five Great Plains populations, hatched bars represent five

Midwestern populations, and open bars represent the Pennsylvania population. Each of the comparisons are grouped by the corresponding locus.

Figure 5. Expected levels of heterozygosity among populations of *Speyeria idalia.* Estimated heterozygosities were based on H-W equilibrium. Each bar indicates the average expected heterozygosity among populations within the corresponding region. Error bars indicate plus or minus one standard error. Solid bars represent the average among five Great Plains populations, hatched bars represent five Midwestern populations, and open bars represent the Pennsylvania population. Each of the comparisons are grouped by locus.

Figure 6. Observed levels of heterozygosity among populations of *Speyeria idalia.* Observed heterozygosities were determined based on direct count. Each bar indicates the average observed heterozygosity among populations within the corresponding region. Error bars indicate plus or minus one standard error. Solid bars represent the average among five Great Plains populations, hatched bars represent five Midwestern populations, and open bars represent the Pennsylvania population. Each of the comparisons are grouped by locus.

Figure 1.

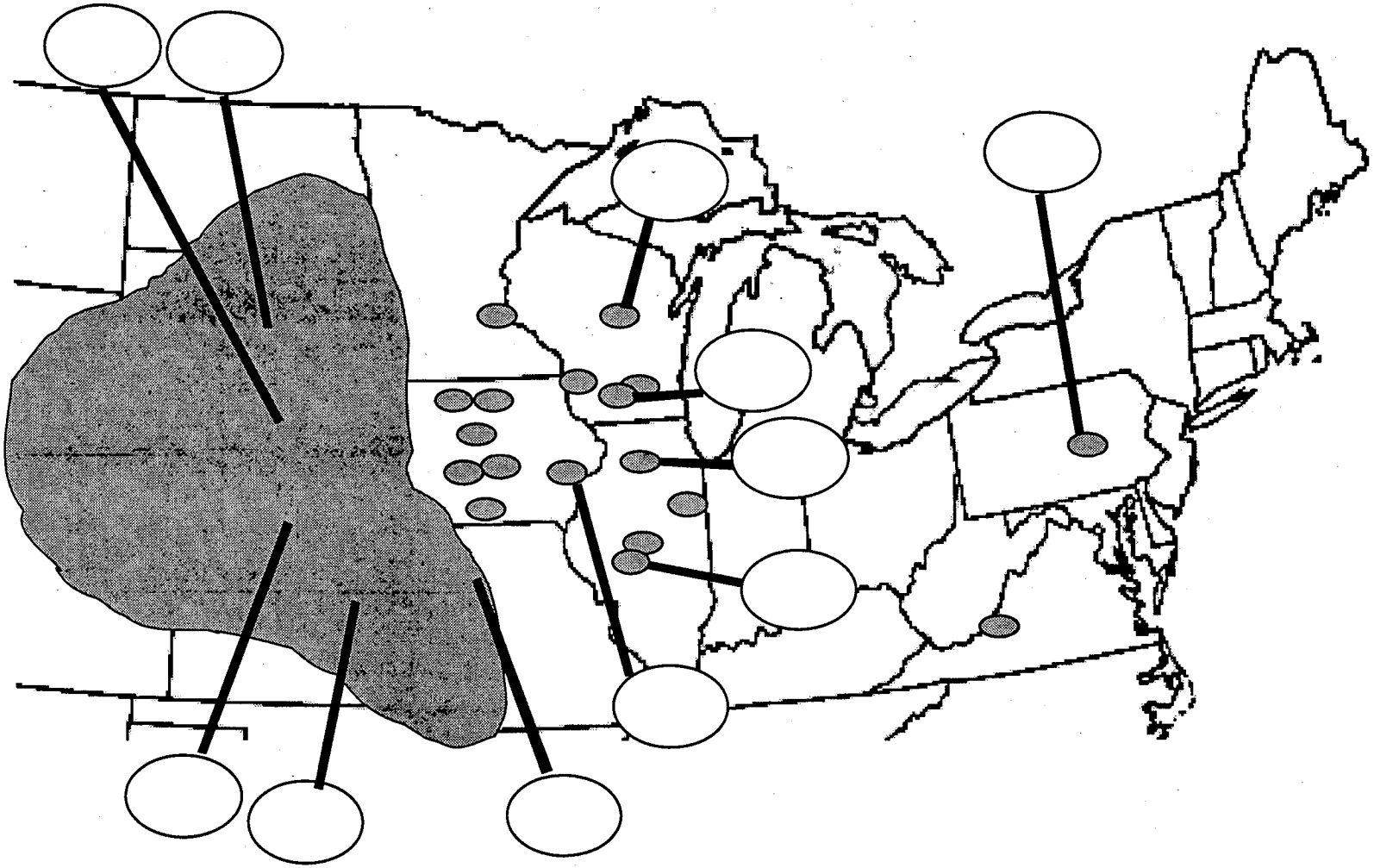


Figure 2.

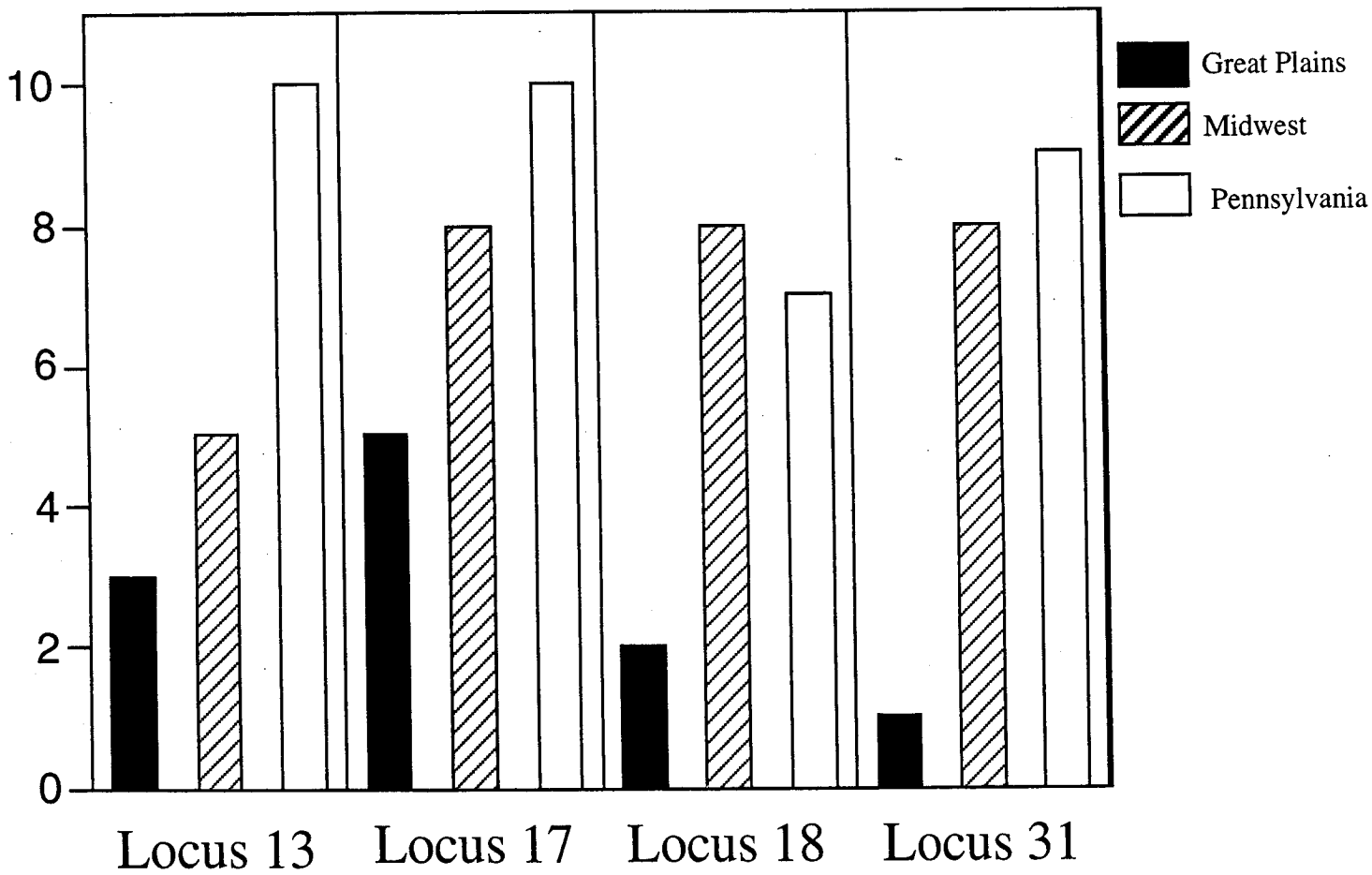

10
8
6
4
2
0
Great Plains
Midwest
Pennsylvania
Locus 13
Locus 17
Locus 18
Locus 31

Figure 3.

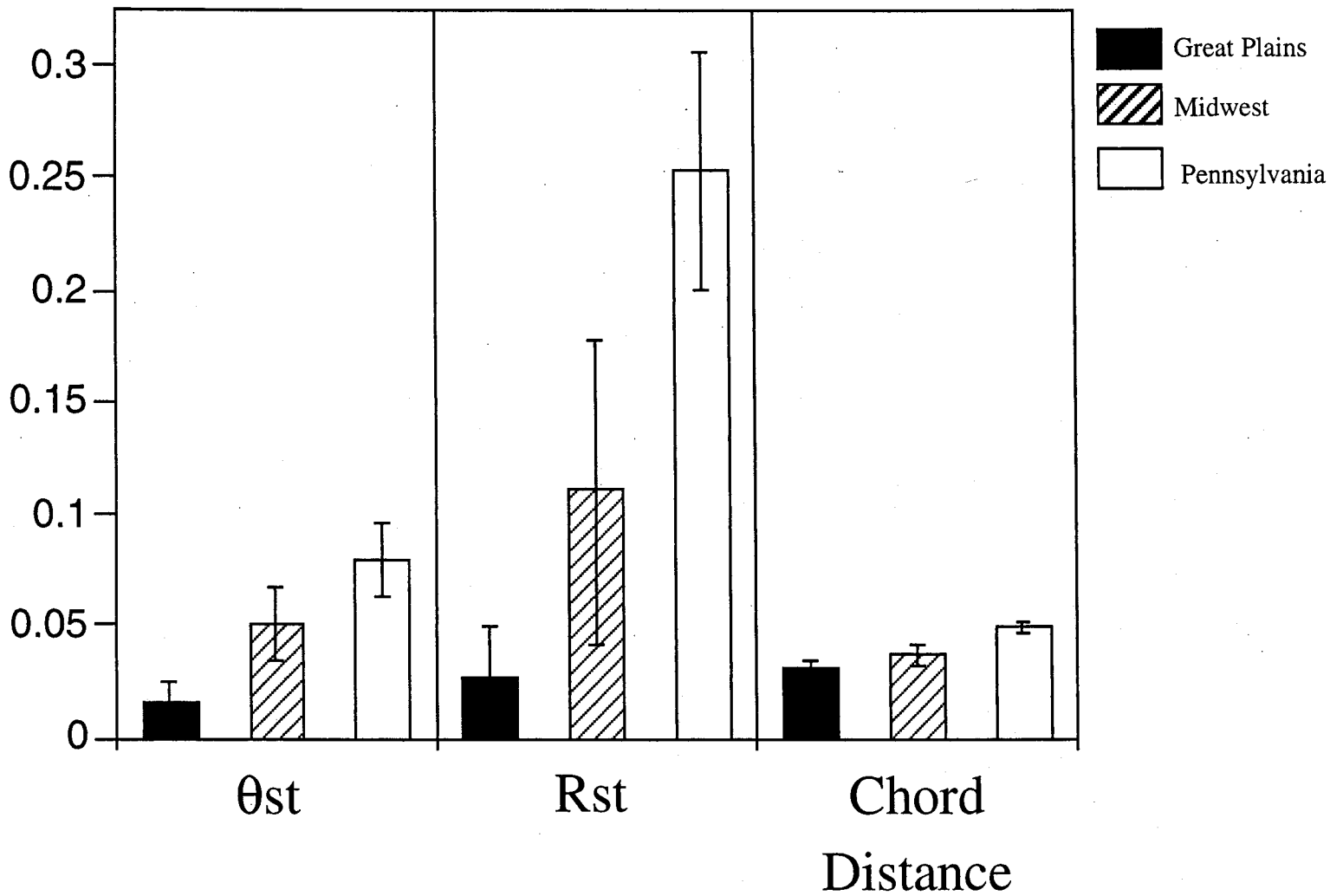

0.3
0.25
0.2
0.15
0.1
0.05
0
Great Plains
Midwest
Pennsylvania
θst
Rst
Chord Distance

Figure 4.

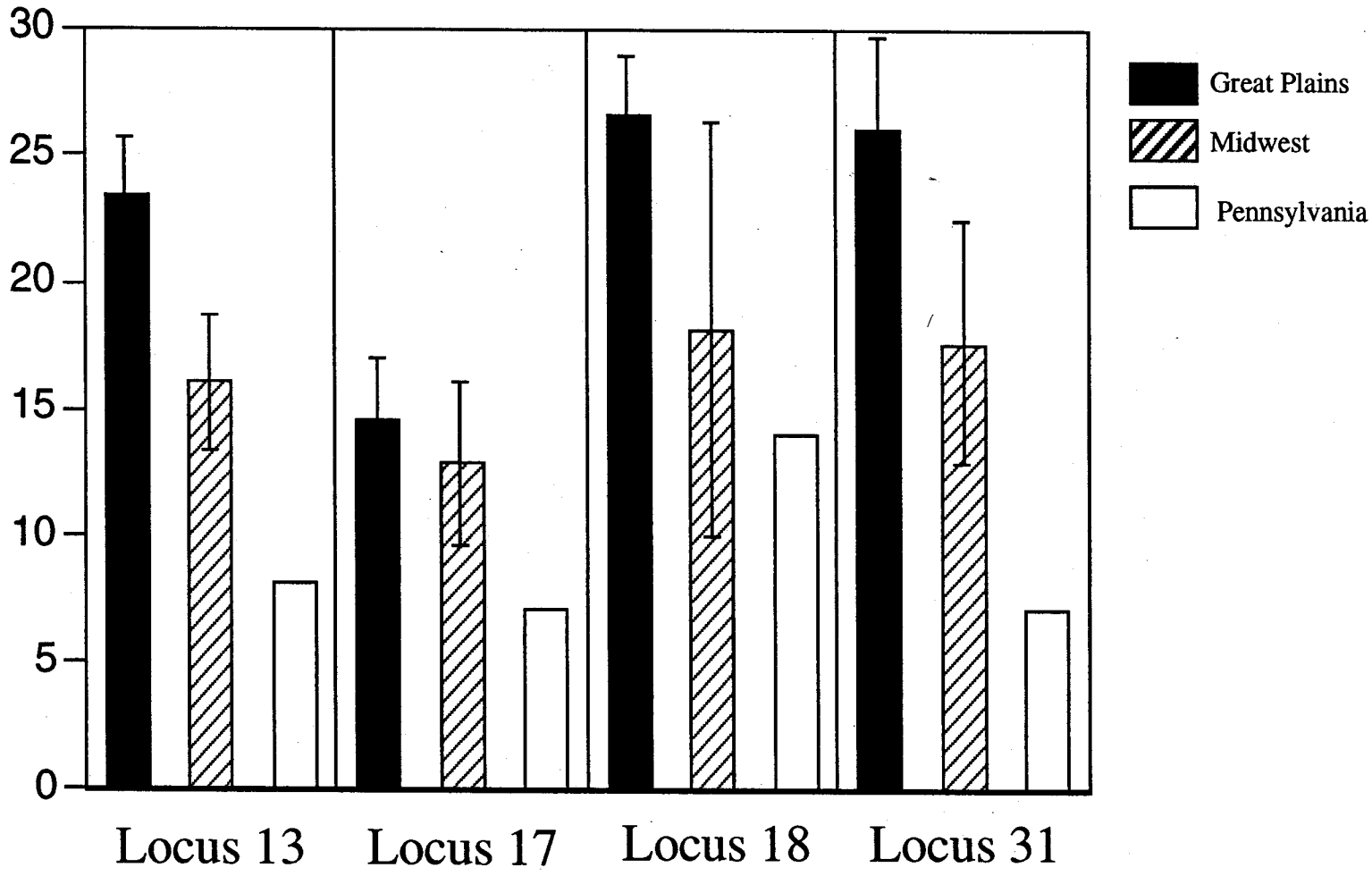

30
25
20
15
10
5
0
Locus 13
Locus 17
Locus 18
Locus 31
Great Plains
Midwest
Pennsylvania

Figure 5.

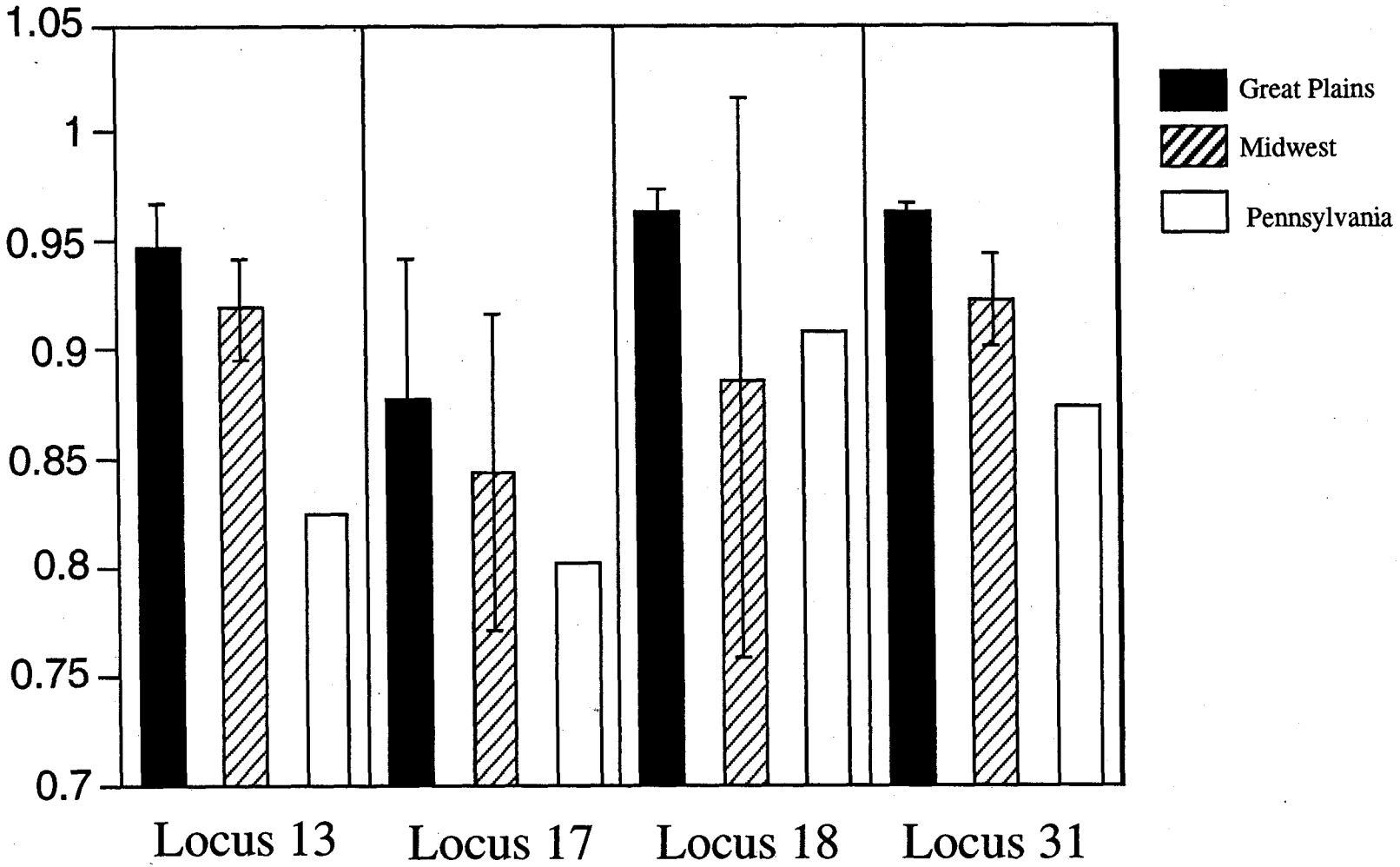
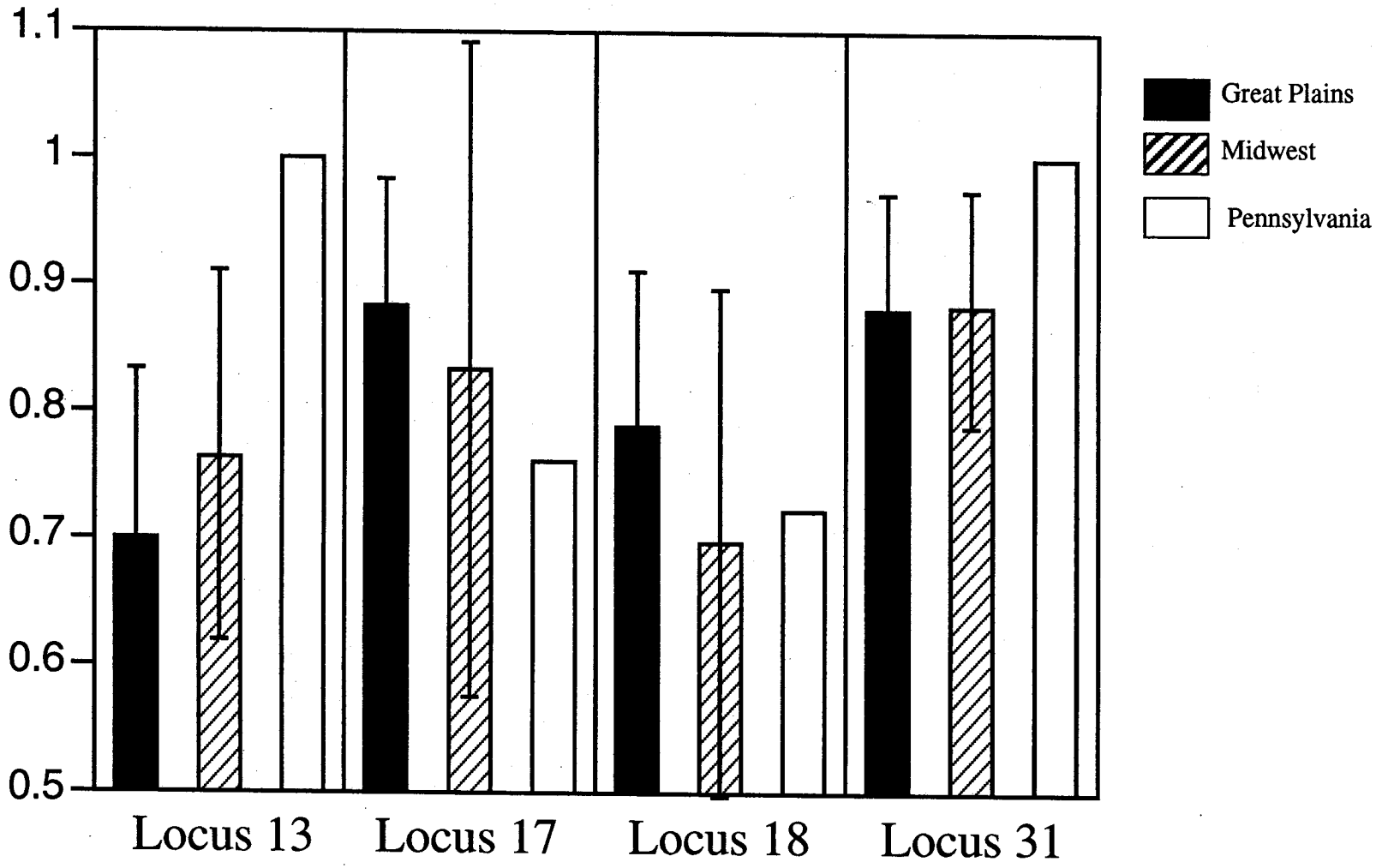

1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
Locus 13
Locus 17
Locus 18
Locus 31
Great Plains
Midwest
Pennsylvania

Figure 6.


1.1
1
0.9
0.8
0.7
0.6
0.5
Locus 13
Locus 17
Locus 18
Locus 31
Great Plains
Midwest
Pennsylvania