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Landscape scale genetic effects of habitat fragmentation on a high gene flow species: *Speyeria idalia* (Nymphalidae)

BARRY L. WILLIAMS,*‡ JEFFREY D. BRAWN† and KEN N. PAIGE*
Department of Animal Biology, University of Illinois, Urbana, IL 61801, USA, †Center for Wildlife Ecology, Illinois Natural History Survey, Champaign, IL 61820 and Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801, USA

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 mitochondrial DNA data from across the current range of *Speyeria idalia* indicated 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, which were recently separated from one another 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 nonfragmented populations. These results suggest that given the appropriate control populations, microsatellite markers can be used to detect the effects of recent habitat fragmentation in natural populations, even at a large geographical scale in high gene flow species.

Keywords: butterfly, conservation, genetic, habitat fragmentation, metapopulation, microsatellite

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Introduction

Anthropogenic habitat fragmentation of previously continuous habitats has been a topic of growing interest (Wilcox & 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 & Barrowclough 1987; Lande 1988; Harrison & 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 & Smith 1997; 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 potentially isolated populations (Harrison & Hastings 1996; Young *et al*. 1996). Ideally, such studies should take additional factors into account. First, historical levels of isolation and differentiation among populations should be determined *a priori* (Bermingham & Avise 1986; Cunningham & Moritz 1998). Historical population structure can have a profound influence on the distribution of genetic variation among contemporary populations such that any observed differentiation may be the result of

Correspondence: B. L. Williams. ‡Present address: Laboratory of Molecular Biology and Howard Hughes Medical Institute, University of Wisconsin, 1525 Linden Drive, Madison, WI 53706, USA. Fax: 608–262–9343; E-mail: bwilliams2@facstaff.wisc.edu

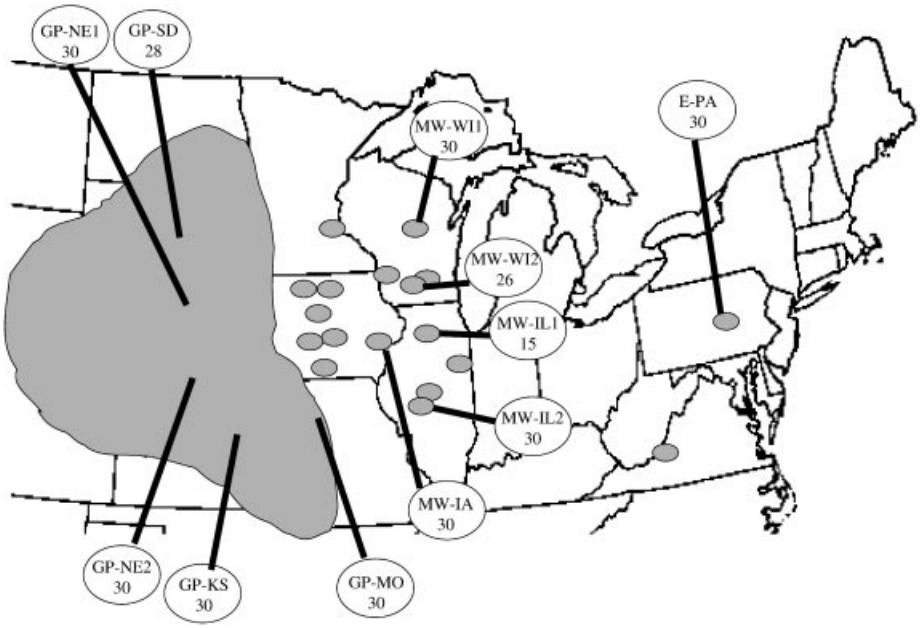

Fig. 1 Range of *Speyeria idalia* and sample locations for each of 11 populations, indicated by open circles. Population names correspond to each of three regions, the unfragmented Great Plains = GP, fragmented Midwestern = MW, and historically isolated Eastern = E. The subsequent labels indicate the state each population was collected in and the sample size for each population; see Williams (2001) for exact collection locations. 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. Unshaded areas indicate no known populations residing within that area.

long-term isolation, rather than recent, anthropogenic fragmentation (Cunningham & Moritz 1998). Alternatively, a lack of differentiation among populations could be the result of shared ancestry among populations, rather than ongoing gene flow among them (Avise *et al*. 1987).

Also, levels of differentiation and genetic diversity among fragmented populations should be compared to populations thought to be undisturbed (Jackson & Pounds 1979; Brawn *et al*. 1996; Van Dongen *et al*. 1998). Such comparisons have been effective at determining the effects of natural isolation among island vs. mainland populations (Baker *et al*. 1990; Brawn *et al*. 1996; Bates 2000; Vucetich *et al*. 2001). However, finding both fragmented and nonfragmented populations can be difficult among naturally occurring populations, often because species are not of conservation concern until only a few, isolated populations remain. Comparisons of fragmented and nonfragmented 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).

Inferring the effects of habitat fragmentation can be especially difficult for high gene flow species because they tend to have relatively low levels of differentiation among populations (Waples 1998). Such low levels of differentiation, even among recently isolated populations, can be difficult to detect and may require the use of genetic markers with greater resolving power, e.g. microsatellites (Hughes & Queller 1993; Waples 1998; Sunnucks 2000; Mossman & Waser 2001). The increased resolution provided by hypervariable markers, like microsatellites, introduces the additional problem of potentially yielding statistically significant levels of differentiation among populations even when the biological relevance of such conclusions is questionable (Goldstein *et al*. 1995; Jarne & Lagoda 1996; Waples 1998; Hedrick 1999; Balloux *et al*. 2000).

The goal of this study was to examine the genetic effects of recent fragmentation on the butterfly *Speyeria idalia* (Lepidoptera: Nymphalidae) Drury using four microsatellite loci (Williams *et al*. 2002). *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 & Krizek 1984; Scott 1986; Barton 1996). The unique biogeographic distribution of this species is ideal for examining the effects of habitat fragmentation. One set of populations, termed Great Plains, are located in relatively continuous habitat (Hammond 1995; Swengel 1997; Debinski & Kelly 1998; Kelly & Debinski 1998; Williams 2002) (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. idaslia* to some extent (Kelly & Debinski 1998; B. Williams personal observation). A second set of populations, termed Midwestern, are located in habitat that has been highly fragmented since, at most, the 1860s (Hammond 1995; Swengel 1997; Warner *et al*. 2000) (Fig. 1). Finally, two extremely isolated populations, termed eastern, are found in eastern Pennsylvania and western Virginia (Barton 1996; Williams 2001, 2002) (Fig. 1). The Virginia population was found in 1997, and estimates based on mark–recapture indicate a population size of less than 100 (Williams 2001); hence, tissue from this population was not available for analysis. This biogeographic distribution provides the unique opportunity to compare levels of genetic differentiation and diversity among fragmented, nonfragmented, and historically isolated populations.

Analyses of mitochondrial DNA (mtDNA) variation among populations indicate that while the eastern population was morphologically and genetically differentiated from all other populations, little genetic structure existed among any of the Great Plains or Midwestern populations (Williams 2001, 2002). These data suggest that *S. idalia* is a high gene flow species and that patterns of genetic differentiation may not be apparent unless they are examined at a large geographical scale (in the order of hundreds of kilometres). Fortunately, 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. Also, the mtDNA data indicate that there is no *a priori* reason to suspect that populations in the Midwest vs. Great Plains should exhibit substantially different patterns of genetic variation at microsatellite loci, with the exception of the effects from recent habitat fragmentation. Alternatively, the eastern 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 when compared to Great Plains populations. Second, is the differentiation of the eastern population observed from mtDNA consistent with patterns observed from nuclear microsatellite loci? Genealogical data indicate that the eastern population should exhibit significant differentiation from all populations as a result of historical isolation, whereas Midwestern and Great Plains populations only differ in the degree of habitat fragmentation (Williams 2002). 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

Tissue samples were collected in the summers of 1997 and 1998, sample sizes and locations are listed in Fig. 1 (see also Williams 2002). The geographical 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 much larger (1483.7 ± 371.1 km). Whole specimens were collected at most locations, with the exception of samples from the states of 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 h in digestion buffer (10 mM Tris–HCl, 10 mM ethylenediaminetetraacetic acid, 50 mM NaCl, 2% sodium dodecyl sulphate, 20 μL dithiothreitol, 0.4 mg Proteinase K), followed by standard organic extraction procedures (Sambrook *et al*. 1989).

Polymerase chain reaction amplification of microsatellites

Microsatellite loci were identified in a previous study (Williams *et al*. 2002), which produced four 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 μL. Each polymerase chain reaction (PCR) was then subjected to an initial denaturation step at 94 °C for 12 min, followed by 35 cycles of amplification at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min. 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 (H_E), and observed heterozygosity (H_O) were calculated according to Nei (1987) as implemented in GENEPOP (Raymond & Rousset 1995). Departures from random associations of allele frequencies between population pairs were tested with the exact test of Raymond & Rousset (1995), with 1000 iterations of the Markov chain method (Guo & Thompson 1992). Critical values were adjusted for multiple statistical tests with the Bonferroni correction (Sokal & 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*. 1995; Bentzen *et al*. 1996; Valsecchi *et al*. 1997). Differentiation among populations was determined using both global estimates and pairwise comparisons of θ_{ST} and R_{ST} values, estimated with FSTAT (Goudet 1995) and MICROSAT (Minch 1996) software packages, respectively, where θ_{ST} is consistent with an IAM (Weir & Cockerham 1984) and R_{ST} is consistent with an SMM (Slatkin 1995). Finally, genetic distances among population pairs were estimated with Cavalli-Sforza & Edwards's (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 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 (Table 1). Allelic differentiation was significant for analyses including comparisons among 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 10 possible pairwise comparisons at each locus, significant allelic differentiation was more common among Midwestern than Great Plains populations (N = 40, mean ± SE = 7.5 ± 1.73 and 3.00 ± 2.16, respectively, averaged across loci), and in almost all 10 pairwise comparisons of the eastern population with all others (N = 40, mean ± SE = 9.25 ± 1.50, averaged across loci). Therefore, exact tests of allelic differentiation were consistent with greater differentiation among Midwestern populations and even greater differentiation for the eastern population.

Measures of genetic differentiation were also consistent with the effects of habitat fragmentation. All three multilocus measures, θ_{ST}, R_{ST}, and chord distances, revealed higher levels of differentiation in pairwise comparisons among the fragmented Midwestern populations vs. nonfragmented Great Plains populations (Tables 1 and 2). This pattern was consistent for each locus individually (e.g. θ_{ST} values across loci, N = 10, mean ± SE, locus 13 = 0.021 ± 0.038 and 0.030 ± 0.017; locus 17 = 0.040 ± 0.038 and 0.065 ± 0.029; locus 18 = 0.007 ± 0.005 and 0.060 ± 0.060 ± 0.048; locus 31 = 0.006 ± 0.004 and 0.048 ± 0.016 among Great Plains vs. Midwestern populations, respectively)

Table 1 Multilocus chord distances are shown below the diagonal, generated by bootstrapping across loci with 100 bootstrap pseudoreplicates. The number of loci that had a significant difference in allele frequency as tested with the exact test of Raymond & Rousset (1995) are above the diagnoal. Comparison among Great Plains populations are within the solid-lined box and comparisons among Midwestern populations are within the dash-lined box and comparisons of the eastern population with all others are shaded

Populations	GP–KS	GP–NE1	GP–NE2	GP–SD	GP–MO	MW–WI2	MW–WI1	MW–IL2	MW–IA	MW–IL1	E–PA
GP–KS	0	1	1	1	1	2	4	1	3	1	4
GP–NE1	0.0276	0	3	1	0	2	3	2	4	2	3
GP–NE2	0.0330	0.0342	0	1	3	1	4	3	3	3	3
GP–SD	0.0284	0.0324	0.0349	0	2	2	4	3	3	1	3
GP–MO	0.0268	0.0254	0.0350	0.0298	0	3	3	1	3	2	4
MW–WI2	0.0303	0.0337	0.0343	0.0377	0.0329	0	3	3	3	2	4
MW–WI1	0.0365	0.0339	0.0412	0.0379	0.0321	0.0328	0	3	4	4	4
MW–IL2	0.0302	0.0308	0.0341	0.0339	0.0305	0.0324	0.0349	0	4	4	4
MW–IA	0.0357	0.0322	0.0423	0.0439	0.0371	0.0368	0.0452	0.0346	0	3	4
MW–IL1	0.0334	0.0319	0.0404	0.0346	0.0364	0.0399	0.0392	0.0388	0.0355	0	4
E–PA	0.0481	0.0451	0.0494	0.0498	0.0545	0.0474	0.0525	0.0486	0.0543	0.0453	0

Table 2 Pairwise, multilocus estimates of F_{ST} are shown below the diagonal, R_{ST} above, both generated by bootstrapping across loci for 100 pseudoreplicates. Comparisons among Great Plains populations are within the solid-lined box, comparisons among Midwestern populations are within the dash-lined box and comparisons of the eastern population with all others are shaded

Populations	GP–KS	GP–NE1	GP–NE2	GP–SD	GP–MO	MW–WI2	MW–WI1	MW–IL2	MW–IA	MW–IL1	E–PA
GP–KS	0	0.0100	0.0630	0.0050	0.0260	0.0090	0.0110	0.0070	0.1300	0.1160	0.2290
GP–NE1	0.0076	0	0.0040	0.0020	0.0170	0.0350	0.0820	−0.0060	0.0450	0.1070	0.2190
GP–NE2	0.0126	0.0206	0	0.0490	0.0350	0.1170	0.1470	0.0260	−0.0100	0.1550	0.2350
GP–SD	0.0017	0.0095	0.0091	0	0.0550	0.0290	0.0720	0.0380	0.1080	0.1340	0.1360
GP–MO	0.0261	0.0069	0.0312	0.0231	0	0.0610	0.0580	0.0240	0.0700	0.0700	0.2840
MW–WI2	0.0134	0.0220	0.0234	0.0205	0.0371	0	0.0270	0.0390	0.1890	0.0600	0.2570
MW–WI1	0.0302	0.0203	0.0408	0.0290	0.0218	0.0404	0	0.0660	0.2180	0.1040	0.2940
MW–IL2	0.0365	0.0203	0.0441	0.0401	0.0124	0.0402	0.0344	0	0.0810	0.1240	0.2850
MW–IA	0.0284	0.0227	0.0416	0.0298	0.0381	0.0282	0.0490	0.0475	0	0.1950	0.2710
MW–IL1	0.0350	0.0441	0.0541	0.0432	0.0644	0.0529	0.0719	0.0815	0.0591	0	0.3250
E–PA	0.0637	0.0648	0.0652	0.0591	0.0931	0.0703	0.0917	0.0930	0.0895	0.1015	0

and for global estimates of θ_{ST} ($N = 10$, mean ± SE = 0.015 ± 0.010 vs. 0.049 ± 0.017) R_{ST} ($N = 10$, mean ± SE = 0.022 ± 0.023 vs. 0.110 ± 0.069) and chord distances ($N = 10$, mean ± SE = 0.031 ± 0.003 vs. 0.037 ± 0.004 Great Plains and Midwestern populations, respectively). Finally, pairwise comparisons of all populations with the eastern population were consistently the highest observed ($N = 10$, mean ± SE = 0.079 ± 0.016, 0.254 ± 0.053, 0.050 ± 0.003 for θ_{ST}, R_{ST}, and chord distance, respectively) (Tables 1 and 2).

For each measure of genetic differentiation, isolation by distance was examined by calculating correlations between geographical and genetic distances (Hutchinson & Templeton 1999). Correlations were calculated for all populations, only Great Plains populations, and only Midwestern populations using θ_{ST}, R_{ST}, 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 entirely a result of the relatively large genetic and geographical distance separating the eastern 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 geographical distance was not apparent in these data.

Table 3 The number of alleles per locus (above) and the observed/expected heterozygosity (below) for each population

Population	Locus 13	Locus 17	Locus 18	Locus 31
GP–KS	24	16	26	30
	0.74/0.95	0.95/0.91	0.82/0.95	0.93/0.97
GP–NE1	27	13	30	26
	0.78/0.96	0.75/0.86	0.89/0.96	0.89/0.96
GP–NE2	21	18	24	21
	0.48/0.94	0.89/0.92	0.85/0.97	0.76/0.96
GP–SD	23	14	25	24
	0.68/0.96	0.81/0.92	0.84/0.98	0.81/0.96
GP–MO	22	12	28	29
	0.82/0.96	1.00/0.77	0.58/0.96	1.00/0.96
MW–WI2	13	17	20	19
	0.52/0.88	0.91/0.92	0.73/0.94	0.83/0.95
MW–WI1	20	13	16	22
	0.92/0.94	0.96/0.80	0.88/0.93	0.97/0.94
MW–IL2	14	12	30	22
	0.80/0.90	1.00/0.76	0.81/0.96	0.82/0.92
MW–IA	16	8	18	13
	0.79/0.92	0.38/0.819	0.71/0.94	1.00/0.92
MW–IL1	17	14	7	12
	0.79/0.94	0.92/0.92	0.36/0.66	0.80/0.89
E–PA	8	7	14	7
	1.00/0.82	0.76/0.80	0.72/0.91	1.00/0.88

Genetic diversity

Levels of allelic variation were consistent with smaller population sizes for Midwestern populations when compared to Great Plains populations ($N = 20$, mean ± SE = 16.15 ± 5.26 and 22.65 ± 5.54, respectively, averaged across loci) (Table 3). Allelic diversity was also lowest in the Pennsylvania population ($N = 4$, mean ± SE = 9.0 ± 3.37, averaged across loci) (Table 3). Levels of expected heterozygosity were lower in Midwestern than in Great Plains populations and were lower again in the eastern population ($N = 20$, 20, and 4, mean ± SE = 0.939 ± 0.048, 0.892 ± 0.076, 0.852 ± 0.051 for Great Plains, Midwestern and Eastern populations, respectively) (Table 3). However, the observed levels of heterozygosity were not always consistent with patterns of expected heterozygosity, had higher variance, and were typically lower than expected

levels based on Hardy–Weinberg equilibrium (N = 20, 20, and 4, mean ± SE = 0.813 ± 0.129, 0.795 ± 0.184, 0.870 ± 0.151) (Table 3).

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 & Barrowclough 1987; Templeton *et al*. 1990; Frankham 1995; Harrison & Hastings 1996; 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 & Hastings 1996; Hutchinson & Templeton 1999). Second, isolated populations may be more likely to experience population bottlenecks, which in turn leads to reduced genetic variability (Wilcox & Murphy 1985; Saunders *et al*. 1991; Frankham 1995; 1998b; Bouzat *et al*. 1998a; 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 & Charlesworth 1987; Frankham 1995; Spencer *et al*. 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 & Moritz 1998; Barratt *et al*. 1999; Clark *et al*. 1999; Seppa & Laurila 1999; Bates 2000; Wolf *et al*. 2000; Vucetich *et al*. 2001) although more commonly, studies focused on recent, anthropogenic habitat fragmentation at relatively small geographical scales (e.g. Gaines *et al*. 1997; Peacock & Smith 1997; Aldrich *et al*. 1998; Gibbs 1998; Van Dongen *et al*. 1998; Dayanandan *et al*. 1999; Gerlach & Musolf 2000; Knutsen *et al*. 2000; Mossman & 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 are 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 & Smith 1997; Bouzat *et al*. 1998b; Gibbs 1998; Van Dongen *et al*. 1998; Gerlach & Musolf 2000; Knutsen *et al*. 2000; Mossman & 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 & Strauss 1998).

High gene flow species, on the other hand, may require extensive gene flow among populations 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 geographical 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 (Table 2). The pattern was also observed regardless of whether or not the method of analysis made assumptions concerning the underlying mutational process observed in microsatellite loci (Table 1). Clearly, habitat fragmentation has disrupted the level of gene flow observed among contemporary Midwestern populations of *Speyeria idalia*. Note that the absolute value of differentiation was low; for example, among Midwestern populations the average θ_{ST} was 0.049 (Table 2). If one was willing to accept the assumptions associated with estimating migration rates from F_{ST} (Wright 1943; Bossart & Prowell 1998; Templeton 1998; Waples 1998; Whitlock & 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 the observed shared ancestry (Williams 2002). 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 (2002) 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 2002) 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 (Table 3). 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. Alternative explanations for the low levels of observed heterozygosity include the presence of null alleles, selection at linked loci, and inbreeding among individuals across most populations. Virtually nothing is known about inbreeding/outbreeding levels for *S. idalia*, or the molecular genetics of these markers, so more data will be required to resolve the issue.

Conservation implications

The effect of fragmentation on populations of *S. idalia* also has management implications. 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 & McCorkle 1984; Nagel *et al*. 1991; Swengel 1997; Debinski & Kelly 1998; Kelly & Debinski 1998). *Speyeria idalia* is dependent 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 a result of 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 & Bragg 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 & Bragg 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 because of 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* to maintain the continued existence of this species in the Midwest.

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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.