Conservation Genetics of the Dakota Skipper (*Hesperia dacotae*)

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Abstract: A range-wide genetic survey of Dakota skipper (*Hesperia dacotae*) populations was carried out to assess levels of genetic variability and geographic scale of population structure in this species of conservation concern. The Dakota skipper is on the Threatened Species list in the state of Minnesota and the Province of Manitoba, Canada, and is currently being considered for US federal protection. It exist on isolated patches of native tall-grass prairie within a highly modified landscape dominated by agriculture. It has been extirpated in the southern portion of its range in Iowa and has suffered range-wide declines.

Nine populations were sampled as follows: five from western Minnesota, two from eastern South Dakota, and two from central Manitoba. The sampling protocol was designed to minimize the demographic effects of removing individuals from these populations. Males were hand-netted and frozen in liquid nitrogen for later analysis. All netted females were released immediately. Proteins were extracted via standard methods and starch gel electrophoresis was carried out on 281 Dakota skippers. Twenty-one allozyme loci were resolved for this study.

Dakota skipper populations were found to be approximately as variable as other lepidopterans with highly fragmented habitats. Mean individual heterozygosity and percentage of loci polymorphic were generally lower in the Dakota skipper than in other lepidopterans (all butterflies) that exist in more continuous habitat. Genetic distances indicated that Manitoba populations were somewhat distinct from the southern ones in Minnesota and South Dakota. Isolation-by-distance was detected range-wide and among the seven southern-most populations in Minnesota and South Dakota. Genetically effective immigration rates were small at both range-wide and regional scales. Effective populations sizes were shown to be low in the sampled populations. These results suggest that Dakota skipper populations are genetically isolated from one another, although they were likely more connected in the recent past. The results also suggest that genetic drift is an important structuring force in these populations. Significant heterozygote deficiencies relative to Hardy-Weinberg expectations and high inbreeding coefficients suggest small-scale structure within sample locations.

Management recommendations include the maximization of effective population size in each Dakota skipper population to offset the effects of drift. Habitat corridors to enhance gene flow between nearby populations may be an option for the Hole-in-the-Mountain, Prairie Coteau, and Starbuck, MN, populations. Habitat management should consider the small-scale (within site) population structure and possible temporal population structure detected in this study by further investigating within-site movements and maintaining all potentially suitable habitat at Dakota skipper sites.

Introduction

The Dakota skipper (Lepidoptera: Hesperidae: *Hesperia dacotae*) is a northern tall-grass prairie obligate species that once occupied an area from southern Iowa, through the eastern Dakotas and western Minnesota, north into Manitoba, Canada (Scott 1986, Figure 1). Details of its ecology can be found in Dana (1991) and Scott (1986). The species has experienced severe habitat fragmentation due largely to agricultural conversion. Dana (1991) cites estimates that indicate that less than two percent of the Dakota skipper's pre-European settlement habitat remains. The species probably has been extirpated from southern parts of its range in Iowa (Dana 1991). Populations that remain exist on remnant patches of native prairie habitat on state, provincial, private, and Nature Conservancy holdings.

The Dakota skipper is on the state Threatened Species List in Minnesota and the provincial Threatened Species List in Manitoba. It has been considered for listing as Threatened under the US Endangered Species Act and was a Category 2 species as of 1984 (Dana 1991). Currently, its Federal status is under review (R. Peterson, pers. com.).

The population genetic effects of habitat fragmentation for the Dakota skipper were investigated for this report. These effects are well understood theoretically and have been observed in a variety of taxa (e.g., Frankel and Soulé 1981, Allendorf and Leary 1986, Hedrick and Miller 1992, Avise 1996). Populations on isolated habitat patches are expected to exhibit several genetic characteristics. Estimates of genetically effective population sizes are expected to be small in isolated populations and this will enhance the effects of genetic drift. Genetic drift reduces the number of alleles in populations and decreases mean individual heterozygosity. Thus, isolated populations that experience drift are expected to be deficient in the frequency of heterozygous individuals relative to Hardy-Weinberg expectations and to have elevated inbreeding coefficients. The genetic structure of populations will also be effected by habitat patchiness. Gene flow between populations will be reduced in a fragmented landscape leading to population differentiation. Currently isolated populations may show isolation-by-distance (Slatkin 1993) in which there is a negative correlation between geographic distances between populations and their genetic similarity. This can be the case for currently isolated populations that, like the Dakota skipper, once occupied continuous habitat (Slatkin 1993). Population isolation leads to genetic differentiation such that more geographically separate populations are expected to be the most divergent genetically.

Starch-gel electrophoresis of proteins was used to estimate levels of genetic variability and population structure in the Dakota skipper across its entire current geographic distribution. Population structure was investigated at three levels: range-wide, regional, and within habitat patch. The implications of these results for the management of the Dakota skipper are discussed below.

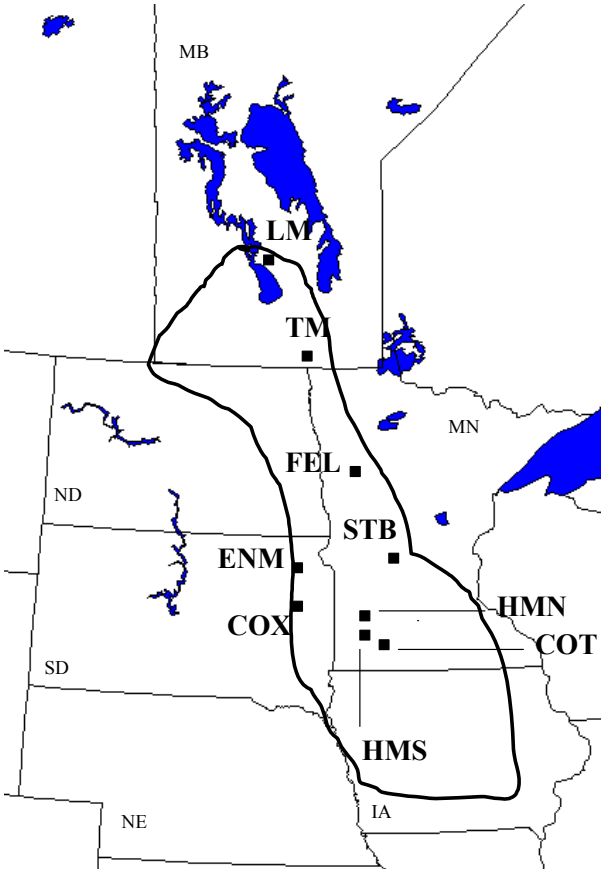

Figure 1. Map of the historic distribution of *Hesperia dacotae* (adapted from Scott 1986) and collection localities.

Materials and Methods

Field Collection

Potential collection sites for Dakota skippers were identified in Minnesota from previous survey efforts (Dana 1991, R. Baker, pers. com). Potential South Dakota and Manitoba sites were identified via personal contact with individuals familiar with Dakota skipper distributions in these locations (R. Royer and P. Klassen). Several potential collection sites were identified in North Dakota for collection in 2001, but population sizes at these locations were apparently too small to sustain sufficient collecting (R. Royer, pers. comm.). Collection protocols were developed with the help of MN DNR personnel to minimize the demographic impacts of sampling on target populations while providing statistically appropriate numbers of individuals for genetic analysis. The collection protocol was as follows:

1) All collecting was done by hand netting.
2) Only males were collected. Any females unintentionally netted were released immediately.
3) Males were placed in glassine envelopes. The first 20 males netted were held in a cooler. The time spent and area covered in collecting these first males was noted. If the effort needed to collect the first 20 males was judged to be excessive, collecting ceased and all captured males were released.
4) An additional 10 males were collected. If the time spent collecting the last 10 males exceeded the length of time needed to collect the first 20, all skippers were released.
5) Once collected, all males were placed in a 5l Dewar flask containing liquid nitrogen for transport to University of South Dakota. Skippers were stored in an ultracold freezer (-80ºC) until processed for gel electrophoresis.

Collection dates and locations of all sites surveyed for Dakota skippers (Figure 1) are given in Table 1.

Electrophoretic Analysis

Dakota skippers were processed in batches of five or six. The entire protein extraction process was carried out on ice. Wings were removed from each skipper and placed in a wing envelope containing a collection site code and a unique specimen number. The whole body was placed in a labeled 12 mm X 75 mm disposable glass culture tube to which was added 0.25 ml cold extraction buffer (0.05 M tris HCL, pH 7.0, see May 1992). Each skipper was crushed in the cold buffer with a teflon rod that was rinsed and wiped dry between each skipper. The tubes were then stored at 4°C for 15 – 20 min. Cold tubes were then centrifuged at 12,000 rpm for 5 min. Supernatant containing the extracted water-soluble proteins was pipetted from each tube into a 1.5 ml microcentrifuge tube labeled with collection site and specimen number. Culture tubes containing the solid fraction were discarded. Microcentrifuge tubes were put into labeled racks that were stored at -80°C until electrophoretically analyzed.

Table 1. *Hesperia dacotae* collection and survey locations (site code), dates, and collector identification.

Year	Date	Location (code)	Latitude	Longitude	Number collected	Collector*
1998	7/7	Prairie Coteau SNA (COT)	45.5N	95.5E	30	JG
	7/5 & 7/7	Hole-in-the Mountain South(HMS)	44.2N	96.3E	20	JG
	7/2	Hole-in-the-Mountain North (HMN)	44.2N	96.3E	30	JG
	7/8 & 7/9	Chippewa Prairie	45.2N	96.0E	0	JG
		Felton Prairie SNA (FEL)	47.0N	95.5E		
	7/12	Bluestem Unit			10	JG
	7/13	Bicentennial Unit			10	JG
	7/13	Felton Prairie			20	JG
		Starbuck (STB)	45.5N	95.5E		
	7/9	R. Eversman Property			3 (released)	JG
	7/16				4 (released)	JG
	7/21				0	JG
	7/21	R. Anderson Property			0	JG
1999		Starbuck (STB)				
	7/2	R. Anderson Property			28	JG
	7/5	Enemy Swim Lake, SD (ENM)	45.4N	97.3E	44	JG
	7/6	Cox WPA, SD (COX)	44.7N	95.5E	29	JG
2000	6/29 - 7/1	Vicinity of Tolstoi, MB	49.1N	96.8E	12 (released)	HB
	6/30	Vicinity of Stuartburn, MB	49.2N	96.7E	0	HB
	7/1	Tolstoi, MB (TM)	49.1N	96.8E	28	HB
	7/1	Lundar, MB (LM)	50.7N	98.1E		
		East			9	HB
	7/2	East			23	HB
	7/1 - 7/3	Airport			0	HB

* JG = Joseph Glasford, HB = Hugh Britten

Extracted proteins were thawed in their microcentrifuge tubes and applied to 5 mm X 10 mm filter paper (Whatman no. 3) wicks that were inserted into a slot cut into a 14% (starch weight to buffer volume) Sigma starch gel. Gels typically contained 20 individual skipper samples and three or four previously run samples as scoring standards. Gels were connected to power supplies using wicks soaked in the appropriate electrode buffer (May 1992). Gels were run four to six hours at 75 milliamps (approximately 200 volts). Progress of the run was tracked using a blue food-coloring marker placed at the origin with the samples. Each gel was sliced horizontally upon completion of the run yielding five to six slices per gel. Each slice was stained for a specific protein (isozyme) using standard histochemical staining procedures (May 1992). Table 2 provides a list of the enzyme loci resolved in this study and the buffers used for each. Individual genotypes at each isozyme locus were obtained by direct count of phenotypes (electromorphs) on the gels, with a common electromorphs being scored as "C," faster ones scored as "B," then "A," slower ones being scored as "D," etc.

Data Analysis

Genotypic frequency data from Minnesota and South Dakota skipper populations were initially analyzed using BIOSYS-1 (Swofford and Selander 1981). These analyses provided summary statistics for these seven populations including estimates of allele frequencies, percent polymorphic loci, mean individual heterozygosity and tests for conformance to Hardy-Weinberg equilibrium, F-statistics, and genetic distances (Nei 1973) between all pairs of populations. An UPGMA phenogram based on genetic distances was also estimated. This analysis shows patterns of genetic similarity among the sampled populations on a tree-like diagram.

The final dataset, including two samples from Manitoba, was analyzed using Tools for Population Genetic Analysis (TFPGA, Miller 1997). TFPGA supports the analyses listed above from BIOSYS-1, but also includes the capability to calculate confidence intervals for Hardy-Weinberg expectations and F-statistics via bootstrap resampling. TFPGA uses the formulations of Weir (1996) to calculate θ, a multi-locus form of F_{st}. TFPGA also calculated bootstrap values for the nodes on the UPGMA phenogram. These provide a means of evaluating the robustness of each branchpoint on the tree. Data from all three years of sampling were used for all analyses under the assumption that allele frequencies do not substantially change in one generation, except in populations with very small genetically effective sizes (Hartl and Clark 1997).

TFPGA was also used to carry out Mantel tests (Manly 1991) for isolation-by-distance (Slatkin 1993) on the entire dataset and on the Minnesota and South Dakota data excluding the Manitoba data. In this test, all pairwise genetic distances are used as the dependent variable in a regression analysis with pairwise geographic distances as the independent variable. A significant regression suggests a pattern of decreasing genetic similarity among Dakota skipper populations with increasing distance between them. The Mantel test, as implemented in TFPGA, randomizes the two distance matrices and recalculates the regression analysis 1,000 times. A *p*-value for the relationship between

Table 2. Isozyme loci assayed in *Hesperia dacotae* populations including Enzyme Commission numbers and buffer systems used.

Locus	Enzyme Name	EC Number	Buffer[1]
AAT (1,2)	Aspartate aminotransferase	2.6.1.1	C, R
GAPDH	Gylceraldehyde-3-phosphate dehydrogenase	1.2.1.12 4	4
GPI	Glucose phosphate isomerse	5.3.1.9	R
G3P	Glycerol-3-phosphate dehydrogenase	1.1.1.8	R
G6PDH	Glucose-6-phosphate dehydrogenase	1.1.1.49	4
HBDH	Hydroxybuterate dehydrogenase	1.1.1.30	C
IDH (1,2)	Isocitrate dehydrogenase	1.1.1.42	4
LDH	Lactate dehydrogenase	1.1.1.27	C
MDH (1,2)	Malate dehydrogenase	1.1.1.37	4
ME (1,2)	Malate dehydrogenase ($NADP^+$)	1.1.1.40	4
MPI	Mannose phosphate isomerase	5.3.1.8	4
PEPLA (1,2)	Peptidase (leucyl-alanine)	3.4.13.-	R
PGD	Phosphogluconate dehydrogenase	1.1.1.43	4
PGM	Phosphoglucomutase	5.4.2.2	C
SOD (1,2)	Superoxide dismutase	1.15.1.1	C,4

[1]Buffer systems from May 1992.

genetic and geographic distances is assigned based on the distribution of *Z*-scores from the 1,000 bootstrap runs. The Mantel test is necessary in analyzing pairwise data such as these because each population's allele frequencies are used in several (six for the dataset excluding Manitoba data and eight for the overall dataset) pairwise comparisons, thus violating the assumption of data independence required to assign *p*-values in standard regression analyses.

Finally, I used MIGRATE (Beerli and Felsenstein 1999, Beerli and Felsenstein 2001) to estimate effective population sizes and genetically effective immigration (*Nm*) for the nine sampled Dakota skipper populations included in the study. This method uses a randomization technique in conjunction with coalescent theory to estimate asymmetric immigration rates for all pairs of populations in a study. That is, the number of genetically effective migrants coming into each population from the other eight populations can be individually estimated. Previous methods based on estimates of F_{st} assumed that the exchange, in numbers of migrants, between pairs of populations is equal. MIGRATE is not constrained by this assumption. MIGRATE was run three times with the same set of start conditions (i.e., 500 trees sampled, 100 trees recorded, 2 short chains and 3 long chains used for the Markhov chain settings). CPU time varied from 24 – 26 hours per run. Effective population sizes and immigration rates for each skipper population were averaged over the three runs.

Results

Field Collection

A total of 281 Dakota skippers collected over three years was included in the genetic analyses. Skippers previously reported as Dakota skippers collected by J. Glasford from Chippewa Prairie, MN, in July 1998 (Progress Reports to MN DNR 12/17/98 and 3/01) proved to be mostly Delaware skippers (*Atrytone logan*) and were not included in the genetic analyses. Table 1 provides dates, locations, numbers collected, and collector identification for Dakota skippers during the study. Most successful collecting occurred in early July. Sites on private land near Starbuck, MN, reported to support Dakota skippers did not provide adequate samples in 1998, but did in 1999 (Table 1). Similarly, several sites in the vicinity of Tolstoi, MB, were surveyed in late June and early July 2000 but did not provide enough Dakota skippers to meet the collection protocol (Table 1). The few Dakota skippers that were netted from these sites were released. The Tolstoi, MB, population required the most sampling effort among the sites sampled for this study. Approximately five kilometers of continuous linear habitat along an abandoned railway right-of-way was sampled during collection at this site. Few female Dakota skippers were encountered during the study and all were released upon removal from the net.

Protein Electrophoresis – Genetic Variability

A total of 21 isozyme loci was resolved for this study. Table 2 provides a list of these loci and the electrophoretic conditions used to assay them. All but one locus (G3P, Table

3) showed at least one alternative allele segregating in at least one population. Ten low-frequency unique alleles, alleles found segregating in only one population, were found with frequencies ranging from 0.0116 to 0.0682 (Table 3). Across all populations, 48 percent of the loci were polymorphic using the 95% criterion (i.e., a locus is not considered polymorphic unless the frequency of the most common allele is < 0.95). Polymorphism estimates for individual populations ranged from 24% at Starbuck, MN, to 52% at Lundar, MB (Table 3). Observed mean individual heterozygosity ranged from 0.07 at Lundar and Tolstoi, MB, to 0.11 at Hole-in-the Mountain North, MN (Table 3). Mean individual heterozygosity as expected under Hardy-Weinberg equilibrium was higher than the observed heterozygosity in all populations and ranged from 0.11 for Starbuck, MN, to 0.18 for Lundar, MB (Table 3).

Protein Electrophoresis – Large-Scale Structure

Mean F_{st} (calculated as Weir's [1996] θ) was 0.32 (0.12 – 0.54, 95% CI). This value accounted for an unexpectedly low proportion of F_{it} which was estimated at 0.52 (0.35 – 0.70, 95% CI). Nei's (1978) unbiased genetic distances between all pairs of populations are given in Table 4 along with all pairwise geographic distances. Genetic distances range from low values between MN and SD populations (e.g., 0.0007 between HMN and HMS, Table 4) to high values between southern populations and the two Manitoba populations (e.g., 0.1942 between ENM and TM, Table 4). The UPGMA phenogram (Figure 2) shows the genetic similarity among the southern seven sampled populations and greater dissimilarity with the two Manitoba populations. Additional inferences about genetic relationships based on the UPGMA phenogram should be made with caution, however, as the bootstrap values suggest that most of the nodes have little statistical support. Specifically, the pattern of genetic similarity among the Minnesota and South Dakota populations cannot be discerned with confidence from Figure 2. The node that places Felton Prairie with the rest of the Minnesota and South Dakota populations has the highest bootstrap support among these populations at only 61%. By convention, a node should have at least 70% bootstrap support to be considered robust. In contrast, the node separating Manitoba populations from the southern ones has 99% bootstrap support and the node that places the two Manitoba populations together has 95% bootstrap support. Two Mantel tests were performed to investigate the relationship between geographic and genetic distances for these populations. This technique revealed an overall pattern of isolation-by-distance in the entire dataset ($r = 0.82$, $p = 0.017$, Figure 3). Similarly, a pattern of isolation-by-distance was revealed on a finer geographic scale among the populations sampled in Minnesota and South Dakota, excluding the Manitoba populations ($r = 0.70$, $p = 0.016$, Figure 4).

MIGRATE (Beerli and Felsenstein 1999, Beerli and Felsenstein 2001) provided estimates of effective population sizes and immigration for each population surveyed. MIGRATE reports effective population sizes, N_e, as θ (not to be confused with Weir's [1996] multi-locus F_{st} estimate) which is estimated as $\theta = 4N_e\mu$, where μ = per locus mutation rate. An average of θs from the three MIGRATE runs was used for interpretation as suggested in the software documentation (see Beerli and Felsenstein 2001). The average estimates of θ ranged from 0.35 for Tolstoi, MB, to 0.73 for Lundar,

Table 3. Allozyme frequencies, sample sizes (N), percentage of loci polymorphic (%P), Hardy-Weinberg expected heterozygosity (H_e), and observed heterozygosity (H_o) for sampled *H. dacotae* populations. Names and locations of populations are given in Table 1. Bolded allele frequency denotes a unique allele.

	Population								
Locus	STB	COX	FEL	ENM	HMS	HMN	COT	TM	LM
AAT1									
B	0.0156	0.0345	0.0000	0.0114	0.0200	0.0000	0.0526	0.6200	0.0000
C	0.8594	0.8276	0.8462	0.9091	0.9000	0.8542	0.7632	0.3800	0.9219
D	0.1094	0.1379	0.1410	0.0682	0.0800	0.1458	0.1842	0.0000	0.0781
E	0.0156	0.0000	0.0128	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000
AAT2									
B	0.0345	0.0263	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000	0.0000
C	1.0000	0.9655	0.9737	1.0000	1.0000	0.9828	1.0000	1.0000	0.9677
D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0161**
E	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0161**
GAPDH									
B	0.0000	0.0000	0.0000	0.0000	**0.0179**	0.0000	0.0000	0.0000	0.0000
C	1.0000	1.0000	1.0000	0.9884	0.9821	1.0000	1.0000	1.0000	1.0000
D	0.0000	0.0000	0.0000	**0.0116**	0.0000	0.0000	0.0000	0.0000	0.0000
GPI									
A	0.0625	0.0690	0.0385	0.0227	0.0179	0.0000	0.0000	0.4000	0.5469
B	0.0625	0.1034	0.0513	0.0227	0.0893	0.1071	0.0200	0.5600	0.4531
C	0.7344	0.7414	0.8590	0.8664	0.8393	0.8750	0.8800	0.0400	0.0000
D	0.1406	0.0690	0.0513	0.0568	0.0536	0.0179	0.1000	0.0000	0.0000
E	0.0000	0.0172	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000
G3P									
C	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3, continued.

G6PDH									
B	0.0000	0.0000	0.0000	0.0000	0.1731	0.0862	0.0000	0.3750	0.1000
C	1.0000	1.0000	1.0000	0.9773	0.8269	0.9138	1.0000	0.5500	0.8667
D	0.0000	0.0000	0.0000	0.0227	0.0000	0.0000	0.0000	0.0750	0.0333
HBDH									
B	0.0000	0.0000	0.0128	0.0116	0.0000	0.0000	0.0208	0.0000	0.1053
C	0.9844	0.9655	0.8590	0.8140	0.8654	0.7955	0.9167	1.0000	0.8947
D	0.0156	0.0345	0.1282	0.1512	0.1346	0.1364	0.0625	0.0000	0.0000
E	0.0000	0.0000	0.0000	0.0233	0.0000	0.0682	0.0000	0.0000	0.0000
IDH1									
B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208	0.0400	0.0000
C	0.9688	0.9286	0.9744	0.8636	0.9259	0.9310	0.9400	0.9200	0.8387
D	0.0312	0.0714	0.0256	0.1136	0.0741	0.0690	0.0400	0.0400	0.1613
E	0.0000	0.0000	0.0000	**0.0227**	0.0000	0.0000	0.0000	0.0000	0.0000
IDH2									
B	0.0162	0.0000	0.0000	0.0114	0.0000	0.0172	0.0208	1.0000	0.6875
C	0.9839	0.9655	1.0000	0.9886	0.9815	0.9828	0.9792	0.0000	0.3125
D	0.0000	0.0345	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000
LDH									
B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0455	0.9600	0.8333
C	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8409	0.0400	0.1333
D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.0000	0.0333
MDH1									
A	0.0312	0.0000	0.0256	0.0000	0.0185	0.0000	0.0000	0.0200	0.0000
B	0.0156	0.0345	0.0256	0.0341	0.0185	0.0172	0.0200	0.9400	0.9375
C	0.9531	0.9483	0.9487	0.9659	0.9630	0.9655	0.9800	0.0400	0.0625
D	0.0000	0.0172	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000

Table 3, continued.

MDH2									
C	1.0000	0.9828	1.0000	1.0000	1.0000	0.9828	0.9600	1.0000	1.0000
D	0.0000	0.0172	0.0000	0.0000	0.0000	0.0172	0.0200	0.0000	0.0000
E	0.0000	0.0000	0.0000	0.0000	0.0000	0.0200	0.0200	0.0000	0.0000
ME1									
B	0.0000	0.0000	0.0000	0.0227	0.0000	0.0000	0.0417	0.0000	0.0000
C	1.0000	1.0000	1.0000	0.9091	1.0000	1.0000	0.9583	1.0000	1.0000
D	0.0000	0.0000	0.0000	**0.0682**	0.0000	0.0000	0.0000	0.0000	0.0000
ME2									
C	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9800	1.0000	1.0000
D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0200**	0.0000	0.0000
MPI									
A	0.0167	0.0000	0.0132	0.0000	0.0000	0.0000	0.0000	0.0000	0.1552
B	0.0833	0.2407	0.3421	0.0357	0.0385	0.0714	0.1190	0.5435	0.4310
C	0.6000	0.7037	0.3158	0.6190	0.8269	0.6786	0.6905	0.3913	0.3276
D	0.2333	0.0556	0.3158	0.3095	0.1154	0.2143	0.1905	0.0652	0.0690
E	0.0667	0.0000	0.0132	0.0357	0.0192	0.0357	0.0000	0.0000	0.0172
PEPLA1									
B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0400	0.0000
C	1.0000	0.9828	1.0000	1.0000	1.0000	1.0000	1.0000	0.9600	1.0000
D	0.0000	**0.0172**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEPLA2									
B	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0172**	0.0000	0.0000	0.0000
C	1.0000	1.0000	1.0000	0.9884	1.0000	0.9483	1.0000	0.9400	0.8906
D	0.0000	0.0000	0.0000	0.0116	0.0000	0.0345	0.0000	0.0600	0.0313
PGD									
B	0.0000	0.0000	0.0000	**0.0114**	0.0000	0.0000	0.0000	0.0000	0.0000
C	0.9500	0.9483	0.9868	0.9773	1.0000	1.0000	1.0000	1.0000	1.0000
D	0.0500	0.0517	0.0132	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000

Table 3, continued.

PGM									
A	0.3125	0.2759	0.1447	0.2614	0.1250	0.1739	0.1400	0.6200	0.0345
B	0.1875	0.3621	0.2105	0.5000	0.2143	0.3696	0.2600	0.2600	0.3793
C	0.3750	0.2241	0.3816	0.1932	0.3214	0.3043	0.3400	0.1200	0.5517
D	0.0781	0.0690	0.2105	0.0114	0.2857	0.1522	0.1200	0.0000	0.0345
E	0.0469	0.0345	0.0395	0.0341	0.0536	0.0000	0.1400	0.0000	0.0000
F	0.0000	0.0345	0.0132	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SOD1									
C	1.0000	1.0000	1.0000	1.0000	0.9615	1.0000	1.0000	1.0000	1.0000
D	0.0000	0.0000	0.0000	0.0000	**0.0385**	0.0000	0.0000	0.0000	0.0000
SOD2									
C	1.0000	1.0000	1.0000	1.0000	0.9815	1.0000	1.0000	1.0000	1.0000
D	0.0000	0.0000	0.0000	0.0000	**0.0185**	0.0000	0.0000	0.0000	0.0000
N	32	29	39	44	28	29	25	25	32
%P	24	33	29	33	33	38	33	38	52
H_e	0.11	0.12	0.12	0.12	0.12	0.12	0.13	0.15	0.18
H_o	0.10	0.10	0.09	0.10	0.09	0.11	0.09	0.07	0.07

Table 4. Nei's (1978) unbiased genetic distances below diagonal and approximate geographic distances (km) above diagonal for nine *Hesperia dacotae* populations.

	STB	COX	FEL	ENM	HMS	HMN	COT	TM	LM
STB	---	157	167	139	75	74	67	415	450
COX	0.0016	---	229	79	85	84	91	525	710
FEL	0.0046	0.0069	---	224	207	206	213	250	440
ENM	0.0047	0.0040	0.0083	---	79	78	89	430	610
HMS	0.0046	0.0045	0.0091	0.0067	---	1	10	605	810
HMN	0.0027	0.0021	0.0048	0.0011	0.0007	---	11	585	790
COT	0.0013	0.0017	0.0049	0.0042	0.0027	0.0010	---	615	810
TM	0.1825	0.1761	0.1876	0.1942	0.1913	0.1892	0.1861	---	200
LM	0.1274	0.1244	0.1281	0.1373	0.1358	0.1322	0.1310	0.0388	---

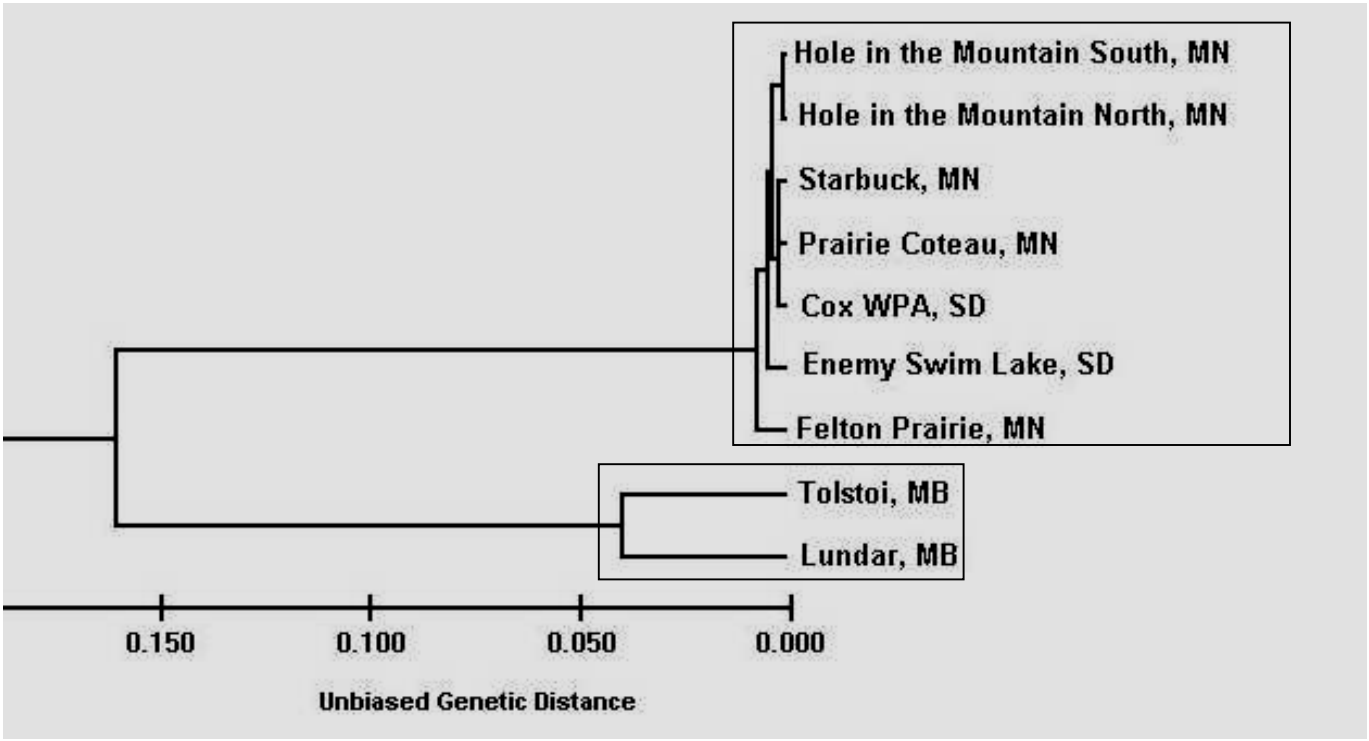

Figure 2. UPGMA phenogram based on Nei's (1978) unbiased genetic distances for nine *Hesperia dacotae* populations. Boxed branches are supported by bootstrap analysis at ≥ 95%.

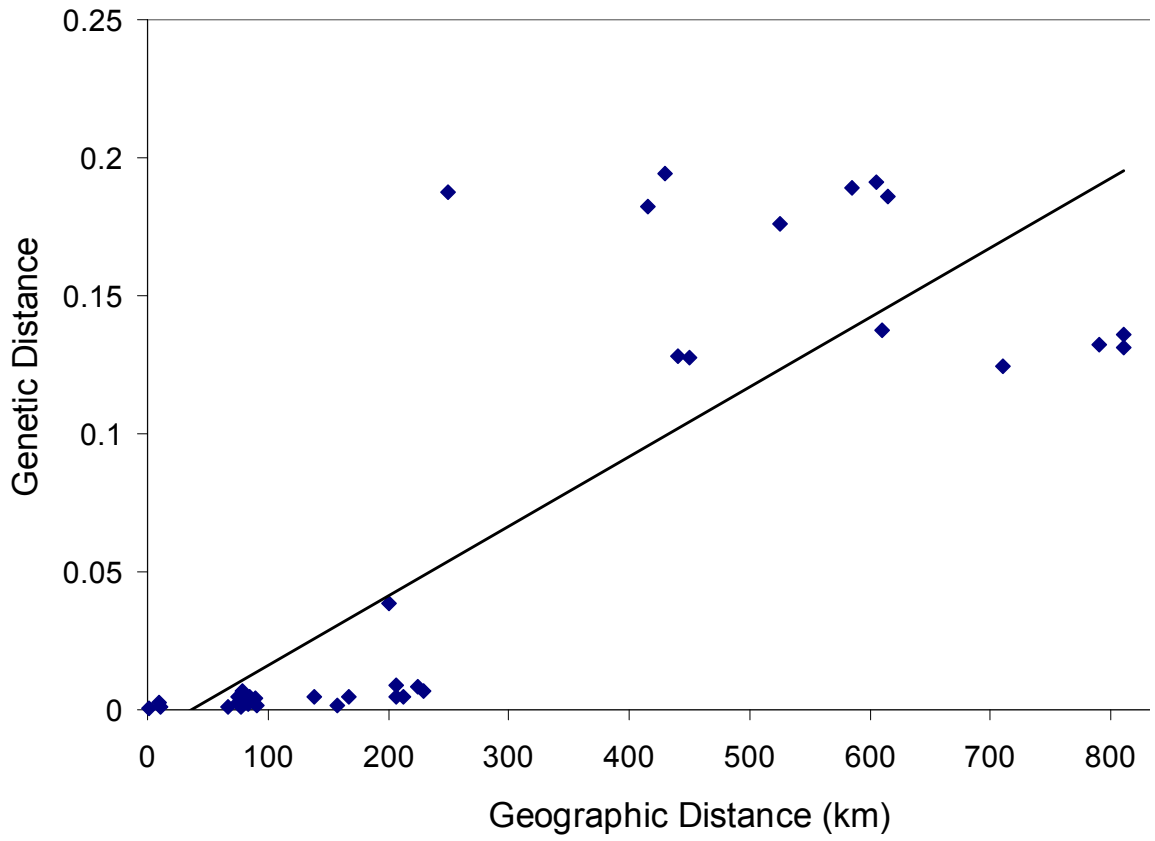

Figure 3. Pairwise unbiased genetic distances plotted against pairwise geographic distances for nine populations of *Hesperia dacotae* from Minnesota, South Dakota, and Manitoba. Standard regression line is shown as a trendline ($r = 0.82$, $p = 0.017$, Mantel test).

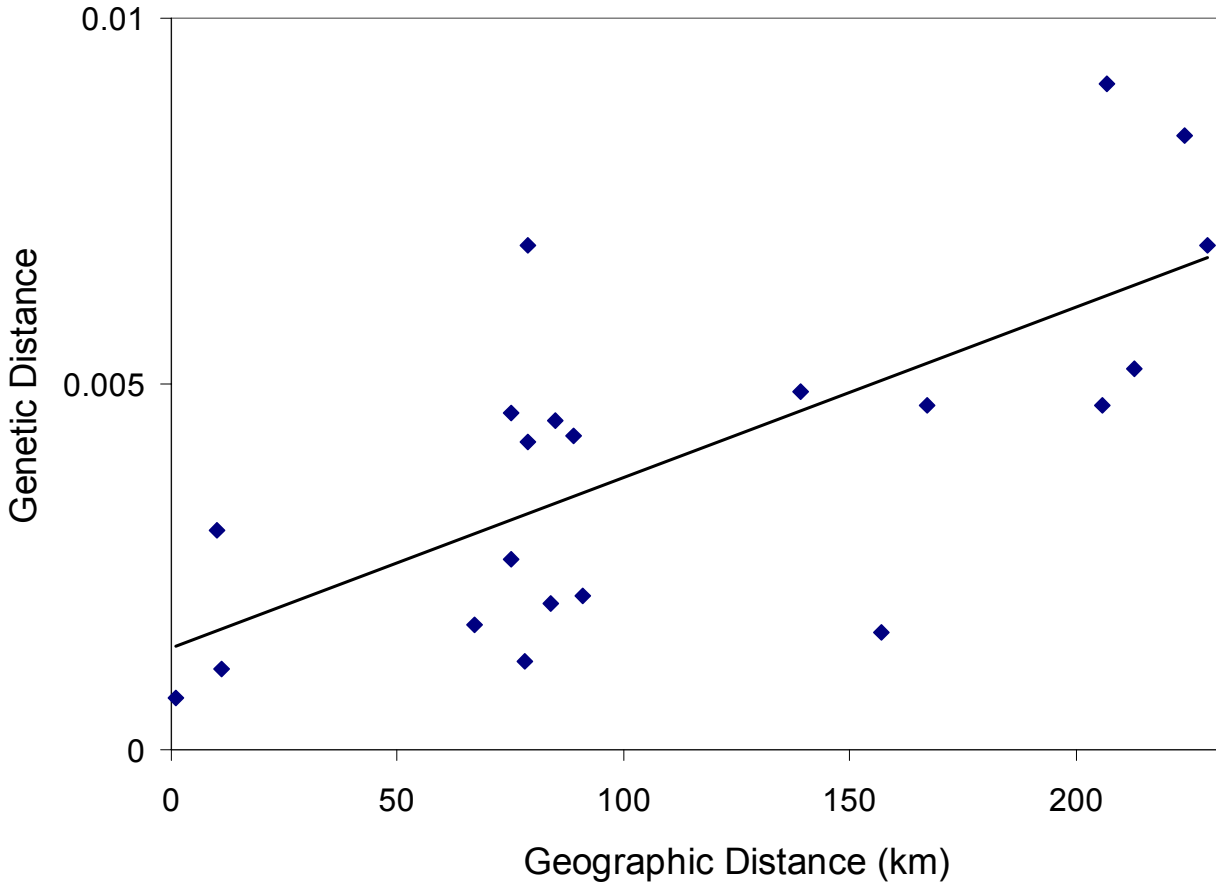

Figure 4. Pairwise unbiased genetic distances plotted against pairwise geographic distances for seven populations of *Hesperia dacotae* from Minnesota and South Dakota. Standard regression line is shown as a trendline ($r = 0.70$, $p = 0.016$, Mantel test).

MB. Thus, genetically effective population sizes for the Dakota skipper are small. Average estimates of genetically effective immigration (*Nm*) were all small as well (Table 5). All populations received on average fewer than one genetically effective migrant from the other eight populations each generation (Table 5) as estimated in MIGRATE. Interestingly, the highest immigration rate was from Tolstoi, MB, into Lundar, MB. Among the lowest immigration rates were those into the Tolstoi, MB, population, while those into the more northerly Lundar, MB, population were consistently higher (Table 5).

Table 5. Maximum likelihood estimates of gene flow (*Nm*) from source populations (columns) into receiving populations (rows) for nine populations of *Hesperia dacotae*.

	Source Population									
	STB	COX	FEL	ENM	HMS	HMN	COT	TM	LM	Mean
STB	----	0.53	0.68	0.83	0.56	0.70	0.57	0.49	0.64	0.63
COX	0.50	----	0.61	0.83	0.66	0.62	0.76	0.41	0.76	0.64
FEL	0.75	0.69	----	0.64	0.84	0.54	0.76	0.35	0.69	0.66
ENM	0.68	0.74	0.61	----	0.46	0.86	0.71	0.35	0.47	0.61
HMS	0.63	0.58	1.05	0.72	----	0.54	0.71	0.38	0.36	0.62
HMN	0.38	0.50	0.52	0.65	0.37	----	0.68	0.36	0.49	0.49
COT	0.68	0.59	0.73	0.73	0.56	0.69	----	0.24	0.37	0.57
TM	0.28	0.27	0.37	0.37	0.22	0.29	0.25	----	0.45	0.31
LM	0.74	0.71	0.65	0.85	0.44	0.81	0.87	1.48	----	0.82
Mean	0.58	0.58	0.65	0.70	0.51	0.63	0.66	0.51	0.53	----

Protein Electrophoresis – Small Scale Structure

The collection protocol used to minimize the demographic effects on sampling Dakota skipper populations largely precluded the collection of large enough samples to make within-site comparisons. Thus, I pooled the data from the three Felton Prairie, MN, subunits to provide a single statistically robust sample from that location. Although the Tolstoi, MB, sample was collected from an area that consisted of several kilometers of linear habitat, the total sample of 28 skippers was pooled into a single population for analysis.

Six of the nine sampled populations (COX, ENM, HMS, HMN, TM, and, LM) showed two or more loci out of Hardy-Weinberg equilibrium. All the deviations were heterozygote deficiencies. The remaining three populations (STB, FEL, and COT) all had one locus that was deficient in heterozygotes given Hardy-Weinberg expectations. F-statistics also suggest a pattern of within-site structuring. The inbreeding coefficient, F_{is}, accounted for a large proportion of the overall structure in these populations, F_{it} = 0.52 and F_{is} = 0.29. Furthermore, a comparison of the 95% CI around F_{is} (95% CI = 0.24 – 0.38) and F_{st} (95% CI = 0.12 – 0.54) suggests that the two estimates are statistically indistinguishable.

Discussion

Historically, two general arguments have been made for preserving genetic diversity in populations (see Britten and Baker 2001). First, evolution by natural selection cannot occur without heritable differences in fitness among individuals in populations. Populations can adapt to the selective pressure of a changing environment, if they have been able to gain and maintain the necessary genetic diversity. Second, the frequency and dispersion of genes within populations can record the history of populations over fairly long intervals of time. Patterns of isolation and changes in population sizes can often be reconstructed from present-day genetic data. These patterns can then inform management in ways that are not possible with real-time ecological study of the populations. These two arguments provide the rationale for managing populations to maximize genetic diversity (average heterozygosity and level of genetic polymorphism within populations). Although the preservation of genetic diversity may not be the direct goal of management, it is difficult to justify not taking the genetic implications of any given management plan into consideration. The goals of this study were to provide data on levels of genetic variability for the relatively under-studied Dakota skipper (*Hesperia dacotae*) and to infer the species' population structure, or the pattern of genetic connections among populations across the landscape. These patterns may reflect both present-day genetically effective movements of individuals (gene flow) and those in the recent past.

The significance of molecular (including allozyme) polymorphism and heterozygosity in the persistence of species is still in dispute (e.g., Lande 1988, Hedrick and Miller 1992). However, it is well accepted that isolated populations will experience a loss of neutral genetic variability such as was assayed in the current protein electrophoretic survey via random processes, collectively termed genetic drift (e.g. Hartl and Clark 1997). The selective neutrality of allozyme markers appears to be a general phenomenon across taxa (Britten 1996) and will be assumed herein. This assumption allows one to make inferences about population structure without the additional complication of natural selection.

Below, I will compare levels of allozyme heterozygosity and polymorphism as determined for the Dakota skipper in the current study with those of other Lepidoptera, some of which are philopatric and, like the Dakota skipper, confined to relatively isolated patches of suitable habitat in a fragmented landscape. These comparisons will provide

insight into the genetic implications of the Dakota skipper's current conservation status. Patterns of isolation-by-distance, genetic similarities among the sampled populations, and estimated levels of gene flow as determined in the present study will be discussed in the context of large-scale geographic structure of the Dakota skipper. F-statistics and levels of conformance to Hardy-Weinberg expectations will be discussed in the context of small-scale geographic structure in these populations. Finally, management implications of the results will be discussed.

Genetic variability

Hesperia dacotae populations sampled for this study have levels of variability similar to those found in other Lepidoptera that occur on isolated habitat patches (Table 3). For example, Britten et al. (1994a) sampled 13 Great Basin populations of *S. nokomis apacheana* and found of range of polymorphism estimates from 4 to 20 % and observed heterozygosity estimates from 0.014 to 0.044. Like *H. dacotae*, *S. n. apacheana* exists in discrete populations on isolated habitat patches. Britten et al. (1995) sampled 18 populations of the butterfly *Euphydryas editha* from montane habitat patches across the Great Basin and the Gunnison Basin in the Rocky Mountains. Estimated mean observed heterozygosity levels for these populations were from 0.03 to 0.09. Mean expected heterozygosity levels were estimated for two moth species (*Hyalophora euryalus* and *H. columbia*) sampled from a hybrid zone in the Sierra Nevada of California (Collins et al. 1993). The range of Hardy-Weinberg expected heterozygosity estimates was 0.03 to 0.093 for five populations of moths (Collins et al. 1993). *Hyalophora* spp. moths apparently exist in small populations in the area studied (Collins et al. 1993). The arctic/alpine butterfly *Boloria improba improba* occurs on somewhat discrete patches of its larval foodplant (*Salix* spp.) in western Canada. Britten and Brussard (1992) estimated that Canadian populations of *B. improba* had heterozygosity levels that ranged from 0.041 to 0.127 in relatively continuous habitat. A narrowly endemic supbspecies, *B. improba harryi*, which is found only in the Wind River Range of Wyoming, had lower observed heterozygosity (0.001 and 0.013) for two sampled populations (Britten and Brussard 1992). Its sister taxon, the narrowly endemic endangered butterfly *B. acrocnema*, was found to have an observed heterozygosity of 0.031 (Britten and Brussard 1992). Both *B. acrocnema* and *B.i. harryi* occur on isolated patches of *Salix nivalis*, whereas suitable habitat is apparently much less patchy for northern *B.i. improba.*

In contrast, Brittnacher et al. (1978) estimated heterozygosity for a number of taxa within the widespread butterfly genus *Speyeria* in California. Observed heterozygosity ranged from 0.067 in *S. atlantis* to 0.141 in *S. coronis coronis*. Finally, Brussard et al. (1989) did an extensive survey of butterflies in the *Euphydryas chalcedona* group in western North America. Mean heterozygosity estimates for these populations ranged from 0.17 to 0.26.

Large-scale population structure

Isolation-by-distance is expected when enough time has elapsed for genetic drift within populations to reach an equilibrium with gene flow among populations (Slatkin 1993). The equilibrium results in a balance between genetic drift that erodes genetic variability

(e.g., heterozygosity) in populations and gene flow among populations that tends to homogenize allele frequencies and prevent the loss of genetic variability.

Isolation-by-distance in the Dakota skipper was found among all nine populations and among the seven populations sampled in Minnesota and South Dakota excluding the Manitoba populations (Figures 3 and 4). This suggests that Dakota skipper populations exist in somewhat isolated populations of small genetically effective size. Dana (1991) found that Dakota skipper populations consisted of about 2,000 – 3,000 adults at Hole-in-the-Mountain North in 1979 and 1981. Estimates of genetically effective population size (N_e) for the Dakota skipper are consistent with this finding. Frankham (1995) found that the ratio of effective to census populations sizes (N_e/N) was in the order of 0.1 to 0.01 for a variety of organisms. These results were based on a review of several studies that used a variety of demographic and genetic methods to estimate N_e (Frankham 1995). The θs calculated in the present study by MIGRATE are likely to be several orders of magnitude less than N_e as they include an estimated average mutation rate for the allozyme loci assayed. This means that Dakota skipper N_es are likely at least two to three orders of magnitude greater than the θs. The number of genetically effective migrants exchanged between populations each generation is small enough to expect that genetic drift is the dominant force in structuring Dakota skipper populations on the scale of the entire study and for the southern populations alone (Mills and Allendorf 1996). Similar patterns of isolation-by-distance have been observed in other Lepidoptera with comparable geographic separation between populations (e.g., Britten et al. 1994a, Britten et al. 1995) and are attributable to historic isolation among populations. The UPGMA phenogram based on genetic distances did not show complete congruence with the geographic distribution of the sampled populations (Figure 2). The overall genetic similarity among Dakota skipper populations (i.e., small genetic distances, Table 4) in Minnesota and South Dakota did not allow a statistically robust branching pattern for these seven populations. The two Manitoba populations are clearly differentiated on the phenogram reflecting their greater geographic and temporal separation from the southern sites and from each other.

Small-scale population structure

Heterozygote deficiencies relative to Hardy-Weinberg expectations and high inbreeding coefficients (F_{is}) suggest population structure within sampled populations. Three possible explanations for these observations are discussed below. First, given a complete lack of population structure within sites, these results would indicate that Dakota skipper populations are inbred. Inbreeding can be a serious problem for populations as the increase in homozygosity that results can lead to inbreeding depression, or a decrease in general population performance (Hartl and Clark 1997). Second, there may be genetic structuring on a small (within site) geographic scale at Dakota skipper sample locations. Dana's (1991) study at Hole-in-the-Mountain North (HMN) suggested this possibility. Dakota skipper movement within four subsites at HMN (A, B, C, and D) were considerably more frequent than movement between the four subsites based on mark-release-recapture data (Dana 1991). Thus, individuals within each subunit are likely to be more closely related to individuals in the subunit than they are to individuals in the

other subunits. It was necessary during the current study to sample from all four HMN subsites and to pool these individuals into a single HMN sample. Given that individuals do not move as much between subsites as they do within them, the HMN population likely consisted of an unknown number of potentially genetically different subpopulations when they were sampled. I am assuming that this subpopulation structure is also present at the other Dakota skipper sites that were sampled for this study. Subpopulation structure can result in the Wahlund effect (Hartl and Clark 1997) in which subpopulations with slightly different alleles frequencies show heterozygote deficiencies compared to Hardy-Weinberg expectations when pooled into a single population for analysis. Finally, we may have encountered a "temporal Wahlund effect" during Dakota skipper sampling at these sites. Dakota skipper flight seasons last two to three weeks (Scott 1986, Dana 1991). Our sampling took place over one, or rarely, two days at each site. Thus, it is possible that close relatives (siblings and half-siblings) may have been disproportionately sampled during the relatively short sample periods as we inadvertently sampled family units (broods). Slightly different allele frequencies among broods sampled on any given day could have resulted in a Wahlund effect and lower than expected frequencies of heterozygous individuals in our samples.

Management recommendations

Several management recommendations are suggested by the genetic results. On a species-wide geographic scale these results show that Dakota skipper populations are approximately as genetically variable as other lepidopterans with similar patchy or fragmented habitats, several of which (e.g., *Speyeria nokomis apacheana*, *Boloria improba harryi*, *B. acrocnema*) are also of conservation concern. Some, possibly considerable, degree of population isolation is suggested by the low *Nm* estimates. Six of the nine sampled populations contain at least one unique allele not detected in the other populations (Table 3), again suggesting some degree of population isolation. In contrast, recent historical connections are clearly indicated by the small genetic distances between the southern seven populations sampled.

Results for the Manitoba populations point to historical connections among the two sites, most likely through a series of geographically intermediate populations. In addition, the two Manitoba populations are clearly distinct from the more southern populations, but may have been genetically connected to them through North Dakota. The Lundar, MB, population is of particular interest because it is at the northern edge of the species' range yet has apparently experienced strong genetic connections with other Manitoba populations and has the highest immigration rate noted in the study (from Tolsoi, MB, Table 5).

The isolation-by-distance noted for Dakota skipper populations also suggests historical connections across a landscape that once supported more continuous habitat. Given these results and those of Dana (1991), management should be based on the assumption that Dakota skipper populations are largely isolated from one another. Each separate population will thus experience genetic drift that will erode its genetic variability over time. Fairly small genetically effective population sizes will speed this process. Thus,

management should strive to maintain Dakota skipper populations at high (genetically effective) numbers at each site to ameliorate the erosive effects of genetic drift. This is particularly important for the more geographically isolated populations in Manitoba, but also for the two South Dakota populations, Felton Prairie, MN, and the sites near Starbuck, MN. Management for genetic connectivity between the two Hole-in-the-Mountain units and Prairie Coteau may be possible through the establishment of habitat corridors. These sites are geographically close (Figure 1), although gene flow estimates among the three are not exceptionally high (Table 4). A similar management strategy might be considered for the private properties near Starbuck, MN.

The apparent subpopulation structure within Dakota skipper populations at each site suggests several small-scale management strategies. First, field studies similar to Dana (1991) at other Dakota skipper locations could reveal habitat subunits that may support semi-isolated subpopulations. Subunits may be initially delineated based on the topography of each site. Second, once identified, each subunit should be managed in ways that maximize population size for all subunits while maintaining potential connectivity among subunits. Finally, the detection of a possible "temporal Wahlund effect" indicates that management activities such as burning that may cause Dakota skipper mortality should be carefully timed to impact the fewest breeding adults. Dana's (1991) study showed that, like many Lepidoptera, Dakota skipper phenology is somewhat annually variable. Peak flight times occur within a span of about 10 days in early July each year. This period is particularly important in maintaining genetic variability in Dakota skipper populations because the largest number of broods are represented in mating adults at this time. Any potentially disruptive management activities should be postponed until at least after the peak flight period, or preferably, after the entire flight period. At this point, the fewest broods will be impacted and the effective size of the (sub)populations will be maintained. Management impacts on larvae should be considered important at all times regardless of adult flight phenology.

Several observations were made during field work for this study that provided at least anecdotal evidence for making an additional management recommendation. A number of sites that were reported to contain Dakota skippers were visited over the three years of field work for this study that did not support adequate (or any) samples (Table 1). These included Chippewa Prairie, private property near Starbuck, MN, and several locations in southern Manitoba. Sites near Starbuck, MN, were found to contain Dakota skipper populations large enough to sample the following year (Table 1). These results may be due, in part, to the timing of collection relative to Dakota skipper phenology. On the other hand, they are consistent with the results from Dana (1991) that showed yearly fluctuations in population size. Similar fluctuations in annual population sizes have been shown in other lepidopterans. For example, Britten et al. (1994b) noted the apparent extirpation of *Boloria acrocnema* from its type locality in 1987 only to have it "reappear" in 1988. Likewise, *Speyeria nokomis apacheana* populations experienced turnover rates up to 30 percent in the Toiyabe Range of Nevada (Fleishman 1998). These events may be due to the cryptic presence of butterflies on the site during collection, or recolonization from unknown source populations. In any case, these observations imply

that once occupied, but currently "vacant," habitat patches should be suitably maintained for the Dakota skipper for a number of years in case reoccupation is possible.

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