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RECOGNITION OF WESTERN POPULATIONS OF *SPEYERIA IDALIA* (NYMPHALIDAE) AS A NEW SUBSPECIES

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ABSTRACT. Western populations of *Speyeria idalia* are described as separate subspecies, ***S. idalia occidentalis*, new subspecies.** Eastern and western populations can be diagnosed morphologically by differences in the size of spots on the underside of the hindwings. Furthermore, mitochondrially encoded cytochrome oxidase I and II genes reveal five synapomorphies for an extant eastern population in Pennsylvania, indicating a unique genetic diversity possessed by this population. Recognition of subspecies status for the eastern population may lead to a petition for an emergency listing under the Endangered Species Act of 1973.

Additional key words: Mitochondrial DNA, morphology, conservation, taxonomy.

Taxonomy within the genus *Speyeria* Scudder has long been a troublesome issue among lepidopterists (dos Passos & Grey 1945, 1947, Moeck 1957, Arnold 1983). No consensus has been reached on the number of species within the genus and relationships among subspecies are even less well resolved (Howe 1975, Arnold 1983, Hammond 1985, Scott 1986). Confusion among taxa stems from numerous examples of polymorphism, sexual dimorphism, convergence and clines (Hovanitz 1941, 1943, Moeck 1957, Rindge 1987, Grey 1989, Hammond 1991). Most of the variation within the genus is found among western North American species and subspecies, whereas eastern taxa are perceived as relatively well resolved. However, because previous research has been focused on the problematic western species, intraspecific variation in the eastern fauna may have been overlooked.

Speyeria idalia (Drury) is an example of an eastern species that has not been an issue of contention (Moeck 1957, Hovanitz 1963). Because *S. idalia* is so easily identified among *Speyeria* species, intraspecific variation may not have been thoroughly examined. The original range of *S. idalia* extended from the plains of North Dakota and Colorado, east to Virginia and Maine (Howe 1975, Scott 1986). However, within the last century, populations of *S. idalia* have been extirpated over most of the species' range due to habitat destruction (Hammond & McCorkle 1984, Hammond 1995, Swengel 1997). Only two populations are known to exist east of Illinois, one in Pennsylvania and the other in Virginia. Hence, if differences do exist between eastern (=Pennsylvania and Virginia) and western (=all other) populations, taxonomic status could have important implications for the conservation of the remaining eastern populations. The purpose of this research is to examine mitochondrial DNA (mtDNA) and morphological variation among populations of *S. idalia* to determine if: (1) significant intraspecific variation exists, (2) there is a pattern to the variation, and (3) any patterns of variation are worthy of taxonomic recognition.

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MATERIALS AND METHODS

Morphological variation was examined via measurements of the size of white spots on the underside of the hindwings. All measurements were taken from museum specimens at the American Museum of Natural History (New York, NY). Sample sizes and collecting localities are provided in Table 1. No specimens from counties adjacent to the extant Pennsylvania population could be found in the American Museum collections; therefore, analyses of eastern populations used specimens from extinct populations from nearby counties in Pennsylvania, New York, and New Jersey to represent morphological variation of the extant Pennsylvania population (Table 1). Traits selected for analyses (Table 2, Fig. 1) were those determined by a pilot study to be most variable and therefore most likely to provide information on patterns of intraspecific variation. Maximum diameter of each spot was measured using a hand held digital micrometer. To account for allometric relationships all measures were transformed using the equation $X' = \log(X/Y)$, where Y = size measure. The size measure used was the length of the second cubital vein. An alternative measure for size, wingspan, was not incorporated because not all specimens had both wings and because some specimens had wings folded for display. A regression of second cubital vein length on wingspan was significant ($r^2 = 0.62$, $p < 0.001$), suggesting that second cubital length is a valid size measure. Analysis of morphological data included both a MANOVA, incorporating all 11 traits as a group, and univariate F-tests, to examine variation at each trait independently. Both analyses tested the effects of region (eastern vs. western; Table 1) and sex on patterns of variation. All analyses were completed using Systat v. 5.0.

Mitochondrial DNA analysis used samples collected from extant populations found across the range of the species. Sample sizes and locales are provided in Table 3. Collection of tissue from those populations of special

TABLE 1. Sample sizes and collection locales for morphological measurements from museum specimens.

State	County	Male	Female
Eastern populations			
Pennsylvania	Montgomery	2	1
	Alleghany	6	3
	Butler	11	3
New York	Renssalaer	2	0
	Albany	1	5
	Columbia	1	0
	Westchester	12	6
	New York	6	5
	Unknown	2	6
New Jersey	Passiac	4	3
	Essex	9	3
	Morrris	14	6
	Hunterdon	2	1
	Unknown	3	3
Total eastern		75	45
Western populations			
Nebraska	Pawnee	2	0
	Otoe	5	0
	Lancaster	14	1
	Douglas	3	3
	Stanton	2	0
	Dixon	4	3
	Cedar	0	3
	Knox	1	1
	Keyapaha	4	0
	Cherry	5	0
	Douglas	2	2
	Johnson	3	0
	Riley	2	0
Iowa	Dickinson	3	1
	Plynouth	3	0
	St. Clair	2	4
	Franklin	8	6
	Polk	5	0
	Guthric	6	0
	Pocahantas	3	0
Illinois	Cook	21	5
	Mercer	3	3
	Iroquois	7	0
Total western		108	32

conservation concern (Pennsylvania, Illinois, Iowa, and Wisconsin) consisted of the removal of the anterior leg on the right side so that specimens could be released alive. Anecdotal observations of seven captive females suggested no decrease in survivorship or oviposition ability following leg removal. These results indicate

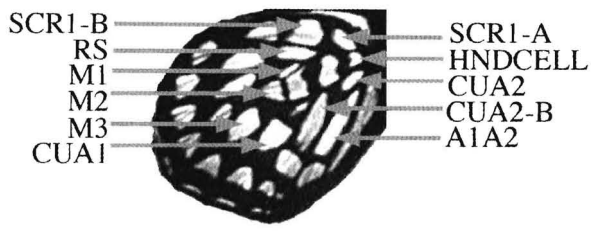

FIG. 1. Arrows indicate underside hindwing spots measured for morphological analysis. Trait abbreviations correspond with Table 1.

TABLE 2. Traits selected for analysis and the corresponding abbreviations used in Figs. 1–3. Cell names follow Scott (1986) (Figure 53, p. 146).

Trait	Abbreviation
Basal-most spot in cell 1A + 2A	1A2A
Basal spot in cell CuA2	CUA2-A
Median spot in cell CuA2	CUA2-B
Basal-most spot in cell CuA1	CUA1
Basal-most spot in cell M3	M3
Basal-most spot in cell M2	M2
Basal-most spot in cell M1	M1
Basal-most spot in cell Rs	RS
Basal-most spot in cell Sc + R1	SCR1-A
2nd most basal spot in cell Sc + R1	SCR1-B
Basal-most spot in the hindwing cell	HCELL

that this method may be useful for future genetic studies in other insect species of conservation concern, although more thorough, species specific, studies should be conducted. DNA extractions were carried out by digesting homogenized tissue at 65°C for 3–12 hours in: 10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, 20 µl dithiothreitol, and 0.4 mg Proteinase K. Nucleic acids were extracted with an equal volume of phenol, the aqueous phase was transferred to a new microcentrifuge tube and the organic extraction repeated with 24:1 chloroform:isoamyl alcohol. Total nucleic acids were precipitated with the addition of 1/10 volume 3 M sodium acetate and 3 volumes of cold 95% ethanol. Nucleic acids were resuspended in 50–100 µl of sterile water.

Mitochondrial DNA sequences were generated by direct sequencing of PCR products from partial cytochrome oxidase I and II genes (COI and II). Primers used for PCR amplification were: C1-J-2183 (alias Jerry) 5′-CAACATTTATTTTGATTTTTTGG-3′ (Simon et al. 1994) and TK-N-3772 5′-GACCATTACTTGCTTTCAGTCATCT-3′ (listed as TK-N-3782 in Sperling & Hickey 1995). PCR reactions were carried out using: 60 ng genomic DNA, 20 mM Tris-HCl, 50 mM KCl, 3 µM $MgCl_2$, 0.25 mM of each dNTP, 2 µM each primer, 1 Unit Taq DNA polymerase, and water to a final volume of 20 µl. Each PCR reaction was then subjected to 30 cycles of amplification at the following conditions: 94°C for 30 seconds, 45°C for 30 seconds, 72°C for 1 minute. PCR reactions were purified using Qiagen (Valencia, CA) PCR purification columns, following manufacturers recommendations.

Sequencing reactions used two internal primers, sequencing in opposite directions, with 30 base pairs of overlap. Sequencing primers were: TL2-N-3014 (alias PAT) 5′-TCCAATGCACTAATCTGCCATATTA-3′ (Simon et al. 1994), modified to 5′-TCCATTACATATAATCAGCCATATTA-3′ and C1-J-2983 (alias LANAE)

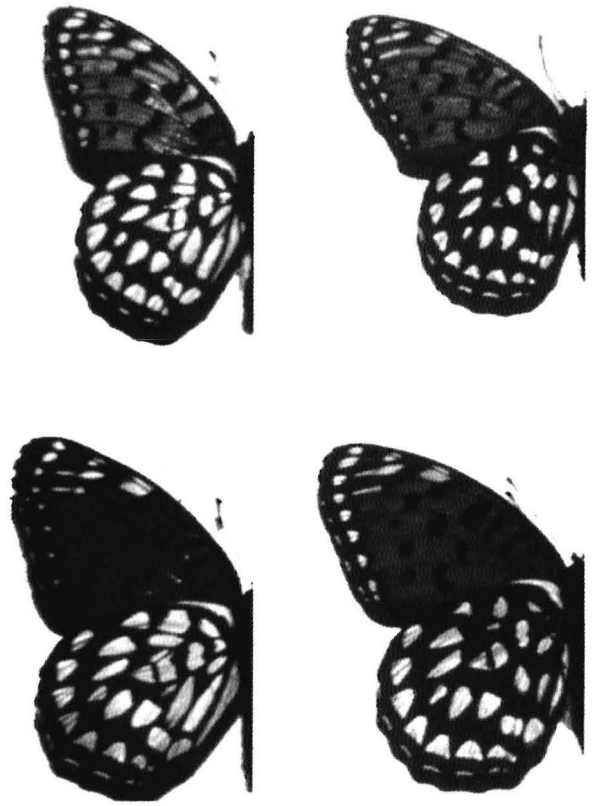

FIG. 2. Comparison of similarly-sized males (upper) and females (lower) from western (left) and eastern (right) populations. Western specimens were from Nebraska and eastern specimens from New York.

5′-TACCTCCTGCTGAACATTCT-3′. Sequencing reactions were carried out using the Perkin Elmer (Foster City, CA) Big Dye cycle sequencing kit, following manufacturer recommendations. Sequences were visualized on an ABI 377 automated sequencer at the University of Illinois Biotechnology Sequencing Center. All sequences were edited using EditView V1.0.1 and aligned using Sequencher V3.0.

Phylogenetic hypotheses were constructed using PAUP* V4.0b3 (Swofford 1998) via maximum parsimony analysis with a heuristic search and tree bisection and reconnection (TBR) branch swapping. Sequences generated from *Speyeria cybele* (Fabricius) and *S. nokomis* (Edwards) were used as outgroups to root the tree. Phylogenetic reconstructions were without weighting schemes because all substitutions but one within *S. idalia* were transitions. Statistical support for nodes was estimated using 100 bootstrap replicates.

RESULTS

Morphological analyses revealed that all traits examined were significantly different between eastern and western populations in both the multivariate test (Wilks' lambda = 0.381, F = 36.006, df = 11, 244, p < 0.001) and all univariate F-tests (p < 0.001 for all traits). As an example, Fig. 2 shows a male and female from an eastern population next to a similarly-sized male and female from a western population. Only one of the traits, the basal-most spot in the third median cell, was significantly different between the sexes (Univariate F = 7.523, df = 1, 254, p = 0.007); however, the multivariate results suggest that as a group, the traits did not differ between the sexes (Wilks' lambda = 0.946, F = 1.254, df = 11, 214, p = 0.252). Figure 3 graphically represents the differences in the trait means between eastern and western populations for the non-transformed data.

Results of mtDNA sequence analysis revealed 30 variable sites, 18 of which were parsimony-informative (Genbank accession number AF295040). Within the COI gene there were 11 parsimony-informative sites: three first-position and one second-position non-synonymous substitutions and seven third-position synonymous substitutions. Within the COII gene there were seven parsimony-informative sites, all third-position synonymous substitutions. Maximum parsimony analysis resulted in five most-parsimonious trees of 126 steps. All individuals in the Pennsylvania population shared the same unique haplotype, which has five synapomorphies (Fig. 4). All other populations sampled revealed a total of 22 unique haplotypes with no apparent geographic associations among them, with the possible exception of some Wisconsin haplotypes (Fig. 4). Also, 45 out of 84 individuals sampled from the 17 western populations shared the same haplotype (haplotype 1, Fig. 4), suggesting little genetic structuring among those populations.

Speyeria idalia idalia (Drury [1773])

See dos Passos and Grey (1945, 1947) for a description and type specimens of *S. i. idalia*. Diagnostic characters that separate subspecies are provided below.

***Speyeria idalia occidentalis* Williams, new subspecies**

Diagnostic characters. *Speyeria idalia occidentalis* can usually be diagnosed by eye because the hindwing spots are usually much larger than in *S. i. idalia*. The basal-most diffuse white spot (not included as a trait used in this study) in the second cubital cell (Figs. 1, 2) is often entirely absent in *S. i. idalia* but usually present in *S i. occidentalis*. While all of the ventral hindwing spots included in this study are significantly larger in *S. i. occidentalis*, the most pronounced differences are in cells A1A2, M1 and RS (Figs. 3, 4). In *S. i. occidentalis* the spot in A1A2 is usually greater than 10 mm, whereas *S. i. idalia* it is usually less than 8 mm (these numbers are general rules of thumb for quick reference and therefore do not incorporate size or sex differences). For M1, *S. i. occidentalis* is usu-

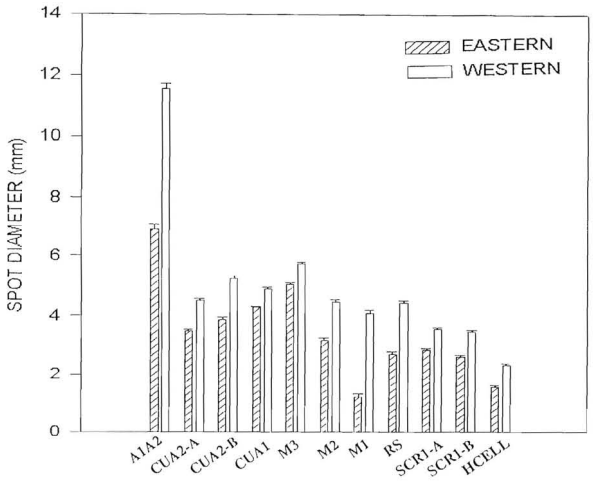

FIG. 3. Means and standard errors of underside hindwing cell spots for raw data. Trait abbreviations correspond with Table 1 and Fig. 1.

ally >3 mm, whereas *S. i. idalia* is usually <3 mm. For RS, *S. i. occidentalis* is usually >4 mm, whereas *S. i. idalia* is usually <4 mm.

Description. Male wingspan 63–92 mm (N = 108). Dorsal surface of forewing with orange background coloration and the usual *Speyeria* pattern of black spots and bars. Black outer marginal border with a row of white spots. Hindwing is orange with hints of blue-black basally and entirely with blue-black background color from the submedian to outer margin. A postmedian row of orange or fulvous spots, a submarginal row of white spots and an outer marginal row of white spots. Ventrally, forewing lighter orange background with submarginal and outer margin rows of white spots. Also, varying numbers of diffuse apical and subapical white spots. Hindwing is unique to *Speyeria idalia*. Background is entirely yellow to black with white spots, either single or multiple, in every cell. Female wingspan 71–110 mm (N = 33). Dorsal surface, forewing with larger marginal black border than in males and a marginal row of white spots. Black apical and subapical background color with white spots. Hindwing as in male except with a post median row of white spots, rather than orange. Ventrally, same as the male except for a deeper fulvous background coloration in the forewing, more black background coloration apically and in the margin.

Types. HOLOTYPE: (male), 1 mile S.E. of Crete, IL, 7 July 1965 (R.R. Irwin). ALLOTYPE: (female), same location data as holotype, 23 August 1965 (R.R. Irwin). PARATYPES: Two males, one female, all with same collection and data as holotype. Deposition of specimens: All specimens at the Illinois Natural History Survey collections.

DISCUSSION

Morphological data suggest that eastern and western populations were distinct entities; in that specimens from eastern populations had spots on the underside of the hindwing that were significantly smaller. The genetic basis for these morphological traits has not been determined, so the potential exists for differences between eastern and western populations to be correlated with as of yet undetermined environmental variables, rather than indicative of a unique evolutionary history. However, mtDNA sequence analysis suggests that the

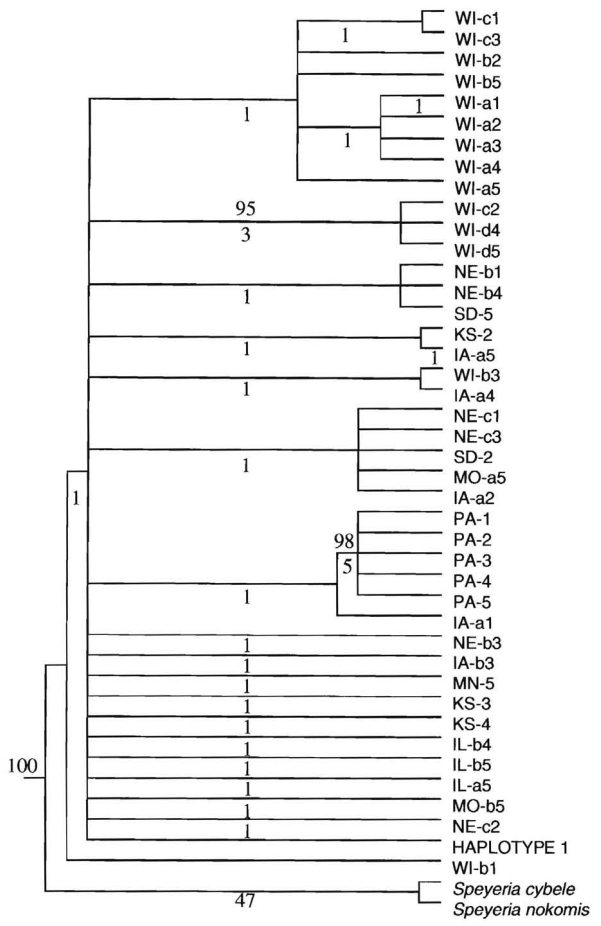

FIG. 4. Strict consensus of the five most-parsimonious trees resulting from maximum parsimony analysis of mitochondrial DNA sequence data from cytochrome oxidase I and II genes (126 steps, consistency index = 0.96). Bootstrap values >60 are shown above branches and the number of synapomorphies for each clade are shown below branches. Forty-five specimens that shared a single haplotype were collapsed together into "haplotype 1" to conserve space. Sample labels correspond with collection locations in Table 3 and numbers in parentheses indicate which of the five individuals per sample correspond to a given haplotype.

Pennsylvania population is a distinct evolutionary lineage that can be diagnosed with 5 synapomorphies, which are unique and fixed in the population. Hence, mtDNA support, in part, differentiation of the eastern populations. Based on these data I recommend that the eastern (=Pennsylvania) and western (=all other) populations be recognized as separate subspecies, and the morphological characters be used as a means for external diagnosis of each subspecies. Fortunately, the subspecies of *S. idalia* named here can be identified on the basis of easily quantified measures (spot size), which make diagnosis relatively straightforward when compared with other *Speyeria* species, in which subspecies are typically differentiated by slight differences in basal

TABLE 3. Sample sizes and collection locales for mitochondrial DNA tissue collection.

State	County	Sample Size
Pennsylvania	Lebanon	30
Nebraska – a	Keyapaha	5
Nebraska – b	Greeley	5
Nebraska – c	Kearney	5
Kansas	Riley	5
Iowa – a	Muscatine	5
Iowa – b	Plymouth	5
Illinois – a	Ogle	5
Illinois – b	Mason	5
Illinois – c	Cass	4
Wisconsin – a	Crawford	5
Wisconsin – b	Iowa	5
Wisconsin – c	Dane	5
Wisconsin – d	Portage	5
Missouri – a	St. Clair	5
Missouri – b	Vernon	5
Minnesota	Lincoln	5
South Dakota	Hughes	5
Total sample size		114

coloration of the hindwing discal area (Howe 1975, Scott 1986). Figure 3 can be used as a guide to distinguish among *S. idalia* subspecies. For example, the maximum diameter of the A1A2 cell spot used in this study (Fig. 1) is less than 8 mm in *S. i. idalia* but greater than 10 mm in *S. i. occidentalis* (Fig. 3).

If the relationship between mtDNA, morphology and longitude were to be generalized, then the Virginia population would be recognized as the same subspecies as the Pennsylvania population. However, the formal status of the Virginia population will remain undetermined until data from this population can be included in the analyses. Assuming that the neotype designation for *S. idalia* (New York, NY) of dos Passos and Grey (1945) applies to the eastern populations in general, the name for the Pennsylvania subspecies becomes *Speyeria idalia idalia*. Western populations therefore fall under the subspecies name *Speyeria idalia occidentalis*, new subspecies.

An initial examination of the male genitalia from five eastern and 25 western specimens did not result in any distinguishable differences (unpubl. data). However, other anecdotal evidence of differentiation does exist. Previous descriptions of habitat use note that eastern populations are typically found in xeric habitats whereas western populations are found in mesic habitats (Scudder 1889, Opler & Krizek 1984). Also, Barton (1996) notes that *Viola saggitatta* is the preferred host plant for the Pennsylvania population, whereas other studies focusing on western populations have noted *V. pedatifida* and *V. pedata* as preferred host plants (Swengel 1997, Kelly & Debinski 1998).

Subspecies status for the eastern population has important conservation implications. The Pennsylvania population is found on a military installation where current land use practices threaten to destroy the remaining *S. i. idalia* habitat (Barton 1996). Because this population resides on federally owned land, formal protection can only be afforded under federal legislation, i.e., the Endangered Species Act of 1973 (ESA). Therefore, the designation of *S. i. idalia* may result in a petition for an emergency listing under the ESA.

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