Population biology of an endangered butterfly, *Lycaeides melissa samuelis* (Lepidoptera; Lycaenidae): genetic variation, gene flow, and taxonomic status

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Abstract: We present data from 34 allozyme loci to test whether the Karner Blue butterfly is specifically differentiated from the Melissa Blue. Furthermore, as the Karner Blue is an endangered organism of low vagility that occurs predominantly in small, widely separated populations, we investigated (*i*) whether the Karner Blue is depauperate in genetic variation and (*ii*) whether gene flow between sampled populations is unusually low. Genetic identities between New York and Wisconsin populations of the Karner Blue and a sample of Melissa Blue from Minnesota are all statistically indistinguishable. Neither genetic identity data nor application of the phylogenetic species concept support formal recognition of the Karner Blue as a species separate from the Melissa Blue. Nonetheless, the data indicate that gene flow among the samples was very low compared with that among populations of other Lepidoptera. Heterozygosity estimates for all three samples were comparable to data for other Lepidoptera and indicate that the Karner Blue populations surveyed are not under immediate threat of extirpation due to loss of genetic diversity. Although the available data are limited, if the Karner Blue is to be managed as an evolutionarily significant unit, then the eastern and western populations should probably be treated independently and each should receive high conservation priority.

Résumé : Nous présentons ici les résultats d'une analyse des allozymes à 34 locus entreprise dans le but de déterminer si le Bleu de Karner est une espèce distincte du Bleu melissa. De plus, comme le Bleu de Karner est un organisme menacé, de faible vagilité, qui forme surtout de petites populations très dispersées, nous examinons également si (*i*) le Bleu de Karner a une variation génétique appauvrie, et si (*ii*) le flux des gènes entre les populations échantillonnées est particulièrement faible. Les caractéristiques génétiques des populations du Bleu de Karner du New York et du Wisconsin et d'un échantillon du Bleu melissa du Minnesota sont statistiquement indistinctes. Ni les données sur l'identité génétique, ni l'application du concept phylogénétique d'espèce ne permettent d'affirmer formellement que le Bleu de Karner est une espèce différente du Bleu melissa. Néanmoins, les données indiquent que le flux des gènes d'un échantillon à l'autre est très faible par comparaison à celui qui prévaut au sein d'autres populations de lépidoptères. Les estimations de l'hétérozygotie des trois populations sont semblables aux données obtenues chez d'autres lépidoptères et indiquent que les populations échantillonnées du Bleu de Karner ne sont pas immédiatement menacées par perte de diversité génétique. Malgré le peu d'informations disponibles, il semble que, si le Bleu de Karner doit être considéré comme une unité évolutive importante, il faille alors traiter les populations de l'est et de l'ouest indépendamment et assurer la priorité de conservation de l'une et de l'autre séparément. [Traduit par la Rédaction]

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Introduction

The conservation of insects in general, and butterflies in particular, is increasing in importance both in the public eye and in terms of the practical measures being taken to prevent further loss of species (Pollard and Yates 1994; Samways 1994). Nonetheless, there is a surprising lack of genetic information relevant to the conservation biology of these organisms, arguably the most charismatic of "mega-invertebrates," relative to the great deal of attention this has received from students of megavertebrates. Genetic data from wild populations are relevant to conservation in three main areas. First, molecular data are of great utility in ascertaining the taxonomic status of populations (for a review see Avise 1994). Second, estimates of gene flow among remnant populations may provide guidance for the design of conservation programs, as is most frequently used in studbooks for zoo populations. Lastly, the possibility

that populations may suffer genetic consequences of small effective population size and thereby enter an extinction vortex can be addressed, although this remains to be done for any invertebrate.

In this paper we address these three issues with reference to the globally endangered Karner Blue butterfly, *Lycaeides melissa samuelis*. The Karner Blue, currently undergoing a dramatic decline in North America (Andow et al. 1994), is of considerable interest, in part because of its potential role as an indicator of good-quality oak savannah, a habitat that currently occupies only 0.02% of its pre-agricultural settlement range in North America (Nuzzo 1986). It also occurs in "pine bush" habitats, also an endangered environment. However, despite considerable interest in conserving this butterfly, there have been no detailed studies of its taxonomic status.

Six subspecies of *Lycaeides melissa* are recognised (Lane and Weller 1994). The Karner Blue (subspecies *samuelis*) is found from New Hampshire to eastern Minnesota (Baker 1994). It was first described as a distinct subspecies of *Lycaeides melissa* by Nabokov (1944), as explained in his revision of North American *Lycaeides* (Nabokov 1949). In a letter to the New York Times (Nabokov 1975) he stated that he now considered it a distinct species, although this does not constitute formal taxonomic recognition. The nominate subspecies, the Melissa Blue, *Lycaeides melissa melissa,* is the most widespread form, ranging from the prairie provinces of Canada to the arid southwestern U.S.A. (Lane and Weller 1994). The remaining four subspecies have very restricted geographic ranges in North America (Lane and Weller 1994) and will not be considered further here.

The Karner Blue and Melissa Blue differ slightly in several morphological characteristics: a rounder wing in the Karner Blue, the presence of orange crescent-shaped markings on the dorsal surface wing margin of both the fore- and hind-wings in the Melissa Blue, and subtle differences in the male genitalia (Lane and Weller 1994). There are also differences in host-plant and habitat preferences (Lane and Weller 1994). However, no genetic data have been obtained to investigate the possibility of species-level genetic differentiation among the various subspecies of *L. melissa*. Given the considerable current concern in conserving the Karner Blue and re-establishing it in areas from which it has been extirpated, the taxonomic status and genetic health of populations of this butterfly are of more than academic interest. This paper presents electrophoretic data on two widely separated populations of Karner Blue and one population of Melissa Blue butterflies in order to address these issues.

Methods

Samples of Karner Blue butterflies were obtained from New York and Wisconsin; Melissa Blues were obtained from Minnesota. The Melissa Blue sample was obtained from the Tuohy Sand Savanna, Fillmore County, Minnesota (Cuthrell 1990). The Wisconsin Karner Blue sample was taken from the Necedah National Wildlife Refuge and a private site nearby, whereas those from New York were obtained from Saratoga County. All samples were maintained in an ultracold freezer until shipped on dry ice to Toronto, where they remained in an ultracold freezer until used for electrophoresis.

Standard starch gel electrophoretic techniques were used throughout this study (for detailed accounts of the methods and recipes used see Packer and Owen 1989, 1990, 1992). The buffer systems used, enzymes scored, and sample sizes for each locus are provided in Table 1.

The BIOSYS computer program package (Swofford and Selander 1989) was used to analyse the data. Variance estimates for heterozygosity, genetic identity, and distance data (Nei's 1978 unbiased estimators) were obtained using a bootstrap resampling scheme (Efron 1972) details of which have been presented elsewhere (Rosenmeier and Packer 1993). Two hundred bootstrap replicates were performed on individual locus genetic diversity values and 95% confidence limits were obtained from the distribution of resampled estimates. Estimates of gene flow among samples were made using Wright's F statistics (Wright 1978) as implemented in the BIOSYS computer program. There are three such statistics, $F_{IS,}$ which measures the correlation of homologous alleles within an individual in relation to that individual's subpopulation (it can thus be considered a measure of local inbreeding), F_{IT}, which is the correlation between an individual's genes and those of the total population (thus constituting a measure of local inbreeding and population subdivision), and $F_{ST,}$ which is the variance in allele frequencies among subpopulations (and is thus a measure of population subdivision). Individual locus F_{ST} values were bootstrapped to obtain 95% confidence limits as outlined above. F_{ST} can be used to obtain an estimate of the amount of gene flow between populations according to the equation $N_m = (1 - F_{ST})/4F_{ST}$ (Wright 1951), where N_m is the number of migrants between subpopulations per generation.

Results

For 4 of the 34 loci listed in Table 1, sample sizes surveyed consisted of fewer than 10 haploid genomes for one or more populations. Three (*Enol, Pgam*, and *Ugpp*) are highly invariant in other taxa surveyed in the senior author's laboratory (Packer and Owen 1992; L. Packer and J.S. Taylor, unpublished observations), and it is unlikely that any common polymorphism will have been missed, despite the small sample sizes. The remaining locus, *Acy*, was polymorphic in two of the three samples and the allele frequencies quoted may be subject to large sampling error.

Table 2 lists the allele frequencies for all 16 variable loci and compares allele frequencies among the three samples. Approximately 30% of the loci were variable in each population and average expected heterozygosities were also similar, ranging from 0.109 in the Wisconsin Karner Blue sample to 0.112 in the other two samples (Table 3). These values are almost identical with the sample mean for Lepidoptera (Table 4).

No fixed electrophoretic differences were found in any pairwise comparison between samples. Genetic identity and distance data (Nei's (1978) unbiased estimate) for the three populations are summarized in Table 5. The three identity values are statistically indistinguishable from one another. Genetic differences between the three samples are restricted to frequency differences or the presence of some uncommon alleles in one or two of the three samples. Alleles that are uncommon in one sample and absent in others may be missing simply because of inadequate sampling of individuals. Significant allele frequency differences were found among samples for the loci *Aha, Esa, Gpi, Idh*, and *Idth* (Table 2), although with 16 polymorphic loci one might expect one significant departure from Hardy–Weinberg equilibrum at the 5% level by chance alone. Each population seemed to have one locus that differed from the other two samples sufficiently to give a significant result. For *Esa* the Melissa Blue is distinct from either Karner Blue population, the New York population is the

Table 1. Allozyme loci, EC numbers, buffer systems, and sample sizes used in electrophoretic studies of samples of the *Lycaeides melissa* subspecies *samuelis* and *melissa*.

				samuelis		*melissa*
Enzyme	Locus	EC No.	Buffer	N.Y.	Wis.	Minn.
Aspartate aminotransferase	*Aat-2*	2.6.1.1	RSL	18	22	14
Acid phophatase	*Acp-2*	3.1.3.2	I	20	22	22
Aminoacylase	*Acy*	3.5.1.14	RSL	14	8	12
B-*N*-acetylhexosaminidase	*Aha*	3.2.15.2	I	18	26	28
Adenylate kinase	*Ak-1*	2.7.4.3	CAM	22	24	18
	Ak-3			20	28	22
Fructose biphosphate aldolase	*Ald*	4.1.2.13	V	14	14	16
Arginine kinase	*Ark*	2.7.3.3	I	14	18	8
Diaphorase (NADH)	*Dia-1*	1.8.1.	V	20	22	24
	Dia-2			22	28	30
Enolase	*Enol*	4.2.1.11	I	6	14	10
Esterase	*Est**	3.1.1.1	RSL	16	16	18
	Esa†			10	12	12
Fumarate hydratase	*Fum*	4.2.1.2	I	16	18	14
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	1.2.1.12	I	16	18	12
Glycerol-3-phosphate dehydrogenase	*G3pdh*	1.1.1.8	I	14	22	18
Glucose-6-phosphate isomerase	*Gpi*	5.3.1.9	I	22	24	26
B-hydroxyacid dehydrogenase	*Had*	1.1.99.6	I	10	16	14
Hydroxyacylglutathione hydrolase	*Hag-1*	3.1.2.6	V	10	16	14
	Hag-2			22	26	22
Hexokinase	*Hk*	2.7.1.1	I	22	26	22
Isocitrate dehydrogenase	*Idh*	1.1.1.42	V	18	18	18
L-Iditol dehydrogenase	*Idth*	1.1.1.15	V	18	24	28
Leucine aminopeptidase	*Lap*	3.4.11.1	I	12	18	16
Malate dehydrogenase	*Mdh*-1	1.1.1.37	CAM	24	28	28
	Mdh-2			26	32	30
Malate dehydrogenase NADP	*Me*	1.1.1.40	I	24	34	32
Peptidase glycine-leucine	Pep_{gl}	3.4.11.	RSL	10	10	12
Peptidase phenylalanine-proline	Pep_{pp}	3.4.13.8	RSL	10	18	16
Phosphoglycerate mutase	*Pgam*	5.4.2.1	I	4	8	10
Phosphogluconate dehydrogenase	*6Pgd*	1.1.1.43	CAM	24	32	26
Phosphoglucomutase	*Pgm*	5.4.2.2	I	12	14	18
Pyruvate kinase	*Pk*	2.7.1.40	V	16	18	14
Uridine D-glucose pyrophosphorylase	*Ugpp*	2.7.7.9	RSL	8	8	8

* *Est* is an esterase locus detected only in homogenates of thoraces.

† *Esa* was detected only in abdomina.

most differentiated at *Gpi,* while *Idh* differentiates the Wisconsin Karner Blue population. For the other two loci all populations appear to contribute to the overall significance level.

Fixation indices for the three samples are presented in Table 6. The F_{IS} value obtained was not significantly different from 0, based upon the bootstrap resampling variance estimates. However, both F_{IT} and F_{ST} values are significant and positive, indicating significant association among alleles within individuals in relation to the whole sample and among samples. With F_{ST} averaging 0.151 across 16 loci, the estimated number of migrants per generation is 1.4. The 95% confidence limits on these estimates are between 0.090 and 0.165 for F_{ST} and 1.2–2.5 for N_m.

Pairwise sample estimates of F_{ST} are provided in Table 7, along with their 95% confidence limits. Although the estimates of population differentiation between subspecies are both approximately twice as large as those between the two samples of the Karner Blue, there is considerable overlap in the 95% confidence limits for the three pairwise comparisons. All F_{ST}

Table 2. Allele frequencies, heterozygosities, and genetic differentiation for variable loci in the three samples studied.

Locus	Allele	Karner Blue N.Y.	Karner Blue Wis.	Melissa Blue	χ^2	p
Aat-2	s	0.944	1.000	1.000	2.04	>0.36
	f	0.054	0.000	0.000		
	H	0.111	0.000	0.000		
Acy	s	0.000	0.000	0.167	5.67	>0.2
	m	1.000	0.875	0.750		
	f	0.000	0.125	0.083		
	H	0.000	0.250	0.439		
Aha	s	0.389	0.192	0.607	11.6	<0.05
	m	0.000	0.115	0.036		
	f	0.611	0.692	0.357		
	H	0.503	0.489	0.521		
Ak-3	s	1.000	1.000	0.955	2.21	>0.3
	f	0.000	0.000	0.045		
	H	0.000	0.000	0.091		
Esa	s	0.500	0.417	0.083	22.72	<0.001
	m	0.000	0.000	0.500		
	f	0.500	0.583	0.167		
	vf	0.000	0.000	0.250		
	H	0.556	0.530	0.712		
Gpi	s	0.273	0.000	0.000	18.66	<0.001
	m	0.000	0.083	0.000		
	f	0.727	0.917	1.000		
	H	0.416	0.159	0.000		
Hag-1	s	1.000	0.938	1.000	0.68	>0.5
	f	0.000	0.063	0.000		
	H	0.000	0.125	0.000		
Hag-2	s	0.000	0.000	0.045	4.49	>0.3
	m	1.000	1.000	0.900		
	f	0.000	0.000	0.045		
	H	0.000	0.000	0.177		
Idth	s	0.222	0.208	0.000	19.1	<0.001
	m	0.778	0.667	0.536		
	f	0.000	0.125	0.464		
	H	0.366	0.518	0.516		
Idh	s	0.778	0.333	0.889	14.00	<0.001
	f	0.222	0.667	0.111		
	H	0.366	0.471	0.209		
Mdh-2	s	1.000	0.813	0.800	5.85	0.053
	f	0.000	0.188	0.200		
	H	0.000	0.315	0.331		
Me	s	0.083	0.118	0.000	3.81	>0.1
	f	0.917	0.882	1.000		
	H	0.159	0.214	0.000		
Pep_{gl}	s	0.000	0.200	0.000	4.69	0.096
	f	1.000	0.800	1.000		
	H	0.000	0.356	0.000		
Pep_{pp}	s	0.300	0.000	0.063	7.25	<0.03
	f	0.700	1.000	0.938		
	H	0.467	0.000	0.125		
Pgm	s	0.000	0.143	0.056	2.15	>0.3
	f	1.000	0.857	0.944		
	H	0.000	0.264	0.111		
6Pgd	s	0.208	0.000	0.077	38.3	<0.0001
	m	0.792	1.000	0.462		
	f	0.000	0.000	0.462		
	H	0.344	0.000	0.591		

Table 3. Heterozygosity estimates for the three samples studied.

	No. of alleles/locus	% loci polymorphic	Expected heterozygosity
Karner Blue			
N.Y.	1.3	29.4	0.112
Wis.	1.4	32.4	0.109
Melissa	1.5	32.4	0.112

values translate into 1–8 migrants between populations per generation. The total-sample N_m estimate is low compared with others available from the literature for Lepidoptera (Table 8).

Discussion

The electrophoretic data described here are of significance to answering two important questions concerning the conservation biology of the Karner Blue butterfly. First, is the Karner Blue butterfly specifically distinct from the Melissa Blue? Second, is there any evidence that either of the Karner Blue populations is suffering genetic impoverishment from the effects of a small effective population size?

Taxonomic status of the Karner Blue butterfly

The difficulties associated with defining appropriate criteria for species delimitation are proving all but intractable for systematic theorists (Otte and Endler 1989; Nixon and Wheeler 1990). The phylogenetic species concept provides one comparatively simple way out of this conundrum: species-level taxa should be unambiguously diagnosable units with no intermediates or polymorphic populations, and the difference should be heritable rather than environmentally induced. Adoption of this concept would result in an increase in the number of species recognised (Wheeler 1990). For example, Cracraft (1992) found that the number of recognised species more than doubled when the phylogenetic species concept was applied to birds of paradise (Aves; Paradisaeidae).

For electrophoretic data, the phylogenetic species concept suggests that the presence of one fixed allelic difference between samples is sufficient for diagnosing separate species (Davis and Nixon 1992). This would also result in an increase in the number of species recognised. For example, Packer and Taylor (1997) found that the number of bee species recognised would increase by between 50 and 100% if the phylogenetic species concept was adopted.

The phylogenetic species concept as applied to electrophoretic data assumes that both a sufficiently large number of loci and a sufficiently extensive proportion of the geographic ranges of the organisms have been surveyed. We had three widely separated samples for two putative taxa. Such minimal sampling is more likely to result in apparent species-level differences being detected than would a more extensive survey. Similarly, the number of loci used is reasonably large for non-*Drosophila* insect studies and is 50% greater than the average usually reported for Lepidoptera (Table 4). Consequently, our protocol is biased in favour of concluding that we are dealing with species-level taxa when the phylogenetic species concept is used. However, not one fixed difference was discovered between either of the Karner Blue samples and the Melissa Blue sample for any of the 34 loci surveyed, and application

Table 4. Heterozygosity estimates for Lepidoptera.

	Pest?	No. of loci	Heterozygosity	Source
Pectinophora gossypiella	Y	31	0.324	Graur 1985
Solenobia triquetrella	N	16	0.227[a]	Graur 1985
Choristoneura lambertiana	Y	15	0.199	Graur 1985
Ptycholoma virescana	N	15	0.189	Graur 1985
Heliothis virescens	Y	23	0.172	Mallet et al. 1993
Ch. fumiferana	Y	18	0.171	Graur 1985
Ch. retiniana	Y	18	0.164	Graur 1985
Argyrotaenia quercifoliana	N	15	0.155	Graur 1985
Clepsis persicana	N	15	0.155	Graur 1985
Cydia pomonella	Y	15	0.145	Graur 1985
Yponomeuta evonymellus	N	41	0.138	Menken 1987
Speyeria callippe	N	16	0.137	Graur 1985
Sp. mormonia	N	16	0.136	Graur 1985
Ch. occidentalis	Y	17	0.136	Graur 1985
Coenonympha gardetta/arcania	N	19	0.199–0.213	Porter et al. 1995
Co. tullia	N	17	0.135–0.204	Porter and Geiger 1988
Ch. biennis	Y	18	0.134	Graur 1985
Cl. clemensiana	Y	15	0.131	Graur 1985
Y. padellus	N	30	0.127	Menken 1987
Sp. egleis	N	16	0.126	Graur 1985
Melissopus latiferreanus	N	15	0.125	Graur 1985
Y. cagnagellus	N	29	0.125	Menken 1987
Pt. peritana	N	15	0.118	Graur 1985
Anticarsia gemmatalis	Y	28	0.114[b]	Pashley and Johnson 1986
Sp. hydaspe	N	16	0.113	Graur 1985
H. armigera	Y	28	0.113	Daly and Gregg 1985
Archips argyrospilus	N	15	0.112	Graur 1985
Lycaeides melissa	N	34	0.109–0.112	This paper
H. punctigera	Y	28	0.108	Daly and Gregg 1985
Diatraea grandiosella	Y	17	0.107	McCauley et al. 1990
Sp. coronis	N	16	0.101	Graur 1985
Sp. zerene	N	16	0.101	Graur 1985
Cy. caryana	Y	15	0.101	Graur 1985
Malacosoma americanum	Y	37	0.092	Shoemaker et al. 1993
Sp. atlantis	N	16	0.089	Graur 1985
Sp. cybelle	N	16	0.087	Graur 1985
Sp. adiaste	N	16	0.084	Graur 1985
Y. malinellus	N	35	0.081	Menken 1987
Limenitis arthemis arizonensis	N	19	0.065	Porter 1989
Lymantria dispar	Y	20	0.061	Graur 1985
Y. mahalebellus	N	42	0.059	Menken 1987
Boloria titania	N	18	0.058–0.223	Britten and Brussard 1992
Limenitis weidermeyeri angustifascia	N	19	0.056	Porter 1989
Pieris napi species-group	Y/N	21	0.055–0.130[c]	Porter and Geiger 1995
H. zea	Y	23	0.055	Mallet et al. 1993
Li. lorquini	N	18	0.053[d]	Porter 1990
Cy. membrosa	Y	15	0.052	Graur 1985
Li. weidermeyeri	N	18	0.046	Porter 1990
Boloria improba improba	N	20	0.041–0.127	Britten and Brussard 1992

Table 4 (*concluded*).

	Pest?	No. of loci	Heterozygosity	Source
Cy. lautiscula	Y	15	0.031	Graur 1985
B. acrocnema	N	20	0.031	Britten and Brussard 1992
Sp. nokomis	N	16	0.029	Graur 1985
Parnassius mnemosyne	N	23	0.004–0.18[e]	Descimon and Napolitano 1993
B. i. barryi	N	20	0.001–0.013	Britten and Brussard 1992
Y. rorellus	N	45	0.005	Menken 1987
Ecdytolopha mana	N	15	0.000	Graur 1985
Mean (SD)		21.0 (7.4)	0.105 (0.06)	

[a] Average of three estimates ranging from 0.200 to 0.25.
[b] Observed heterozygosity estimate, expected value not provided.
[c] Range among 30 populations surveyed.
[d] Data from the population with the largest number of individuals surveyed are presented.
[e] Range among 24 populations surveyed.

Table 5. Genetic identity (above the diagonal) and genetic distance (below the diagonal) among the three samples studied.

	Karner Blue		
	N.Y.	Wis.	Melissa
Karner Blue			
N.Y.	—	0.989	0.976
Wis.	0.011	—	0.967
Melissa	0.024	0.033	—

Table 7. Pairwise F_{ST} values with 95% confidence limits among the three samples studied.

	Karner Blue		Melissa Blue
	N.Y.	Wis.	Minn.
Karner Blue			
N.Y.	—	0.059	0.098
		0.031–0.094	0.041–0.015
Wis.	—	—	0.125
			0.033–0.145

Table 6. Fixation indices for 16 loci and three populations of *Lycaeides melissa*.

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-2	–0.059	–0.019	0.038
Acy	0.061	0.141	0.085
Aha	–0.089	0.016	0.096
Ak-3	–0.048	–0.015	0.031
Esa	–0.424	–0.143	0.197
Gpi	0.035	0.175	0.145
Hag-1	–0.067	–0.021	0.043
Hag-2	–0.073	–0.023	0.047
Idth	–0.291	–0.142	0.116
Idh	–0.125	0.167	0.259
Mdh-2	0.386	0.432	0.074
Me	0.211	0.242	0.039
Pep_{gl}	–0.250	–0.071	0.143
Pep_{pp}	0.395	0.490	0.157
Pgm	0.682	0.700	0.056
6Pgd	0.036	0.285	0.258
Mean	–0.058	0.101	0.151

of the phylogenetic species concept fails to support species-level status for the Karner Blue butterfly.

A more ambiguous way of identifying species from electrophoretic data is to calibrate levels of genetic identity for pairs of species/populations known or believed to represent different levels of phylogenetic divergence, based upon other criteria. This was the approach used by Brussard et al. (1985) in their study of checkerspot butterflies, which included a literature survey of other genetic divergence values for insects. Their survey showed that genetic-identity values for subspecies ranged from 0.77 to 0.99, whereas those for sibling species ranged from 0.56 to 0.94. Different populations of the same species had genetic identity values ranging from 0.92 to 1.0, with their own data forming an outlier at 0.88, below the range of the remaining values. The lowest genetic identity we found in three pairwise comparisons was 0.97, above and beyond the range reported for sibling species and well within the range obtained for subspecifically differentiated taxa and intraspecific populations.

All species concepts applied to sexually reproducing organisms have the interruption of gene flow among species as part of their basis. Estimates of gene flow among the populations surveyed can be made by using Wright's *F* statistics or Slatkin's private alleles method (Slatkin 1985; Slatkin and Barton 1989). There were too few private alleles in our data set to warrant the use of Slatkin's method. Nonetheless, the N_m value obtained from *F* statistics is one of the lowest recorded for Lepidoptera (Table 8 and see below).

The electrophoretic data, then, provide no evidence to support recognition of the Karner Blue as a species distinct from the Melissa Blue. However, it should be noted that in several electrophoretic studies of reproductively isolated species, comparatively high genetic identity values and no fixed differences have been found (Rosenmeier and Packer 1993 and references therein). However, this does not mean that the Karner Blue is not an "evolutionarily significant unit" (ESU) (Moritz 1994). Moritz's definition requires reciprocal monophyly of mitochondrial DNA (mtDNA) sequences and significant allele frequency differences at nuclear loci.

Table 8. Estimates of the number of migrants among populations of Lepidoptera.

	Scale	Pest?	N_m	Source
Pieris meridionalis	Southern Europe	N	980.0[a]	Porter and Geiger 1995
Coenonympha gardetta/arcania	Local	Y	942.0	Porter et al. 1995
Heliothis virescens	Southeastern U.S.A.	Y	124.8	Korman et al. 1993
Spodoptera exempta	Continental	Y	41.4	Pashley et al. 1985
Alabama argillacea	Intercontinental	Y	35.5	Pashley et al. 1985
Danaus plexippus	Eastern U.S.A	N	27.5	Pashley et al. 1985
Pi. rapae	Continental	Y	17.6	Pashley et al. 1985
Maniola jurtina	Local	N	16.4	Goulson 1993
Parnassius mnemosyne	Local	N	16.4[b]	Descimon and Napolitano 1993
Anticarsia gemmatalis	Continental	Y	11.6	Pashley et al. 1985
Pi. napi	Continental	Y	11.0	Porter and Geiger 1995
H. armigera	Continental	Y	10.6	Daly and Gregg 1985
Yponomeuta cagnagellus	Local	Y	9.0	Menken 1989
Pi. bryoniae	Switzerland	N	8.8	Porter and Geiger 1995
Y. padellus	Local	Y	8.1	Menken 1989
Y. rorellus	Local	Y	8.1	Menken 1989
Di. grandiosella (U.S.A)	Continental	Y	6.5	McCauley et al. 1990
Limenitis weidermeyeri	State	N	6.0	Rosenberg 1989
Malacosoma americanum	Local	Y	5.07	da Costa and Ross 1993
H. virescens	Continental	Y	5.0	Pashley et al. 1985
Li. lorquini	Western U.S.A	N	4.4	Porter 1990
Y. malinellus	Local	Y	4.1	Menken 1989
Co. tullia complex	State	N	3.9–7.8[c]	Porter and Geiger 1988
Cydia pomonella	Global	Y	3.5	Pashley et al. 1985
Li. weidermeyeri	Southwestern U.S.A	N	3.1	Porter 1990
Euphiloptes enoptes	Two States	N	2.9–8.4[c]	Peterson 1996
Spodoptera frugiperda	Continental	Y	2.7	Pashley et al. 1985
Euphydryas chalcedona	Local	N	2.5	Pashley et al. 1985
Y. viginipunctatus	Local	N	2.5	Menken 1989
Pi. adalwinda	Scandinavia	N	2.2	Porter and Geiger 1995
E. editha	Local	N	1.9	Pashley et al. 1985
Pa. mnemosyne	Local	N	1.7[b]	Descimon and Napolitano 1993
Pi. britannica	U.K.	N	1.6	Porter and Geiger 1995
Lycaeides melissa	Continental	N	1.4	This paper
Speyeria nokomis apacheana	Local	N	0.85	Britten et al. 1994
Di. grandiosella (U.S.A. and Mexico)	Intercontinental	Y	0.7	McCauley et al. 1990
Boloria titania	Western U.S.A. and Canada	N	0.29	Britten and Brussard 1992

[a] Populations from mainland Europe only.
[b] Maximum and minimum values are provided.
[c] Range is given.

Legge et al. (1996) applied the ESU concept to Cryan's buckmoth, a day-flying silkmoth. They compared allozyme, mtDNA, and host-performance data for a range of samples of the *Hemileuca maia* complex and found no evidence for differentiation into previously described species units for either allozyme or mtDNA data. There were significant differences in host performance between Cryan's buckmoth, *H. maia,* and *H. nevadensis/maia*, but the data did not give outright, discrete, diagnosable differentiation. Nonetheless, given the marked ecological differences between Cryan's buckmoth and the other populations surveyed, and despite the lack of any allozyme or mtDNA divergence among samples, they concluded that it warranted management as an ESU.

No mtDNA data are available for *L. melissa* and so this component of Moritz's ESU definition cannot be applied. The significant allele frequency differences suggest that all three samples have potential as ESUs. The host plant preference/performance possibilities remain to be tested, although absolute differences seem unlikely, as the Melissa Blue simply has a wider range of larval food plants than the Karner Blue.

As Lane and Weller (1994) noted, further classical morphological analyses are required, using modern cladistic methods, to clarify the taxonomic status of the Karner Blue. Our genetic data indicate that such detailed morphological and morphometric analyses are indeed badly needed. Nonetheless, it would seem sensible to consider management of Karner Blue populations in the east as a separate issue from conservation of those in the west and both should receive high priority.

Genetic variation and gene flow

Levels of genetic variation found in the three samples were very similar and the values obtained are average for Lepidoptera

(Table 4). There is often a negative relationship between the number of loci scored and the heterozygosity measured (Singh and Rhomberg 1987; but see Shoemaker et al. 1993). As we have scored approximately 50% more loci than the average for studies of Lepidoptera, it is possible that our heterozygosity estimate is biased downwards because of the large number of loci surveyed. However, the lepidopteran heterozygosity data do not give a significant negative relationship with number of loci scored ($R^2 = –0.013$, $p > 0.3$, $n = 66$).

Although the Karner Blue is currently considered an endangered species, neither of the populations of this butterfly had significantly lower levels of genetic variation than the generally more common Melissa Blue. However, as the Melissa Blue sample itself was taken from a small, peripheral population (see below), it could be that all three populations surveyed are suffering from a reduction in heterozygosity and that the Karner–Melissa comparison is inappropriate. Inspection of heterozygosity data for a variety of Lepidoptera (Table 4) indicates that the level of genetic variation we find in the three samples is not low, even in comparison with some pest species, which presumably have huge populations. This suggests that the Karner Blue populations have not been chronically small enough, isolated for a sufficient length of time, or gone through a small enough population bottleneck to have suffered a significant impact on levels of genetic variation. What information do we have on population sizes and degree of population isolation in these organisms?

Using methods described by Pollard (1977) and Schweitzer (1994), the Melissa Blue population was estimated at around 30–60 adults in flight daily at the collection site, which is the only locality where the Melissa Blue occurs in southeastern Minnesota. The nearest Karner Blue population is approximately 50 km to the northeast. Studies of the Wisconsin Karner Blue sites indicate that there the butterfly exists as metapopulations ranging from several hundred to 2000 individuals. The New York population surveyed is the largest population of Karner Blues and it has been censused by mark–release–recapture, with estimates of approximately 14 000 individuals in the second generation (Sommers and Nye 1994). All metapopulations of these butterflies are sufficiently isolated from each other by unsuitable habitat for it to be impossible for them to be exchanging genes at present.

It is possible to investigate the expected reduction in heterozygosity resulting from small population size using the formula $H_t = (1 – 1/2N)^t H_0$, where H_t is the heterozygosity level at generation t and N is the effective population size (Hedrick 1983). It is well known that population sizes of the spring generation of the Karner Blue are often approximately one-third those of the summer generation (Packer 1994). If the effective population size of spring and summer generations is set to 250 and 750, respectively, then over 70% of the original heterozygosity remains after 125 years (250 generations). With estimates of second-generation butterflies, based upon mark–release–recapture, of approximately 14 000 (Sommers and Nye 1994), it is probable that the main New York population has considerably exceeded the effective population size of 250 and 750 in spring and summer generations, respectively, for at least most of its recent history. Although most extant Karner Blue populations are much smaller, perhaps by an order of magnitude, it is probable that in most instances they occur (or occurred until recently) as structured populations with migration and gene flow between them rendering estimates of effective population size no more than guesses. However, even for an effective population size of 25 and 75 in spring and summer generations, respectively, a halving of heterozygosity levels is expected through drift only after 25 years (50 generations). The almost identical heterozygosity values obtained for the Karner Blue and Melissa Blue samples and their average placement in the range of such estimates for Lepidoptera suggest that small population size has not yet influenced the levels of genetic variability in the Karner Blue. It therefore seems highly unlikely that genetic impoverishment has been the cause of the recent decline in numbers of the Karner Blue throughout its range. However, the role of genetic impoverishment cannot be ruled out as a factor in the demise of the smallest and most isolated populations. Nonetheless, as has been argued elsewhere (Ehrlich 1983; Murphy et al. 1990), it is more probable that demographic problems cause extirpation of butterfly populations, including those of the Karner Blue.

Estimates of gene flow were compared among lepidopteran species for which suitable data were available from the literature (Table 8). The number of migrants among populations per generation calculated for the three *L. melissa* samples by means of Wright's F statistics was one of the lowest recorded. Only the southwestern corn borer when U.S. and Mexican samples were compared (McCauley et al. 1990) and two butterfly species that persist in fairly small, isolated areas in parts of the Rocky Mountains (Britten and Brussard 1992; Britten et al. 1994) have lower values. However, interpretation of this result is rendered less simple by the fact that most of the other estimates were obtained for pest species which may be expected to have large effective population sizes and comparatively contiguous areal coverage (the distribution of Nm values for pest species is significantly elevated above those available for non-pests, Mann–Whitney U test, $U_s = 232$, $p < 0.05$). The small size of the populations, the large area of unsuitable habitat between the isolated populations, and the apparently limited dispersal ability of *L. melissa* (Lawrence 1994) suggest that gene flow over the large distances separating the sample sites is unlikely to be occurring either directly or indirectly via populations in intermediate locations. This suspicion is borne out by the relatively low N_m values. However, the 95% confidence intervals of these estimates do not overlap zero. This is presumably the result of shared ancestral polymorphisms, the time since separation among populations being too short for the effect of past gene flow to be obscured. Nonetheless, the paucity of data suitable for comparative purposes suggests that studies of gene flow among non-endangered, non-pest lepidopteran species (perhaps especially for Lycaenidae, which typically occur in small, isolated populations) would be very worthwhile.

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References

Andow, D.A., Baker, R.J., and Lane, C.P. (*Editors*). 1994. The Karner Blue butterfly: a symbol of a vanishing landscape. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Baker, R.J. 1994. The Karner Blue butterfly: 1993 and beyond. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 163–169.

Britten, H.B., and Brussard, P.F. 1992. Genetic divergence and the Pleistocene history of the alpine butterflies *Boloria improba* (Nymphalidae) and the endangered *Boloria acrocnema* (Nymphalidae) in western North America, Can. J. Zool. **70**: 539–548.

Britten, H.B., Brussard, P.F., Murphy, D.D. and Austin, G.T. 1994. Colony isolation and isozyme variability of the western seep fritillary, *Speyeria nokomis apacheana* (Nymphalidae) in the western great basin. Great Basin Nat. **54**: 97–105.

Brussard, P.F., Ehrlich, P.R., Murphy, D.D., Wilcox, B.A., and Wright, J. 1985. Genetic distances and the taxonomy of checkerspot butterflies (Nymphalidae; Nymphalinae). J. Kans. Entomol. Soc. **58**: 403–412.

Cracraft, J. 1992. The species of the birds-of-paradise (Paradisaeidae): applying the phylogenetic species concept to a complex pattern of diversification. Cladistics, **8**: 1–43.

Cuthrell, D. 1990. Status of the Karner Blue butterfly, *Lycaeides melissa samuelis* in Minnesota, 1990. Report to the Minnesota Department of Natural Resources, St. Paul.

da Costa, J.T., and Ross, K.G. 1993. Seasonal decline in intracolony genetic relatedness in eastern tent caterpillars: implications for social evolution. Behav. Ecol. Sociobiol. **32**: 47–54.

Daly, J.C., and Gregg, P. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in two pest species *H. armigera* (Hubner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). Bull. Entomol. Res. **75**: 169–184.

Davis, J.I., and Nixon, K.C. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Syst. Biol. **41**: 421–435.

Descimon, H., and Napolitano, M. 1993. Enzyme polymorphism, wing pattern variability, and geographical isolation in an endangered butterfly species. Biol. Conserv. **66**: 117–123.

Efron, B. 1972. The jacknife, the bootstrap and other resampling plans. Monogr. No. 38, CBMS – National Science Foundation Regional Conference Series in Applied Mathematics, SIAM, Philadelphia.

Ehrlich, P.R. 1983. Genetics and the extinction of butterfly populations. *In* Genetics and conservation: a reference for managing wild animal and plant populations. *Edited by* C.M. Schonewald-Cox, S.M. Chambers, B. MacBride, and L. Thomas. Benjamin/Cummings, London. pp. 152–163.

Goulson, D. 1993. Allozyme variation in the butterfly *Maniola jurtina* (L.) (Lepidoptera: Satyrinae): evidence for selection. Heredity, **71**: 86–393.

Graur, D. 1985. Gene diversity in Hymenoptera. Evolution, **39**: 190–199.

Hedrick, P.W. 1983. Genetics of populations. SBI, Boston.

Korman, A., Mallet, J., Goodenough, J.L., Graves, J.B., Hayes, J.L., Hendricks, D.E., Luttrell, R., Pair, S.D., and Wall., M. 1993. Population structure in *Heliothis virescens* (Lepidoptera: Noctuidae): an estimate of gene flow. Ann. Entomol. Soc. Am. **86**: 82–188.

Lane, C.P., and Weller, S.J. 1994. A review of *Lycaeides* Hubner and Karner Blue Butterfly taxonomy. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 5–21.

Lawrence, W.S. 1994. Karner Blue butterfly populations in the Allegan State Game Area, Michigan. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 53–62.

Legge, J.T., Roush, R., Desalle, R., Vogler, A. P., and May, B. 1996. Genetic criteria for establishing evolutionarily significant units in Cryan's buckmoth. Conserv. Biol. **10**: 85–98.

Mallet, J., Korman, A., Heckel, D.G., and King, P. 1993. Biochemical genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and evidence for a founder event in *Helicoverpa zea*. Ann. Entomol. Soc. Am. **86**: 89–197.

McCauley, D.E., Breden, F.J., Chippendale, G.M., and Mihm, J.A. 1990. Genetic differentiation of populations of the southwestern corn borer (Lepidoptera: Pyralidae) from the United States and Mexico. Ann. Entomol. Soc. Am. **83**: 86–590.

Menken, S.B.J. 1987. Is the extremely low heterozygosity level in *Yponomeuta rorellus* caused by bottlenecks? Evolution, **41**: 630–637.

Menken, S.B.J. 1989. Electrophoretic studies on geographic populations, host races and sibling species of insect pests. *In* Electrophoretic studies on agricultural pests. *Edited by* H.D. Loxdale and J. den Hollander. Clarendon Press, Oxford. pp. 181–202.

Moritz, C. 1994. Defining evolutionarily significant units for conservation. Trends Ecol. Evol. **9**: 373–375.

Murphy, D., Freas, K., and Weiss, S. 1990. An environment–metapopulation approach to population viability analysis for a threatened invertebrate. Conserv. Biol. **4**: 1–51.

Nabokov, V. 1944. Notes on the morphology of the genus *Lycaeides* Hubner (Lycaenidae, Lepidoptera). Psyche (Camb.), **50**: 104–138.

Nabokov, V. 1949. The Nearctic members of the genus *Lycaeides* Hubner (Lycaenidae, Lepidoptera). Bull. Mus. Comp. Zool. **101**: 78–541.

Nabokov, V. 1975. Novelist as lepidopterist. New York Times, July 27, 1975. p. 46.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Nixon, K.C., and Wheeler, Q.D. 1990. An amplification of the phylogenetic species concept. Cladistics, **6**: 211–223.

Nuzzo, V.A. 1986. Extent and status of midwest oak savannah: presettlement and 1985. Nat. Areas J. **6**: 6–36.

Otte, D., and Endler, J.A. 1989. Speciation and its consequences. Sinauer and Associates, Sunderland, Mass.

Packer, L. 1994. The extirpation of the Karner Blue butterfly in Ontario. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 143–152.

Packer, L., and Owen, R.E. 1989. Allozyme variation in *Halictus rubicundus* (Christ): a primitively eusocial halictine bee (Hymenoptera: Halictidae). Can. Entomol. **121**: 1049–1058.

Packer, L., and Owen, R.E. 1990. Allozyme variation, linkage disequilibrium and diploid male production in a primitively social bee *Augocholorella striata* (Hymenoptera; Halictidae). Heredity, **65**: 241–248.

Packer, L., and Owen, R.E. 1992. Variable enzyme systems in the Hymenoptera. Biochem. Syst. Ecol. **20**: 1–7.

Packer, L., and Taylor, J.S. 1997. How many hidden species are there? An application of the phylogenetic species concept to genetic data for some comparatively well known bee "species." Can. Entomol. **129**: 587–594.

Pashley, D.P., and Johnson, S.J. 1986. Genetic population structure of migratory moths: the velvetbean caterpillar (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. **79**: 26–30.

Pashley, D.P., Johnson, S.J., and Sparks, A.N. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. **78**: 756–762.

Peterson, M.A. 1996. Long-distance gene flow in the sedentary butterfly *Euphilotes enoptes* (Lepidoptera: Lycaenidae). Evolution, **50**: 1990–1999.

Pollard, E. 1977. A method for assessing changes in the abundance of butterflies. Biol. Conserv. **12**: 115–134.

Pollard, E., and Yates, T.J. 1994. Monitoring butterflies for ecology and conservation. Chapman and Hall, London.

Porter, A.H. 1989. Genetic evidence for reproductive isolation between hybridizing *Limenitis* butterflies (Lepidoptera: Nymphalidae) in southwestern New Mexico. Am. Midl. Nat. **122**: 275–280.

Porter, A.H. 1990. Testing nominal species boundaries using gene flow statistics: the taxonomy of two hybridizing admiral butterflies (*Limenitis*: Nymphalidae). Syst. Zool. **39**: 131–147.

Porter, A.H., and Geiger, H. 1988. Genetic and phenotypic population structure of the *Coenonympha tullia* complex (Lepidoptera: Nymphalidae: Satyrinae) in California: no evidence for species boundaries. Can. J. Zool. **66**: 2751–2765.

Porter, A.H., and Geiger, H. 1995. Limitations to the inference of gene flow at regional geographic scales—an example from the *Pieris napi* group (Lepidoptera: Pieridae) in Europe. Biol. J. Linn. Soc. **54**: 329–348.

Porter, A.H., Schneider, R.W., and Price, B.A. 1995. Wing pattern and allozyme relationships in the *Coenonympha arcania* group, emphasising the *C. gardetta–darwiniana* contact area at Bellwald, Switzerland (Lepidoptera, Satyridae). Not. Lepid. **17**: 155–174.

Rosenberg, R. 1989. Genetic differentiation among populations of Weidermeyer's admiral butterfly. Can. J. Zool. **67**: 2294–2300.

Rosenmeier, L., and Packer, L. 1993. A comparison of genetic variation in two sibling species pairs of haplodiploid insects. Biochem. Genet. **31**: 185–200.

Samways, M.J. 1994. Insect conservation biology. Chapman and Hall, London.

Schweitzer, D. 1994. Prioritizing Karner Blue butterfly habitats for protection activities. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 173–183.

Shoemaker, D.D., da Costa III, J.T., and Ross, K.G. 1993. Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. Heredity, **69**: 573–582.

Singh, R.S., and Rhomberg, L.R. 1987. A comprehensive study of gene variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. Genetics, **120**: 1043–1051

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution, **39**: 53–65.

Slatkin, M., and Barton, N.H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution, **43**: 1349–1368.

Sommers, L.A., and Nye, P.E. 1994. Status, research and management of the Karner Blue butterfly in New York. Minn. Agric. Exp. Stn. Misc. Publ. Ser. No. 84–1994. pp. 129–134.

Swofford, D.L., and Selander, R.B. 1989. BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Urbana.

Wheeler, Q.D. 1990. Insect diversity and cladistic constraints. Ann. Entomol. Soc. Am. **83**: 1031–1047.

Wilson, E.O. 1992. The diversity of life. Belknap Press of Harvard University Press, Cambridge.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. **15**: 323–354.

Wright, S. 1978. Evolution and genetics of populations. Vol. IV. University of Chicago Press, Chicago.