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## GENETIC, PHENETIC, AND DISTRIBUTIONAL RELATIONSHIPS OF NEARCTIC *EUCHLOE* (PIERIDAE, PIERINAE, ANTHOCHARIDINI)

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**ABSTRACT.** This work presents the results of a molecular investigation (mtDNA: COI) of Nearctic *Euchloe* species. The five hitherto accepted species, *E. creusa* (Doubleday & Hewitson, 1847), *E. hyantis* (W.H. Edwards, 1871), *E. guaymasensis* Opler, 1986, *E. olympia* (W.H. Edwards, 1871), and *E. ausonides* (Lucas, 1852), are supported. They are part of the species groups II and V of the existing phylogeny of Palaearctic *Euchloe* species (Back *et al.* 2008). The subspecies so far described, as well as additional populations with respect to ecology and phenotype, show no or little sequence divergences and we conclude that these represent intraspecific variation. This lack of divergence appears for members of the *Euchloe hyantis* complex where we assert that biological and phenotypic characters indicate that species-level isolation has been attained for *E. hyantis*, *E. lotta* Beutenmüller, 1898 and *E. andrewsi* Martin, 1936, respectively. Interestingly, there are also no differences between Nearctic and Siberian populations of *E. creusa*. Despite these findings, *E. ausonides* is supported as a discrete species, while its affiliation to species group V points towards a certain relationship to *E. ausonia* (Hübner, 1804). However, it is evident, that all samples of *E. ausonides ogilvia* Back, including one specimen with a dark color pattern that resembles *E. ausonia naina* Kozhantshikov, are genetically very similar to *E. ausonides*, which leads to consideration of *E. ogilvia* as a subspecies of *E. ausonia*. The occurrence of the Palaearctic species *E. ausonia naina* in Yukon Territory is not treated, because we lacked material suitable for DNA analysis. The species status of the most recently described *E. guaymasensis* was also reaffirmed. Although this species shows some similarities in phenotype and haplotype to *E. hyantis*, our genetic analysis, especially the COI DNA sequences detected a closer relation to *E. olympia*. In fact, there are also specific similarities in phenotype between these two species. *E. olympia* is distinct according to both phenotype and genetic features. Phylogenetic assignment is not explicit. While the more conservative method of nuclear DNA comparison points towards a closer relationship to *E. ausonides*, analysis of the faster-evolving, maternally-inherited mitochondrial COI gene shows a closer relatedness with *E. hyantis* and especially with *E. guaymasensis*. Male *Euchloe* use either ridge and peak patrolling or drainage course patrolling as mate location strategies. Use of these disparate strategies by sympatric species may be most important as a barrier to hybridization and may serve as a mechanism for speciation.

**Additional key words:** North America, Mexico, mitochondrial DNA, barcode, COI gene

In this paper, we present new genetic evidence based on an analysis of the Mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) gene for several hundred individuals of Nearctic *Euchloe*. We compare the genetic distances between taxa and use various clustering methods to construct a molecular cladogram to match with those previously deduced solely by

morphological study. We compare and discuss the new results and revise the North American species accordingly. We assign the Nearctic species to groups previously erected by Back *et al.* (2008) for Palaearctic *Euchloe*. They defined six species groups of *Euchloe* butterflies based on morphological and molecular data. Species group I consists of *E. falloui* (Allerd) a very

peculiar species of Northern Africa and the Near East. Species group II consists of several taxa related to *E. creusa*, which occurs in several subspecies from the Altai as north as Wrangel Island of Russia. Species group III was defined by Southern Mediterranean *E. belemia* (Esper) and related species of the Canary Islands. *E. daphalis* (Moore) is the representative of group IV, and *E. ausonia* (Hübner, 1804) defined species group V. Species group VI was named after *E. crameri* Butler, but also includes *E. insularis* (Staudinger) from Corsica and Sardinia, as well as *E. melanochloros* Röber from Northern Africa.

Six species of Nearctic *Euchloe* are currently recognized (Pelham 2008). The most recent revision by Opler (1966-1974) included only four species (i.e. *E. ausonides*, *E. hyantis*, *E. olympia*, and *E. creusa*), but, subsequently, *Euchloe guaymasensis* (Opler 1986), and *Euchloe ogilvia* (Back 1990), were described as new; later *Euchloe ausonia naina*, a Palaearctic subspecies of *Euchloe ausonia*, was reported from Alaska and the Yukon Territory (Layberry *et al.* 1998). Most recently, this subspecies was found in the Mackenzie Mountains, Northwest Territories (Chris Schmidt pers. comm.). Several authors have considered that *E. ogilvia* constitutes a subjective junior synonym of *Euchloe ausonides* (Layberry *et al.* 1988).

Several studies have made use of molecular markers to clarify taxonomic relationships of species in question (e.g. Hajibabaei *et al.* 2006). "DNA-barcoding," which uses a portion of the mitochondrial cytochrome oxidase gene (COI) to detect population divergences, is becoming accepted as a library of DNA sequence information of all organisms on earth ([www.barcoding.si.edu](http://www.barcoding.si.edu)). This approach revealed, for example, a group of cryptic species in what had been thought a single species of skipper (*Astraptes fulgerator* (Walch)) (Hebert *et al.* 2004). In whites (Pieridae), molecular analyses have been used to present molecular phylogenies (Pollock *et al.* 1998; Braby *et al.* 2006; Chew & Watt 2006). In a previous study we analysed Palearctic Anthocharidini butterflies on a broad scale and confirmed species groups; In addition, we encountered some hitherto undetected species (Back *et al.* 2008). Our objective here is to integrate Nearctic *Euchloe* into an existing species group concept, and to complete molecular investigation of Holarctic *Euchloe*.

#### MATERIAL AND METHODS

**Insect material.** Taxa selection was focused on the completion of an existing dataset (Back *et al.* 2008). Nearctic specimens were selected from the collections of Werner Back and John F. Emmel, as well as those of several institutions—C.P. Gillette Museum of

Arthropod Diversity, Colorado State University; Essig Museum of Entomology, University of California, Berkeley, and the Los Angeles County Museum of Natural History. Other critical specimens were donated or purchased. Samples were stored in absolute alcohol, dried, or pinned and dried. In some cases, specimens had been rehydrated in a relaxer prior to spreading. In most cases, three legs were used for isolation and analysis of nucleic acids.

**DNA extraction, amplification and sequencing.** The extraction of sample DNA, amplification and sequencing was performed by kmbs ([www.kmbioservices.com](http://www.kmbioservices.com)). Tissue from adult individuals was used for the proteinase K procedure with DNeasy tissue kit (QIAGEN), following the manufacturer's protocol for animal tissues with slight modifications. Digestion was performed for 12 hours. Then, DNA was eluted with buffer AE. Prior to PCR amplification, the DNA concentration was measured using a Fluorometer (BioRad, VersaFluor™) and adjusted to 50 ng/μl. A 326 bp fragment of the mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) gene was amplified by PCR (Biometra T professional) using protocols and primers as in Back *et al.* (2008). Direct sequencing of dye-labelled templates (BigDye V 1.1, Applied Biosystems) was carried out using an ABI 377 XL automated sequencer (Applied Biosystems). Single strand sequences were used in both directions to generate a consensus sequence for quality control of electropherograms using Sequence Navigator V 1.0.1 (Perkin Elmer). The sequences were aligned to the reference sequence of *Pieris napi* (Linnaeus, 1758) and *P. brassicae* (Linnaeus, 1758). The new sequences are deposited at EBI/GenBank. The accession numbers are shown in Table 1.

**Sequence data analysis.** The pairwise genetic distances were calculated using the NJ-algorithm of PAUP\* software (Phylogenetic Analysis Using Parsimony 4.0 β 10 version, Swofford 2003). These values were compiled to calculate the ranges of genetic distance between the subgroups and the outgroup, respectively, using the software PAST v. 1.35 (Hammer *et al.* 2005). For each sample, the 25–75 percent quartiles are drawn using a box. The median is shown with a horizontal line inside the box. The minimal and maximal values are shown with short horizontal lines.

Phylogenetic analyses were conducted using the Maximum Parsimony (MP) algorithm of PAUP\*, the settings of a heuristic search (branch swapping, tree bisection and reconnection (TBR) were 10,000 starting trees, random stepwise addition sequences. Within each heuristic search 10 replicates were performed. For ML it is essential to use the most fitting evolution model for

TABLE 1. Sample material. Asterisks in column "Sample-ID" refer to individuals investigated earlier (Back et al. 2008).

Sample-ID	Genus	Species	Subspecies	Author, Year	Locality	GenBank acc. no.
WB010*	<i>Euchloe</i>	<i>ausonia</i>	<i>taurica</i>	Röber, 1907	Armenia, S. Zangezur, Megrinski Khrebet, Shvaizdov valley, 1800m, 22.V.2001, leg. A. Dantchenko.	FM196446
WB025*	<i>Euchloe</i>	<i>putverata</i>		(Christoph, 1884)	Kazakhstan, Ily desert, 10km from S. Bakamas, 6.5.1996.	FM196447
WB026*	<i>Elphinstonia</i>	<i>charltonia</i>		(Donzel, 1842)	S.E. Egypt, border to Sudan, Jabal Hamatah, app. 1500-1900m, early March 2003, leg. G. Müller.	FM196448
WB028*	<i>Euchloe</i>	<i>crameri</i>		Butler, 1869	Spain, Andalusia, Baza, 2.VI.2004, leg. W. Back.	FM196449
WB034*	<i>Iberochloe</i>	<i>tagis</i>	<i>granadensis</i>	(Ribbe, 1910)	Spain, Andalusia, road to Ronda, 15km N. San Pedro, 17.IV.2001, leg. W. Back.	FM196450
WB037*	<i>Euchloe</i>	<i>belenia</i>		(Esper, 1800)	Spain, Andalusia, Baza, 31.III.2005, leg. W. Back.	FM196452
WB042*	<i>Anthocharis</i>	<i>cardamines</i>		(Linnaeus, 1758)	Germany, Bavaria, Freising, e.o. 11.-20.IV.2005, leg. W. Back.	AM262790
WB051*	<i>Euchloe</i>	<i>oghtia</i>		Back, 1990	Canada, Yukon Ter., Dempster Highway, mile 1, e. p. May 1982, leg. July 1981.	FM196455
WB079*	<i>Euchloe</i>	<i>simplonia</i>		(Boisduval, 1828)	France, Ht. Alpes, le Monétier-les-Bains, Briançon, Col du Lautaret, 21.-22.VI.1984 egg, e.o. early IV.1984, leg. W. Back.	FM196457
WB081*	<i>Euchloe</i>	<i>granca-nartensis</i>		Acosta, 2008	Spain, Canary Islands, Gran Canaria, Mogan, 14.III.1992, leg. W. Back.	FM196459
WB087*	<i>Euchloe</i>	<i>persica</i>		Verity, 1908	Iran, Chuzistan, Ramshir, Omidiye, 100m., larva 11.IV.1978, e.l. 10.V.1981, leg. Back & Reissinger.	FM196464
WB088*	<i>Euchloe</i>	<i>daphalis</i>		(Moore, 1865)	Pakistan, SW Himalaya, Indus Kohistan, Kaghantal, Naran, 2400-3000m, 3.-13.VI.1977, leg. de Freina, e.l. 7.VI.1980.	FM196465
WB089*	<i>Euchloe</i>	<i>falloui</i>		(Allerd, 1867)	Morocco, E. Tafraoute, Env. Ait-Abdallah, 1600-1800m, 1.-15.IV.2006, leg. M. Tarrier.	FM196466
WB094*	<i>Euchloe</i>	<i>creusa emioientalis</i>		Verity, 1911	Russia, Siberia, Tuva near Todzha, 11. VI. 1993.	FM196467
WB095*	<i>Euchloe</i>	<i>creusa emioientalis</i>		Verity, 1911	Russia, Siberia, Tuva near Todzha, 11. VI. 1993.	FM196468
WB106*	<i>Euchloe</i>	<i>ausonides ausonides</i>		(Lucas, 1852)	USA, WA, Whitman County, Snake River Canyon, 400m, 2.IV.1992, leg. John Reichel.	FM196472
WB107	<i>Euchloe</i>	<i>oghtia</i>		Back, 1990	Canada, Yukon Ter., Dempster Highway, mile 1, Dawson City, larva 10.VI.1981, e.l. end IV-early V. 1982, leg. N. Tremblay.	FR728196
WB137*	<i>Euchloe</i>	<i>insularis</i>		(Staudinger, 1861)	France, Corsica, Castetau, 500m, 18.IV.2001.	FM196483
WB153*	<i>Euchloe</i>	<i>hesperidum</i>		Rothschild, 1913	Spain, Canary Islands, Fuerteventura, Vega del Rio Palma, 5km S Betancuria, 300m, 9.II.1990, leg. Aistleitner.	FM196494
WB162*	<i>Euchloe</i>	<i>eversi</i>		Stamm, 1963	Spain, Canary Islands, Tenerife, Montaña de la Cruzeta, 2040 m, 24.-29. IV. 2006, leg. Carlo Pensotti.	FM196500
WB177	<i>Euchloe</i>	<i>hyantis lotta</i>		Beutenmüller, 1898	USA, AZ, Pima County, Catalina Highway, milepost 2, Santa Catalina Mts., 22.II.2003, leg. Jim Brock.	FR728197
WB178	<i>Euchloe</i>	<i>hyantis lotta</i>		Beutenmüller, 1898	USA, CO, Delta Co., Black Ridge, 14.IV.2002, leg. M. C. Garhart.	FR728198

TABLE 1. Continued

Sample-ID	Genus	Species	Subspecies	Author, Year	Locality	GenBank acc. no.
WB179*	<i>Euchloe</i>	<i>creusa</i>	<i>orientalis</i>	(Bremer, 1894)	Russia, Jakutia, near Tomtor, 800m, 25.VI.1995, leg. Gashko.	FM196508
WB180*	<i>Euchloe</i>	<i>creusa</i>	<i>orientalis</i>	(Bremer, 1894)	Russia, W. Chukotka, Bilibino, 350-500m, 5.-30.VI.2004, leg. G. Kuleshov.	FM196509
WB187*	<i>Euchloe</i>	<i>ausonia</i>	<i>naina</i>	Kozhantschikoff, 1923	Russia, Siberia, W. Sayan, Aradanski Mts., Aradan r. 5.-8.VII.1998, 2100-2300m, leg. V. Komarov.	FM196514
WB189*	<i>Euchloe</i>	<i>melanochloros</i>		Röber, 1907	Algeria, Biskra, 31.III.1983, leg. Joss.	FM196516
WB199*	<i>Iberochloe</i>	<i>tagis</i>	<i>pechi</i>	(Staudinger, 1885)	Algeria, El Kantara, Dj. Malou Schergij, 850m, egg early IV.1976, e.l. 23.V.1978, leg. W. Back.	FM196521
WB200	<i>Euchloe</i>	<i>hyantis</i>	<i>cf. hyantis</i>	(Edwards, 1871)	USA, CA, Tulare Co., Lamont Meadows, 18.V.2003, leg. Ken Davenport.	FR728199
WB201	<i>Euchloe</i>	<i>hyantis</i>	<i>cf. hyantis</i>	(Edwards, 1871)	USA, CA, Tulare Co., Lamont Meadows, 18.V.2003, leg. Ken Davenport.	FR728200
WB202	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, UT, Utah Co., Big Canyon, US Hwy 6, East Tintic Mnts, 6.2 road mile NE of Eureka, 5400', 2.V.2003, leg. Todd Stout.	FR728201
WB203	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, UT, Utah Co., Big Canyon, US Hwy 6, East Tintic Mnts, 6.2 road mile NE of Eureka, 5400', 2.V.2003, leg. Todd Stout.	FR728202
WB204*	<i>Euchloe</i>	<i>hyantis</i>	<i>hyantis</i>	(Edwards, 1871)	USA, CA, Napa Co., Butts Canyon, 30.III.1972, leg. Oakley Shields	FM196522
WB205	<i>Euchloe</i>	<i>hyantis</i>	<i>hyantis</i>	(Edwards, 1871)	USA, CA, Napa Co., Butts Canyon, 30.III.1972, leg. Oakley Shields	FR728203
WB213	<i>Euchloe</i>	<i>ogilvia</i>		Back, 1990	Canada, SW Yukon Terr. Montana Mtn., 1350m, 3.VII.2002, leg. P. Jacobek.	FR728204
WB214	<i>Euchloe</i>	<i>ausonides</i>	<i>transmontana</i>	Austin & J. Emmel, 1998	USA, CA, Sierra Co., S. Loyalta, Sardine Valley Road, 21.VI.2005, leg. E. Opler.	FR728205
WB215	<i>Euchloe</i>	<i>ausonides</i>	<i>coloradensis</i>	(Edwards, 1881)	USA, CO, Jefferson Co., Coal Creek Canyon, 6.VI.2007, leg. P. A. Opler.	FR728206
WB216	<i>Euchloe</i>	<i>hyantis</i>	<i>hyantis</i>	(Edwards, 1871)	USA, CA, Plumas Co., Round Lake trail, Plumas Natl. For., 28.VI.2003, leg. P.A.&E. Opler (High Sierra Type).	FR728207
WB217	<i>Euchloe</i>	<i>olympia</i>		(Edwards, 1871)	USA, CO, Larimer Co., County Road 80, 5 miles east of US Highway 287, 5600', 29.IV.2007, leg. P.A. Opler & E. Buckner.	FR728208
WB218	<i>Euchloe</i>	<i>olympia</i>		(Edwards, 1871)	USA, CO, Larimer Co., County Road 80, 5 miles east of US Highway 287, 5600', 29.IV.2007, leg. P.A. Opler & E. Buckner.	FR728209
WB219	<i>Elphinstonia</i>	<i>transcaspica</i>		(Staudinger, 1882)	Iran, NO-Karaj, Avgi dareh, 1600m, 10.IV.2002.	FR728210
WB220	<i>Euchloe</i>	<i>creusa</i>		(Doubleday & Hewitson, 1847)	USA, AK, Steese Highway 6, Mile 106, Eagle Summit, 5.VI.1988.	FR728211
WB223	<i>Euchloe</i>	<i>guaymasensis</i>		Opler, 1987	Mexico, Sonora, Highway 58, 9 miles NW Quisape, 25.III.2005, leg. P.A.&E. Opler.	FR728212
WB224	<i>Euchloe</i>	<i>guaymasensis</i>		Opler, 1987	Mexico, Sonora, Highway 58, 9 miles NW Quisape, 25.III.2005, leg. P.A.&E. Opler.	FR728213
WB226	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, CA, Inyo Co., Nine Mile Canyon, 1.5 miles E. Tulare Co. Line, 19.IV.2003, leg. K. Davenport.	FR728214

WB227	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, AZ, Pima Co., Box Canyon, Santa Rita Mts., 21.III.2003, leg. P.A. Opler.	FR728215
WB228	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, AZ, Cochise Co., Carr Canyon, Crump Hill, Huachuca Mts., 20.III.2003, leg. P.A. Opler.	FR728216
WB229	<i>Euchloe</i>	<i>hyantis</i>	<i>andrewsi</i>	Martin, 1936	USA, CA, San Bernardino County, San Bernardino Mts., 2.5 air miles NW of Cedarapines Park, 12.VI.1996, leg. Dr. John F. Emmel.	FR728217
WB230	<i>Euchloe</i>	<i>hyantis</i>	<i>andrewsi</i>	Martin, 1936	USA, CA, San Bernardino County, San Bernardino Mts., 2.5 air miles NW of Cedarapines Park, larva on <i>Streplanthus bernardinus</i> 18.VI.1994, emgd. 5.IV.1995, leg. Dr. John F. Emmel.	FR728218
WB231	<i>Euchloe</i>	<i>hyantis</i>	<i>hyantis</i>	(Edwards, 1871)	USA, CA, Nevada Co., Fordyce Creek, 5700ft., 9.VI.1974 (High Sierra Type), leg. P.A. Opler.	FR728219
WB232	<i>Euchloe</i>	<i>hyantis</i>	<i>hyantis</i>	(Edwards, 1871)	USA, CA, El Dorado Co., Loon Lake N of Riverton, 9.VI.1973 (Low Sierra Type), leg. R.L. Langston	FR728220
WB233	<i>Euchloe</i>	<i>lotta x guajmasiensis</i>			USA, AZ, Cochise Co., Mule Mts., vic. Bisbee, spring 2005, leg. Mike Leski (Hybrid population).	FR728221
WB234	<i>Euchloe</i>	<i>lotta x guajmasiensis</i>			Mexico, Sonora, W of Cuitapa, Highway 2, 23.III.2003, leg. P.A.&E. Opler (Hybrid population).	FR728222
WB236	<i>Euchloe</i>	<i>ogilvia</i>	Back, 1990		Canada, Yukon Terr., near Haines, 22.VI.1987.	FR728223
WB238	<i>Euchloe</i>	<i>ogilvia</i>	Back, 1990		Canada, British Columbia, Pink Mtn., HW 97, Mile 147, 11.VII.1996, leg. N. Tremblay.	FR728224
WB239	<i>Euchloe</i>	<i>ausonides</i>	<i>mayi</i>	(Chermock, 1940)	Canada, Manitoba, Duck Mts., 19.VI.1985, leg. N. Tremblay.	FR728225
WB240	<i>Euchloe</i>	<i>olympia</i>	(Edwards, 1871)		USA, NE, Sioux Co., Sobelly Canyon, Pants Butte, NE of Harrison, 26.V.1990, leg. J.M. Reiser & J. Reichel.	FR728226
WB242	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	Canada, British Columbia, Southern interior B.C., Fountain Ridge, 700m, 21.IV.2001, leg. P. Jakubek.	FR728227
WB243	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, CO, Garfield Co., Ridge W of Roaring, Fork River, 2 km E of Jct. of 125+117 Road, 5 miles S of Glenwood Springs, 7600', 7.V.2002, leg. P.A. Opler & E. Buckner.	FR728228
WB245	<i>Euchloe</i>	<i>creusa</i>	<i>creusa</i>	(Doubleday & Hewitson, 1847)	Canada, British Columbia, Pink Mts., HW 97, Mile 147, 11.VII.1996, leg. N. Tremblay.	FR728229
WB246	<i>Euchloe</i>	<i>creusa</i>	<i>creusa</i>	(Doubleday & Hewitson, 1847)	Canada, British Columbia, Pink Mts., HW 97, Mile 147, 8.VII.1996, leg. N. Tremblay.	FR728230
WB247	<i>Euchloe</i>	<i>creusa</i>	<i>creusa</i>	(Doubleday & Hewitson, 1847)	USA, AK, Steese Highway 6, Mile 106, Eagle Summit, 5.VI.1989.	FR728231
WB254	<i>Euchloe</i>	<i>ausonides</i>	<i>mayi</i>	(Chermock, 1940)	Canada, Manitoba, Duck Mts., 19-20.VI.1984, leg. N. Tremblay.	FR728232
WB255	<i>Euchloe</i>	<i>ausonides</i>	<b>ssp.</b>		Canada, Alberta, East Slope of Rocky Mountains, Forestry Trank Road, 200km South of Nordegg, 19.VI.1980, leg. John Reichel.	FR728233
WB257	<i>Euchloe</i>	<i>lotta x guajmasiensis</i>			USA, AZ, Cochise Co., Mule Mts., vic. Bisbee, spring 2005, leg. Mike Leski (Hybrid population).	FR728234
WB308	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, UT, Utah Co., Big Canyon, US Hwy 6, East Tintic Mnts, 6.2 road mile NE of Eureka, 5400', 2.V.2003, leg. Todd Stout.	FR728235
WB314	<i>Euchloe</i>	<i>ausonides</i>		(Lucas, 1852)	USA, CA, Mariposa Co., Briceburg, 9.III.1983.	FR728236
WB321	<i>Euchloe</i>	<i>hyantis</i>	<i>lotta</i>	Beutenmüller, 1898	USA, CA, San Diego Co., Jacumba, 29-30.III.1993, leg. W. Back.	FR728237
WB322	<i>Euchloe</i>	<i>ausonides</i>		(Lucas, 1852)	USA, CA, Marin Co., Sausalito, 2./3.IV.1993, leg. W. Back.	FR728238
WB323	<i>Euchloe</i>	<i>creusa</i>	<i>orientalis</i>	(Bremer, 1894)	Russia, Siberia, Wrangel-Island, N. Magadan, 29.VI-7.VII.1992, leg. T. S. Churkin	FR728239



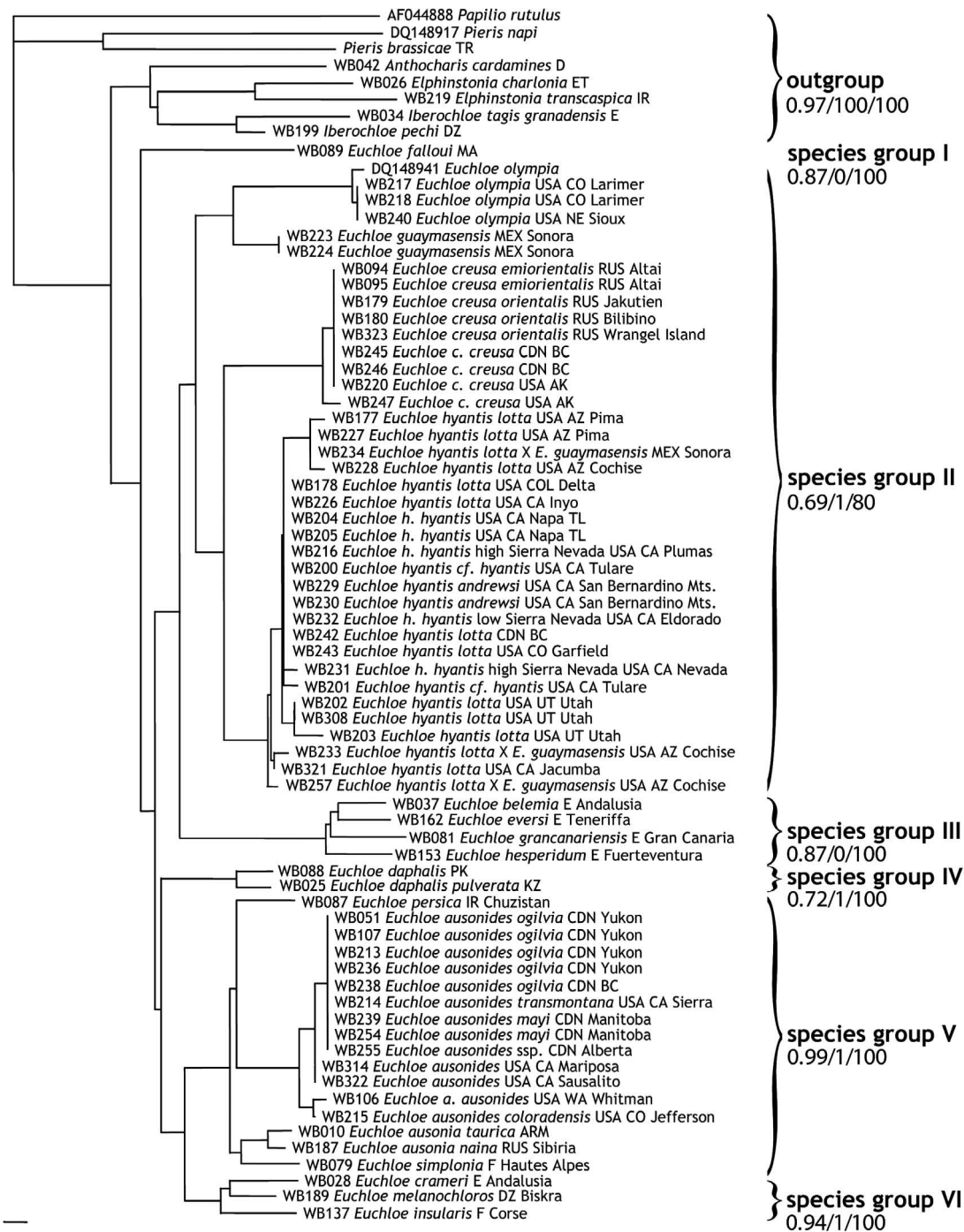


FIG. 1. Neighbor joining tree of *Euchloe* taxa. DNA sequences of mtCOI gene contained 326 bp each. Outgroup rooting was set to *Papilio rutulus*. Representatives of yet established species groups I, III, IV, and VI were only single specimens. CI was 0.494, RI was 0.817. Bar indicates 0.005 changes. Values show support of nodes with posterior probability values of Bayesian analysis/results of Maximum Likelihood: 1 – branch was found; 0 – branch was not found/Maximum Parsimony majority rule consensus. See text for settings of tree search algorithms.

a given dataset. To calculate this, we used Modeltest V. 3.7 (Posada & Crandall 1998). Calculations using the received evolution model were done by the software PhyML (Guindon & Gascuel 2003). Bayesian analysis (BI) was performed using MrBayes V. 3.1.2 (Ronquist & Huelsenbeck 2003) with default settings: two times four chains, MCMC, chain temperature = 0.2, number of generations = 5,000,000.

**Molecular data.** We accepted sequences divergences of 2 % or more as evidence of species-level status. This was in part based on both our previous studies of Anthocharidini (Back *et al.* 2006a, b, 2008) and those of others for other groups (Sperling 2000). Intraspecific variability, i.e. oscillation of sequence divergence within a single population is below 1 %, while we arbitrarily assume subspecies level from 1 to 2%.

RESULTS

All of the sample individuals yielded DNA suitable for PCR. Amplified fragments yielded sequences with the expected 326 base pairs (bp) in length each, and base composition of the resulting nexus file including outgroup specimens showed the bias of A and T as typical for insects (A: 0.28%; C: 0.18%; G: 0.13%; T: 0.41%). Of 326 bp 215 bps were constant, 84 of 111 variable positions were parsimony-informative which means a portion of 25.77% positions with phylogenetic impact.

**Haplotype diversity.** Haplotype identity occurred regularly and was detected even between individuals several hundred miles apart. Within clade II, we found one haplotype in *E. olympia* (n=3, HTe01, ref. specimen WB217), one haplotype in *E. guaymasensis* (n=2, HTeg1, ref. specimen WB223), two haplotypes in *E. creusa* (n=9, HTec1, ref. specimen WB220, HTec2, ref. specimen WB247), and 11 haplotypes in *E. hyantis* (n=23, HTeh1, ref. specimen WB177, HTeh2, ref. specimen WB227, HTeh3, ref. specimen WB228, HTeh4, ref. specimen WB204, HTeh5, ref. specimen WB321, HTeh6, ref. specimen WB201, HTeh7, ref.

specimen WB202, HTeh8, ref. specimen WB203, HTeh9, ref. specimen WB233, HTeh10, ref. specimen WB321, HTeh11, ref. specimen WB257.

Clade V bears four different haplotypes within *E. ausonides* (n=13): HTea1, ref. specimen WB051, HTea2, ref. specimen WB314, HTea3, ref. specimen WB106, and HTea4, ref. specimen WB215. Of all newly investigated nearctic specimens (n=50), 19 different haplotypes in 5 nominal taxa were detected.

**Distance analysis.** Genetic p-distances were from 0.0% between specimens of one population to 17.9% between least related taxa. Mean genetic distances of outgroup specimens of other or the respective family was about 15%, distances to specimens of genera of the family Pieridae, tribe Anthocharidini was approximately 10% (Fig. 1). The least genetic distance was found between *Euchloe* and *Iberochloe*, the closest relative to genus *Euchloe*.

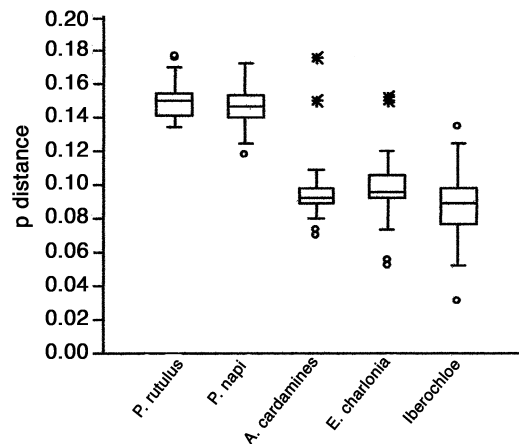


FIG. 2. Box plots of genetic distances. Box 1: genetic distance of outgroup taxon *Papilio rutulus* (AF044888, Papilionidae) to *Euchloe* taxa. Box 2: genetic distance of *Pieris napi* (DQ148917, Pieridae) to *Euchloe* taxa. Box 3: genetic distance of *Anthocharis cardamines* to *Euchloe* taxa. Box 4: genetic distance of *Elphinstonia charltonia* to *Euchloe* taxa. Box 5: genetic distance of *Iberochloe* species to *Euchloe* taxa. Outliers are shown with circles or asterisks when they exceed the 95% confidence interval.

TABLE 2. Species groups of genus *Euchloe*. Hitherto known members of respective group (Back *et al.* 2008) and new results.

species groups and reference taxon	known ingroup taxa	taxa new to species groups
I ( <i>E. falloui</i> )	<i>E. falloui</i>	
II ( <i>E. creusa</i> )	<i>E. creusa</i>	<i>E. olympia</i> , <i>E. guaymasensis</i> , <i>E. hyantis</i> complex
III ( <i>E. belemia</i> )	<i>E. belemia</i> , <i>E. hesperidum</i> , <i>E. eversi</i> , <i>E. grancanariensis</i>	
IV ( <i>E. daphalis</i> )	<i>E. daphalis</i>	
V ( <i>E. ausonia</i> )	<i>E. ausonia</i> , <i>E. simplonia</i> , <i>E. persica</i>	<i>E. ausonides</i>
VI ( <i>E. crameri</i> )	<i>E. crameri</i> , <i>E. melanochloros</i> , <i>E. insularis</i>	



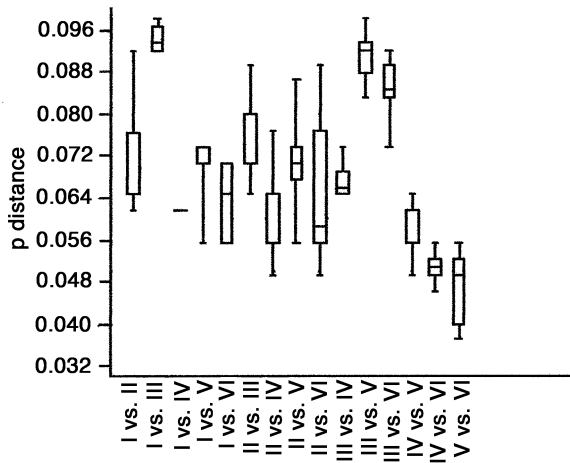


FIG. 3. Box plots of genetic distances within *Euchloe* species groups. I vs. II, I vs. III, I vs. IV, I vs. V, I vs. VI, II vs. III, II vs. IV, II vs. V, II vs. VI, III vs. IV, III vs. V, III vs. VI, IV vs. V, IV vs. VI, V vs. VI.

Based on our sequence divergence results, we accept five species of Nearctic *Euchloe* as clearly resolved and genetically defined. These species are *E. ausonides*, *E. creusa*, *E. hyantis*, *E. olympia*, and *E. guaymasensis*. Genetic distance analysis clusters these species clearly into the species groups defined by Back *et al.* (2008) (Table 2).

Genetic distances of outgroup taxa vs. *Euchloe* specimens gradually decreased with phylogenetic relatedness. Values seem to reach a maximum at approximately 15% sequence divergence, as there is no significant difference between the *Papilio* and the *Pieris* individuals when comparing p-values (Fig. 2). At the genus level, values vary around 10% sequence divergence, when comparing genera of the same tribe (Anthocharidini). Noteworthy is the increasing fluctuation in combination with increasing relation to the ingroup.

Within the selected taxa of Nearctic *Euchloe* we compared genetic distances of the respective groups. Distances ranged from 3.68% to 9.02% between groups. Mean values varied from 4.72% for the closest related group V and VI to 9.43% for the most distantly related group I and II (Fig 3, table 3).

Genetic variation within groups was calculated solely for groups II, and V, while further splitting of group II was performed to compare values of species or species complex. Based on the tree we assigned specimens WB217, WB218, WB240, and DQ148941 to *E. olympia*, WB223 and WB224 to *E. guaymasensis*, WB094, WB095, WB179, WB180, WB220, WB245, WB246, WB232, and WB247 to *E. creusa*, WB177, WB227, WB234, WB228, WB178, WB200, WB204,

TABLE 3. Comparison of genetic distances between species groups.

pairs of species groups	mean p-distance	standard deviation
I vs. II	0.07005668	0.00973179
I vs. III	0.09432515	0.00254342
I vs. IV	0.06134969	0.00000000
I vs. V	0.07073259	0.00520469
I vs. VI	0.06339469	0.00630308
II vs. III	0.07407029	0.00561397
II vs. IV	0.05905487	0.00765592
II vs. V	0.06982519	0.00585621
II vs. VI	0.06473578	0.01143300
III vs. IV	0.06710123	0.00323088
III vs. V	0.09058102	0.00373733
III vs. VI	0.08486707	0.00506109
IV vs. V	0.05728979	0.00419014
IV vs. VI	0.05061350	0.00293690
V vs. VI	0.04721521	0.00589193

WB205, WB216, WB226, WB229, WB230, WB232, WB242, WB243, WB231, WB201, WB202, WB308, WB203, WB233, WB321, and WB257 to *E. hyantis*. Within group V, all nearctic *Euchloe* specimens (WB051, WB107, Wb213, WB214, WB236, WB238, WB239, WB254, WB255, WB314, WB322, WB106, and WB215) were assigned to *E. ausonides*.

**Genetic diversity within species groups: group II.** Species group II is comprised of *E. olympia*, *E. guaymasensis*, *E. creusa*, and the *E. hyantis* complex. Genetic diversity between these taxa varies from the closest between *E. guaymasensis* and *E. hyantis* ( $p=0.03374233$ ) to the most distant between *E. olympia* and *E. hyantis* ( $p=0.06724877$ ). Mean genetic distances between species is  $p=0.04656292$ , which supports well differentiated species at the COI level (Fig. 4, Table 4).

**Genetic diversity within species groups: group V.** Species group V is comprised of *E. ausonia*, *E. simplonia*, *E. ausonides*, and *E. persica*. Genetic diversity was calculated between *E. ausonides* and their conspecifics. P-values vary from  $p=0.02760736$  in closest neighbors (*E. ausonia* -*E. ausonides*, and *E. ausonia* -*E. persica*) to  $p=0.0398773$  in most distant ones (*E. simplonia* -*E. ausonia*) (Fig. 5, Table 5). Mean genetic distances between all species of this group is at  $p=$

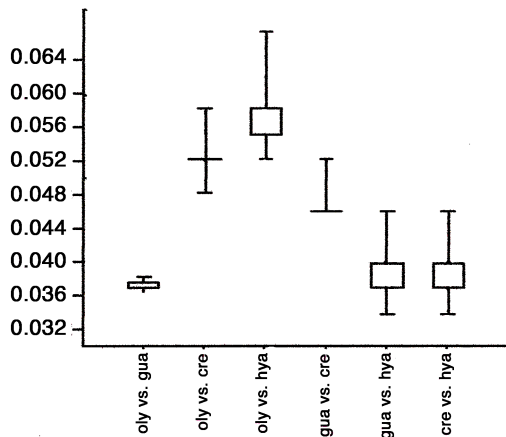


FIG. 4. Box plots of genetic distances of species within *Euchloe* species group II.

0.03441089, which supports the respective species distinctiveness.

**Phylogenetic analysis.** Phylogenetic signal is found at several nodes of the NJ tree. The majority rule consensus tree of the 10 trees retained from MP search supported the monophyly of the genus *Euchloe*. All of the six groups were retained with highest support, with the exception of group II, which included group III and IV as sister group to a clade containing *E. olympia* and *E. guaymasensis*, respectively.

**ML:** The best fitting model of evolution as defined by Modeltest was the GTR+I+G model with a likelihood-value of  $-\ln L = 1934.2101$ . Monophyly of *Euchloe* failed in ML analysis, when *E. falloui* clustered in a clade containing *Elphinstonia*, *Anthocharis* and *Iberochloe*. Besides this, all the other groups were retained and the topology was identical to the NJ tree.

**Bayes:** Bayesian inference confirmed monophyly of *Euchloe* with *E. falloui* as sister to all other *Euchloe*. Topology resembles most the NJ tree with the exception

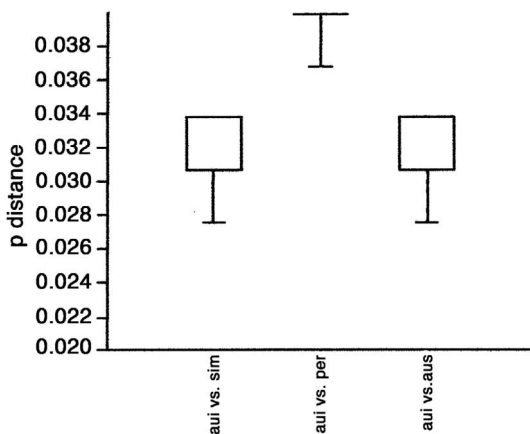


FIG. 5. Box plots of genetic distances of species within *Euchloe* species group V.

TABLE 4. Comparison of genetic distances between species of group II.

pairs of species	mean p-distance	standard deviation
<i>E. olympia</i> vs. <i>E. guaymasensis</i>	0.03717561	0.00063356
<i>E. olympia</i> vs. <i>E. creusa</i>	0.05185511	0.00257368
<i>E. olympia</i> vs. <i>E. hyantis</i>	0.05682268	0.00371462
<i>E. guaymasensis</i> vs. <i>E. creusa</i>	0.04669393	0.00192804
<i>E. guaymasensis</i> vs. <i>E. hyantis</i>	0.03867698	0.00370084
<i>E. creusa</i> vs. <i>E. hyantis</i>	0.03876590	0.00332918

of an unresolved basal trichotomy, containing group IV, a clade with group V and VI, and a clade with group II and III.

DISCUSSION

We realize that use of the COI barcode method is most suitable for the discrimination of genetic divergence of genera, species and subspecies, and one should not make firm conclusions with only a mitochondrial gene about phylogenetic relationships and lineages. Nevertheless we feel our analysis is novel and somewhat informative and may stand until more inclusive studies are performed. The limitation of this type of analysis was shown in our earlier paper (Back *et al.* 2006b), as the magnitude of sequence divergence was lower between *E. bazae* Fabiano and *E. penia* (Freyer) and not, as had been expected, between *E. bazae* and *E. charlonia* (Donzel). On the other hand, separation of putative species was considerably higher than that for other butterflies, and in general agreement with the magnitude of divergence found in earlier studies (e.g. Pollock *et al.* 1998).

**Taxonomy.** The Nearctic *Euchloe* are included below in species groups II and V of the extant cladogram for Palaearctic *Euchloe* (Back *et al.* 2008). In the following paragraphs, we will discuss how these placements agree or disagree with the species groups

TABLE 5. Comparison of genetic distances between species of group V.

pairs of species	mean p-distance	standard deviation
<i>E. ausonides</i> vs. <i>E. simplonia</i>	0.03916942	0.00129241
<i>E. ausonides</i> vs. <i>E. persica</i>	0.03256253	0.00191695
<i>E. ausonides</i> vs. <i>E. ausonia</i>	0.03150071	0.00200566

presented by Opler (1971) and others which were based on both larval and adult phenotypic characters, biology, and ecological occurrence.

**Species group II.** This group is represented by the Holarctic *E. creusa*. When investigating individuals from Siberia it was noticed that there was no genetic diversity at all between populations at the investigated COI fragment, even though the dispersion of populations exceeded ranges of several 1000km and were as distant as Wrangel Island (Russia). In the present project we found that individuals from both British Columbia and Alaska fit perfectly into this previously described cluster, suggesting relatively recent trans-Beringian genetic exchange between these populations. An exception was an Alaskan individual (WB247) that showed some genetic divergence, but we considered it within the range of intraspecific sequence divergence.

The placement of *E. creusa* in this species group differs from the results of Opler (1971) who placed *E. creusa* with *E. ausonides* and *E. olympia* in the *Ausonides* species group based on adult characters: white scales in forewing discal black bar, valval shape, and juxta configuration. Moreover, both *E. olympia* and *E. ausonides* have nearly identical larval color patterns (Opler 1974).

*Euchloe hyantis* is another distinct species of species group II. Its association with species group II of *Euchloe* is not entirely unanticipated, because the butterflies show phenotypic similarities to *E. creusa*, leading to an occasionally proposed conspecificity of these two species (Talbot 1932-1935). Because of phenotypic similarities to *Iberochloe tagis* (e.g. the adult appearance, univoltinism, larvae green with violet and white lateral stripes) it would not have been surprising, if *E. hyantis*, as well as *E. creusa*, were assigned to the newly erected genus *Iberochloe* Back, Kneibelsberger & Miller, 2008. The genetic data strictly rejects this with high support, as the genetic distance is close to 10%. However, it appears that larval color pattern is relatively unstable and should not be used for phylogenetic considerations. A case in point is shown by the larvae of closely related species *Anthocharis belia* (Linnaeus) and *A. euphenoides* (Staudinger), which show a similar range of variability (Back 1977).

We investigated the *Euchloe hyantis* group or complex most intensively, because apparent local variation, phenotypic differences, larval host use, and adult behavior all suggested that more than one species might be involved (Opler 1971, unpublished; J. Emmel personal communication). Many individuals throughout most of the range were selected for genetic analysis. However, we found only slight genetic differences

between the populations that we examined with our fragment of COI. For example, we tried to discover whether the separation *E. hyantis* and *E. lotta* is justified (Beutenmüller 1898; Opler 1999). We found that close to nomenotypical individuals of *E. hyantis* from Napa Co., California, (type locality Mendocino, Mendocino Co., California) are of an identical haplotype as one individual *E. hyantis lotta* from near Glenwood Springs, Garfield Co., Colorado (WB243). We refer this Colorado individual as closest to toptypical *E. lotta* (Kanab, Kane Co., Utah) (Opler, 2008).

Interestingly, a series of 20 individuals from a population at Eureka, Juab Co., Utah, had the forewing black discal patch especially broad in both sexes and a noticeable clear white base color, differing slightly from Arizona, Colorado, and Nevada populations, which possess a more creamy white dorsal ground color. These individuals closely resemble the lectotype of *E. h. lotta* from Kanab. The genetic differences are less than 1% (0.92%) and within our standard for subspecific range of sequence diversity. We consider this population to be within the range of toptypical *lotta* (Kanab, Utah) because of both its geographic and apparent genetic proximity. It is possible that some other populations that occur east of the Cascade – Sierra Nevada crest may eventually be described as subspecies as there is notable variation in some regions.

Likewise, the population from Jacumba, San Diego Co., California, close to the Mexican border (Peninsular Ranges Segregate) showed small sequence divergence from other *E. hyantis/E. lotta*. Since some populations are found along the western edge of the Colorado Desert, one might consider these to be *E. h. lotta*, however, extending upward from the desert, this population blankets the east side of the Laguna Mountains to its summit. These have been considered closest to nomenotypic *E. hyantis* by Opler (1971, 1999) because of their narrow black discal patches on the forewings and the lack of pearly sheen in the white areas on the ventral hindwing.

In contrast, the two specimens of *E. hyantis andrewsi* were identical to the most common haplotype of *hyantis/lotta*-specimens. Somewhat more distinct are the populations found in southeastern Arizona. These belong to the *E. h. lotta* phenotype, but their forewings have much narrower black discal bars (Opler 1971). There seems to be a cline extending northward as the average bar width gradually increases as one approaches southern Utah. The Arizona specimens are 0.6-1.8% different from all the remaining *E. hyantis* populations.

Some populations of *Euchloe hyantis* in southeastern Arizona, in fact, show lowered proportion of green

marbling on the ventral hindwings, reminiscent of *Euchloe guaymasensis*. This is noticeable in the Mule Mountains of Cochise County and the Santa Rita Mountains of Santa Cruz County, and may represent introgression from populations of *E. guaymasensis* in Sonora, Mexico. In fact, one population located west of Cuitapa, Sonora, Mexico, appears to be a hybrid swarm between *E. hyantis lotta* and *E. guaymasensis*. Most individuals in this population appear widely variable between the two parental phenotypes. We included a few individuals *hyantis lotta* x *guaymasensis* from this population in our analysis. In this area the ranges of *E. hyantis lotta* and *E. guaymasensis* are now parapatric. We believe this represents secondary contact of the species intermediated by the spread of weedy *Descurainaea pinnata* (Walt.) Britt. (Brassicaceae), which thrives in historically overgrazed, disturbed habitats. Further south in Sonora, *E. guaymasensis* uses only *Dryopetalum runcinatum* A. Gray var. *laxiflorum* Rollins, a native crucifer, even though *Descurainaea pinnata* is present at the same sites where we found no evidence of its use (Brock & Opler unpublished). According to the results of maternally inherited COI the hybrids cluster within the *hyantis*-clade from SW-Arizona and from the Sonoran Desert. An investigation of nuclear Histone3-gene (H3) revealed, that, besides the slower mutation rate of this gene, the genotype of the hybrids is identical to those of *E. hyantis* and *E. guaymasensis*, respectively. This result points towards the actual hybrid identity of these two species.

Because of the sensitivity of mtDNA sequences with respect to recognition of distinct species, the consequences of the results are the following. *E. hyantis* is a genetically robust species, and gene flow between populations is still possible and visible. However, because of the phenetic, biological, and spatial occurrence we feel that recognition as separate subspecies is justified, i.e. *hyantis*, *lotta*, *andrewsi* being quite reasonable. It is possible that when additional genes are sequenced, these taxa may in fact foster their genetic distinctiveness. Almost all populations of *E. hyantis* from the Pacific Divide and westward feed solely on *Streptanthus* species as their larval hosts; *Streptanthus tortuosus* Kellogg (Brassicaceae) is used in most instances (Opler 1974). To the east of the Pacific Divide, populations assignable to *E. h. lotta* are catholic in their host use and several unrelated genera of Brassicaceae are selected and consumed. *Euchloe h. andrewsi*, first described by Martin (1936) as a subspecies of *Euchloe ausonides*, is endemic to a small area of the San Bernardino where its sole host is the endemic *Streptanthus bernardinus* (Greene) Parish (Brassicaceae) (C. Henne personal communication).

The large size and phenotype of *E. h. andrewsi* is distinctive and shows no evidence of blending with adjacent populations of *E. h. lotta* or *E. h. hyantis*, which occur nearby in the same mountain range. For that, some authors, including Opler, argue that this population looks and behaves like a species separate from other *hyantis* populations (Chris Schmidt pers. comm.) and therefore should be treated as distinct species.

The fact that *E. guaymasensis* also belongs to species group II had been expected due to the phenotype (butterfly, caterpillar, chrysalid [Brock in Interactive Listing of Mexican Butterflies]), but the distinct gap of 3.7% from the closest species (*E. olympia*) shows that its separation from the other *Euchloe* is of long-standing. However, these butterflies, despite their present-day long distance disjunct occurrence of roughly 1400 km, bear a slight similarity to *E. olympia*. In particular, both share the evanescent rosy pink shade along the costal margin of the ventral hindwing, have a sparse distribution of green marbling on the ventral hindwings, and a convex forewing outer margin. Moreover, no other *Euchloe* species share the rosy-pink iridescence due to an unstable pteridine pigment that changes after death.

*Euchloe olympia* also belongs to species group II, according to our results. This was surprising, as was the relatively high degree of similarity to *E. guaymasensis*, especially since the shape and color pattern of the caterpillar and chrysalid pointed towards inclusion with species group V. In fact, Opler (1971) had *E. olympia* as part of the *ausonides* species group. Given the short length of the COI fragment used in our study, such association may not be justified. A comparison with a more conservative, nuclear DNA (H3) shows, in contrast, that *E. olympia* is in fact more similar to *E. ausonides* (data not shown). Apart from that, the three samples are concordant despite their different localities. Likewise, the GenBank COI sequence DQ148941 from Larimer Co., CO (Chew & Watt 2006), shows only minor sequence divergence. Moreover, in a study of *Euchloe olympia* by Opler & Clench (1983), detailed phenetic analysis argued against the recognition of any subspecies throughout its range.

In contrast to this apparent relationship are other characters shared between *E. olympia* and *E. ausonides* including identical larval color pattern and genitalia features. The existence of individuals showing intermediate phenotypes between *E. olympia* and *E. ausonides* from Jefferson Co., Colorado and Natrona Co., Wyoming suggests periodic matings between these species (C.P. Gillette Museum, Colorado State University). These seem to be rare individuals and no



evidence of retrogression is seen in either parent population.

Species group V. Our results strongly support the inclusion of *E. ausonides* in species group V. Scott (1986) included *E. ausonides* as a subspecific taxon under *E. ausonia*, apparently due to its superficial similarity, but our analysis shows it separated by at least 2.76% of genetic divergence in our fragment of COI.

The molecular analysis of numerous *E. ausonides* throughout its distribution resulted in a unitary outcome, similar to that for *E. hyantis*. The maximum value of sequence divergence was 1.2%. The main grouping, consisting of specimens from several localities from Yukon (*ogilvia*), Sierra Co., California (*transmontana*), Manitoba (*mayi*), British Columbia and Alberta, respectively, showed the same haplotype. A small magnitude of divergence from the above cluster was encountered solely in specimens from Mariposa County, California and Sausalito, Marin County, CA (0.3%), as well as individuals from Jefferson County, Colorado and Whitman County, Washington (0.9-1.2%).

We interpret these differences to be infrasubspecific genetic divergences and point mutations, leading to the assumption that *E. ausonides* populations have not been isolated since their occurrence and only slight genetic and phenotypic divergence have occurred to date. However, discrimination at the subspecific level because of distinct phenotypic characters of the four infraspecific taxa *E. a. ausonides*, *E. a. mayi*, *E. a. coloradensis* and *E. a. ogilvia* (e. g. different shape of chrysalid in *E. a. ogilvia*: Back 1990) seems reasonable to us. The validity of the subspecies *E. a. insulana* Guppy & Shepard and *E. a. paleoreis* K. Johnson was not tested as no individuals from these described taxa were included in our study.

**Mating barriers.** Behavioral mating strategies in butterflies have been detailed by Scott (1974, 1986) as perching or patrolling, and it is clear that all *Euchloe*, in common with all temperate Pierinae, have males that patrol in search of mates. Overlain on these mate location modes is the topographic character on which mate location takes place. In western North America, a clear division between use of peaks and ridges versus drainage courses may be made (Scott 1974). Shields (1967) detailed hill-topping behavior of many butterfly species at one site in California. In Anthocharidini, mate-seeking takes place almost entirely before noon, and the individuals wander more widely afterwards. Newly emerged females of hilltopping species fly to ridges and peaks for mating (Opler personal obs.), while females of drainage followers may be more random in orientation.

*Euchloe* populations and species may also be characterized as either hilltopping or drainage following.

*Euchloe olympia* throughout its range is a ridge and hilltop follower, while *Euchloe ausonides* is a drainage follower in almost all cases. These two species are broadly sympatric and synchronic along the foothills of the Rocky Mountain front in Colorado, Wyoming, and Montana. Since these two species may hybridize (as shown by rare intermediates), we feel that the prime importance of the disparate mate-location venues is a mostly effective mating barrier. After mating, females scatter and seek nectar sources and, most importantly, their respective hosts for oviposition. In the case of the above species pair, oviposition choices are primarily *Arabis* species, although other Brassicaceae may also be selected (Opler 1974; Opler & Clench 1983).

For other North American *Euchloe*, hilltopping is the primary mate location strategy we have observed (unpublished), especially for *Euchloe hyantis* and northernmost *Euchloe guaymasensis* populations. Exceptions are some populations of *Euchloe hyantis* in the western Sierra Nevada foothills where males patrol the stands of their *Streptanthus* (Brassicaceae) hosts along steep cliffs (Shapiro pers. comm.), and more southern populations of *Euchloe guaymasensis* whose males patrol shaded hillsides (Brock & Opler pers. obs.). In northern Sonora where both *Euchloe hyantis lotta* and *Euchloe guaymasensis* use hilltopping behavior, the result may be open mating and hybrid swarms. We have no information on the mate-location strategy of *Euchloe creusa*.

**Conclusions:** 1. The COI gene bar code method is most suitable for the discrimination of genetic divergence of genera, species and subspecies, and one should not make firm conclusions about phylogenetic relationships and lineage based on only a single mitochondrial gene. Nevertheless, we feel our analysis is novel and somewhat informative and may stand until more inclusive studies are performed.

2. Nearctic *Euchloe* are placed in species groups II and V of the cladogram for Palaearctic *Euchloe* (Back *et al.* 2008). We discuss how these placements compare with the species groups presented by Opler (1971) and others that are based on both larval and adult phenotypic characters.

3. In the investigated Nearctic specimens (n=50), 19 different haplotypes in 5 nominal taxa were detected.

4. Species group II is represented by the Holarctic *E. creusa*, *E. olympia*, *E. hyantis*, and the *E. guaymasensis* complex. There is almost no genetic divergence between Siberian and North American *E. creusa* strongly suggesting recent trans-Beringian exchange of populations. Affinities of *E. olympia* and *E. guaymasensis* are shown genetically and by shared phenotypic characters. A hybrid zone between *E.*



*guaymasensis* and *E. hyantis* is probably the result of secondary contact through their use of the weedy larval host *Descurainia pinnata*.

5. The *Euchloe hyantis* group shows little divergence in COI showing their strong relatedness. Because of the phenetic, biological, and distributional integrity, the taxa *hyantis*, *lotta*, and *andrewsi* may be treated as geographic subspecies. We believe that when additional genes are sequenced, these taxa may in fact display further genetic distinctiveness.

6. Because of its phenetic distinctness and close parapatry with other *E. hyantis* populations, with a concomitant lack of intermediacy, we feel that *E. andrewsi* can be treated as a local endemic species with strong need for conservation concern and action.

7. Scott placed *ausonides* as a subspecies of *E. ausonia*, our analysis shows at least 2.76% genetic divergence between the two taxa.

8. Mating barriers may be affected by the mate-location systems described by Scott in several papers. The vast majority of North American Pierinae utilizes patrolling mate location, and the Anthocharini are no exception. Further, sympatric species may be either ridge followers or drainage followers to enhance genetic isolation. Despite the fact that *E. olympia* males are ridge followers, and *E. ausonides* males are drainage followers in their area of sympatry, rare hybrids do occur.

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