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Cover Illustration: *Euchloe olympia*, one of the North American species of marbles. See paper on page 1. Image by Paul Opler.

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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 Palearctic 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). 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). 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, VersaFluorTM) 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 ß 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*	*Euchloe*	*ausonia*	*taurica*	Röber, 1907	Armenia, S. Zangezur, Megrinski Khrebet, Shwaidzov valley, 1800m, 22.V.2001, leg. A. Dantchenko.	FM196446
WB025*	*Euchloe*	*pulverata*		(Christoph, 1884)	Kazachstan, Ily desert, 10km from S. Bakanas, 6.5.1996.	FM196447
WB026*	*Elphinstonia*	*charlonia*		(Donzel, 1842)	S.E. Egypt, border to Sudan, Jabal Hamatah, app. 1500-1900m, early March 2003, leg. G. Müller.	FM196448
WB028*	*Euchloe*	*crameri*		Butler, 1869	Spain, Andalusia, Baza, 2.VI.2004, leg. W. Back.	FM196449
WB034*	*Iberochloe*	*tagis*	*granadensis*	(Ribbe, 1910)	Spain, Andalusia, road to Ronda,15km N. San Pedro, 17.IV.2001, leg. W. Back.	FM196450
WB037*	*Euchloe*	*belemia*		(Esper, 1800)	Spain, Andalusia, Baza, 31.III.2005, leg. W. Back.	FM196452
WB042*	*Anthocharis*	*cardamines*		(Linnaeus, 1758)	Germany, Bavaria, Freising, e.o. 11.-20.IV.2005, leg. W. Back.	AM262790
WB051*	*Euchloe*	*ogilvia*		Back, 1990	Canada, Yukon Ter., Dempster Highway, mile 1, e. p. May 1982, leg. July 1981.	FM196455
WB079*	*Euchloe*	*simplonia*		(Boisduval, 1828)	France, Ht. Alpes, le Monétier-les-Bains, Briancon, Col du Lautaret, 21.-22.VI.1984 egg, e.o. early IV.1984, leg. W. Back.	FM196457
WB081*	*Euchloe*	*grancanariensis*		Acosta, 2008	Spain, Canary Islands, Gran Canaria, Mogan, 14.III.1992, leg. W. Back.	FM196459
WB087*	*Euchloe*	*persica*		Verity, 1908	Iran, Chuzistan, Ramshir, Omidiye, 100m., larva 11.IV.1978, e.l. 10.V.1981, leg. Back & Reissinger.	FM196464
WB088*	*Euchloe*	*daphalis*		(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*	*Euchloe*	*falloui*		(Allerd, 1867)	Morocco, E Tafraoute, Env. Ait-Abdallah, 1600-1800m, 1.-15.IV.2006, leg. M. Tarrier.	FM196466
WB094*	*Euchloe*	*creusa*	*emiorientalis*	Verity, 1911	Russia, Siberia, Tuva near Todzha, 11. VI. 1993.	FM196467
WB095*	*Euchloe*	*creusa*	*emiorientalis*	Verity, 1911	Russia, Siberia, Tuva near Todzha, 11. VI. 1993.	FM196468
WB106*	*Euchloe*	*ausonides*	*ausonides*	(Lucas, 1852)	USA, WA, Whitman County, Snake River Canyon, 400m, 2.IV.1992, leg. John Reichel.	FM196472
WB107	*Euchloe*	*ogilvia*		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*	*Euchloe*	*insularis*		(Staudinger, 1861)	France, Corsica, Castetau, 500m, 18.IV.2001.	FM196483
WB153*	*Euchloe*	*hesperidum*		Rothschild, 1913	Spain,Canary Islands, Fuerteventura, Vega del Rio Palma, 5km S Betancuria, 300m, 9.II.1990, leg. Aistleitner.	FM196494
WB162*	*Euchloe*	*eversi*		Stamm, 1963	Spain, Canary Islands, Tenerife, Montana de la Crucita, 2040 m, 24.-29. IV. 2006, leg. Carlo Pensotti.	FM196500
WB177	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	USA, AZ, Pima County, Catalina Highway, milepost 2, Santa Catalina Mts., 22.II.2003, leg. Jim Brock.	FR728197
WB178	*Euchloe*	*hyantis*	*lotta*	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*	*Euchloe*	*creusa*	*orientalis*	(Bremer, 1894)	Russia, Jakutia, near Tomtor, 800m, 25.VI.1995, leg. Gashko.	FM196508
WB180*	*Euchloe*	*creusa*	*orientalis*	(Bremer, 1894)	Russia, W. Chukotka, Bilibino, 350-500m, 5.-30.VI.2004, leg. G. Kuleshov.	FM196509
WB187*	*Euchloe*	*ausonia*	*naina*	Kozhantschikoff, 1923	Russia, Siberia, W. Sayan, Aradanski Mts., Aradan r. 5.-8.VII.1998, 2100-2300m, leg. V. Komarov.	FM196514
WB189*	*Euchloe*	*melanochloros*		Röber, 1907	Algeria, Biskra, 31.III.1983, leg. Joss.	FM196516
WB199*	*Iberochloe*	*tagis*	*pechi*	(Staudinger, 1885)	Algeria, El Kantara, Dj. Malou Schergij, 850m, egg early IV.1976, e.l. 23.V.1978, leg. W. Back.	FM196521
WB200	*Euchloe*	*hyantis*	*cf. hyantis*	(Edwards, 1871)	USA, CA, Tulare Co., Lamont Meadows, 18.V.2003, leg. Ken Davenport.	FR728199
WB201	*Euchloe*	*hyantis*	*cf. hyantis*	(Edwards, 1871)	USA, CA, Tulare Co., Lamont Meadows, 18.V.2003, leg. Ken Davenport.	FR728200
WB202	*Euchloe*	*hyantis*	*lotta*	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	*Euchloe*	*hyantis*	*lotta*	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*	*Euchloe*	*hyantis*	*hyantis*	(Edwards, 1871)	USA, CA, Napa Co., Butts Canyon, 30.III.1972, leg. Oakley Shields	FM196522
WB205	*Euchloe*	*hyantis*	*hyantis*	(Edwards, 1871)	USA, CA, Napa Co., Butts Canyon, 30.III.1972, leg. Oakley Shields	FR728203
WB213	*Euchloe*	*ogilvia*		Back, 1990	Canada, SW Yukon Terr. Montana Mtn., 1350m, 3.VII.2002, leg. P. Jacubek.	FR728204
WB214	*Euchloe*	*ausonides*	*transmontana*	Austin & J. Emmel, 1998	USA, CA, Sierra Co., S. Loyaltea, Sardine Valley Road, 21.VI.2005, leg. E. Opler.	FR728205
WB215	*Euchloe*	*ausonides*	*coloradensis*	(Edwards, 1881)	USA, CO, Jefferson Co., Coal Creek Canyon, 6.VI.2007, leg. P. A. Opler.	FR728206
WB216	*Euchloe*	*hyantis*	*hyantis*	(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	*Euchloe*	*olympia*		(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	*Euchloe*	*olympia*		(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	*Elphinstonia*	*transcaspica*		(Staudinger, 1882)	Iran, NO-Karaj, Azgi dareh, 1600m, 10.IV.2002.	FR728210
WB220	*Euchloe*	*creusa*		(Doubleday & Hewitson, 1847)	USA, AK, Steese Highway 6, Mile 106, Eagle Summit, 5.VI.1988.	FR728211
WB223	*Euchloe*	*guaymasensis*		Opler, 1987	Mexico, Sonora, Highway 58, 9 miles NW Quisape, 25.III.2005, leg. P.A.&E. Opler.	FR728212
WB224	*Euchloe*	*guaymasensis*		Opler, 1987	Mexico, Sonora, Highway 58, 9 miles NW Quisape, 25.III.2005, leg. P.A.&E. Opler.	FR728213
WB226	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	USA, CA, Inyo Co., Nine Mile Canyon, 1.5 miles E. Tulare Co. Line, 19.IV.2003, leg. K. Davenport.	FR728214

WB227	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	USA, AZ, Pima Co., Box Canyon, Santa Rita Mts., 21.III.2003, leg. P.A. Opler.	FR728215
WB228	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	USA, AZ, Cochise Co., Carr Canyon, Crump Hill, Huachuca Mts., 20.III.2003, leg. P.A. Opler.	FR728216
WB229	*Euchloe*	*hyantis*	*andrewsi*	Martin, 1936	USA, CA, San Bernardino County, San Bernardino Mts., 2.5 air miles NW of Cedarpines Park, 12.VI.1996, leg. Dr. John F. Emmel.	FR728217
WB230	*Euchloe*	*hyantis*	*andrewsi*	Martin, 1936	USA, CA, San Bernardino County, San Bernardino Mts., 2,5 air miles NW of Cedarpines Park, larva on *Strepthantus bernardinus* 18.VI.1994, emgd. 5.IV.1995, leg. Dr. John F. Emmel.	FR728218
WB231	*Euchloe*	*hyantis*	*hyantis*	(Edwards, 1871)	USA, CA, Nevada Co., Fordyce Creek, 5700ft., 9.VI.1974 (High Sierra Type), leg. P.A. Opler.	FR728219
WB232	*Euchloe*	*hyantis*	*hyantis*	(Edwards, 1871)	USA, CA, El Dorado Co., Loon Lake N of Riverton, 9.VI.1973 (Low Sierra Type) , leg. R.L. Langston	FR728220
WB233	*Euchloe*	*lotta x guaymasiensis*			USA, AZ, Cochise Co., Mule Mts., vic. Bisbee, spring 2005, leg. Mike Leski (Hybrid population).	FR728221
WB234	*Euchloe*	*lotta x guaymasiensis*			Mexico, Sonora, W of Cuitapa, Highway 2, 23.III.2003, leg. P.A.&E. Opler (Hybrid population).	FR728222
WB236	*Euchloe*	*ogilvia*		Back, 1990	Canada, Yukon Terr., near Haines, 22.VI.1987.	FR728223
WB238	*Euchloe*	*ogilvia*		Back, 1990	Canada, British Columbia, Pink Mtn., HW 97, Mile 147, 11.VII.1996, leg. N. Tremblay.	FR728224
WB239	*Euchloe*	*ausonides*	*mayi*	(Chermock, 1940)	Canada, Manitoba, Duck Mts., 19.VI.1985, leg. N. Tremblay.	FR728225
WB240	*Euchloe*	*olympia*		(Edwards, 1871)	USA, NE, Sioux Co., Sobelly Canyon, Pants Butte, NE of Harrison, 26.V.1990, leg. J.M. Reiser & J. Reichel.	FR728226
WB242	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	Canada, British Columbia, Southern interior B.C., Fountain Ridge, 700m, 21.IV.2001, leg. P. Jakubek.	FR728227
WB243	*Euchloe*	*hyantis*	*lotta*	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	*Euchloe*	*creusa*	*creusa*	(Doubleday & Hewitson, (1847)	Canada, British Columbia, Pink Mts., HW 97, Mile 147, 11.VII.1996, leg. N. Tremblay.	FR728229
WB246	*Euchloe*	*creusa*	*creusa*	(Doubleday & Hewitson, (1847)	Canada, British Columbia, Pink Mts., HW 97, Mile 147, 8.VII.1996, leg. N. Tremblay.	FR728230
WB247	*Euchloe*	*creusa*	*creusa*	(Doubleday & Hewitson, (1847)	USA, AK, Steese Highway 6, Mile 106, Eagle Summit, 5.VI.1988.	FR728231
WB254	*Euchloe*	*ausonides*	*mayi*	(Chermock, 1940)	Canada, Manitoba, Duck Mts., 19.-20.VI.1984, leg. N. Tremblay.	FR728232
WB255	*Euchloe*	*ausonides*	**ssp.**		Canada, Alberta, East Slope of Rocky Mountains, Forestry Trannk Road, 200km South of Nordegg, 19.VI.1980, leg. John Reichel.	FR728233
WB257	*Euchloe*	*lotta x guaymasiensis*			USA, AZ, Cochise Co., Mule Mts., vic. Bisbee, spring 2005, leg. Mike Leski (Hybrid population).	FR728234
WB308	*Euchloe*	*hyantis*	*lotta*	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	*Euchloe*	*ausonides*		(Lucas, 1852)	USA, CA, Mariposa Co., Briceburg, 9.III.1983.	FR728236
WB321	*Euchloe*	*hyantis*	*lotta*	Beutenmüller, 1898	USA, CA, San Diego Co., Jacumba, 29.-30.III.1993, leg. W. Back.	FR728237
WB322	*Euchloe*	*ausonides*		(Lucas, 1852)	USA, CA, Marin Co., Sausalito, 2./3.IV.1993, leg. W. Back.	FR728238
WB323	*Euchloe*	*creusa*	*orientalis*	(Bremer, 1894)	Russia, Siberia, Wrangel-Island, N. Magadan, 29.VI.-7.VII.1992, leg. T. S. Churkin	FR728239

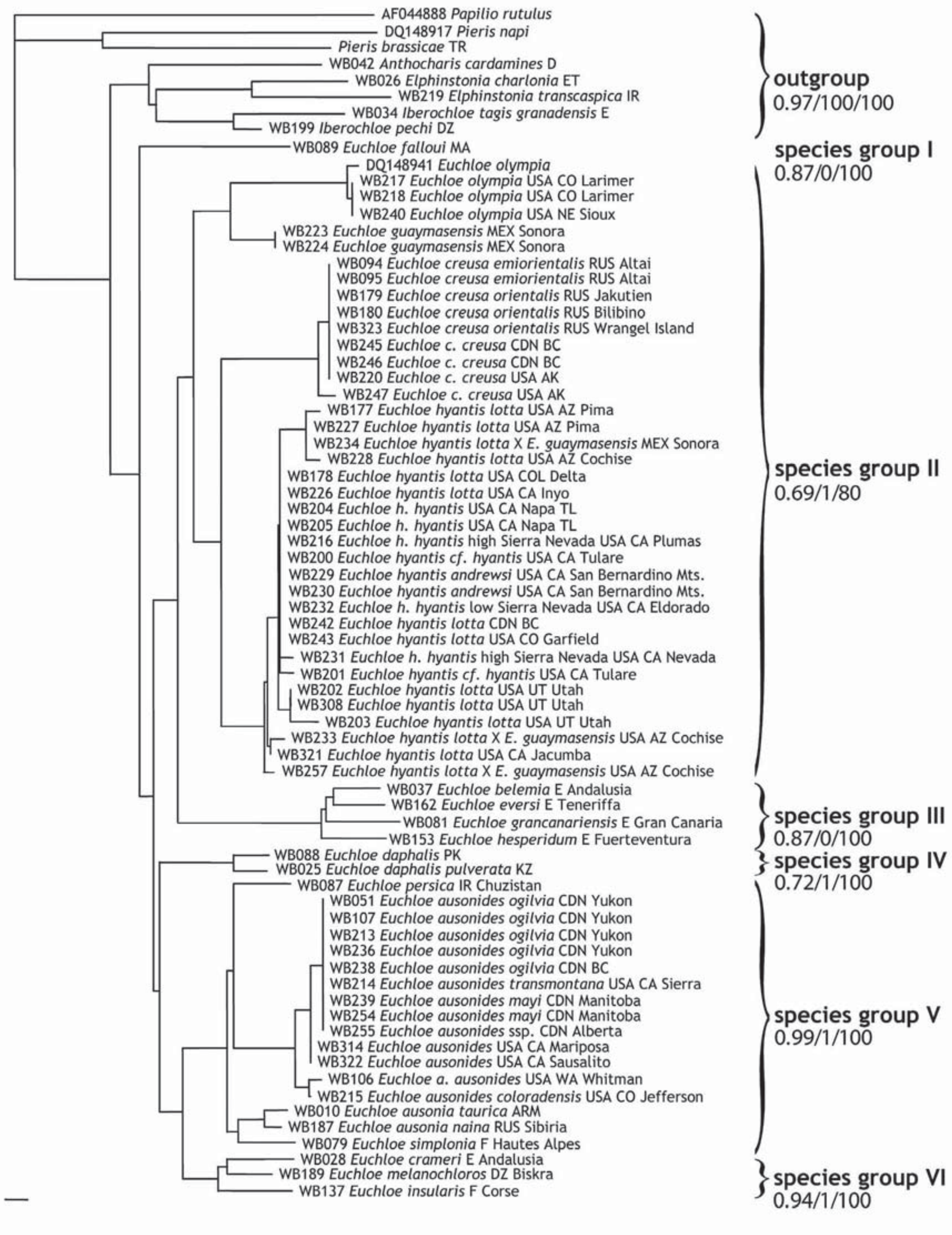

FIG. 1. Neighbor joining tree of *Euchloe* taxa. DNA sequences of mtCOI gene contained 326 bp each. Outgroup rooting was set to *Papilio rutulus*. Representatives of yet established species groups I, III, IV, and VI were only single specimens. CI was 0.494, RI was 0.817. Bar indicates 0.005 changes. 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, HTeo1, 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*.

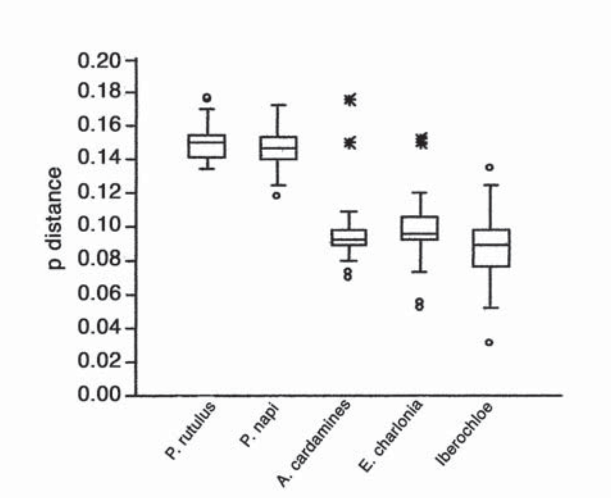

FIG. 2. Box plots of genetic distances. Box 1: genetic distance of outgroup taxon *Papilio rutulus* (AF044888, Papilionidae) to *Euchloe* taxa. Box 2: genetic distance of *Pieris napi* (DQ148917, Pieridae) to *Euchloe* taxa. Box 3: genetic distance of *Anthocharis cardamines* to *Euchloe* taxa. Box 4: genetic distance of *Elphinstonia charlonia* 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 (*E. falloui*)	*E. falloui*	
II (*E. creusa*)	*E. creusa*	*E. olympia, E. guaymasensis,* *E. hyantis* complex
III (*E. belemia*)	*E. belemia, E. hesperidum,* *E. eversi, E. grancanariensis*	
IV (*E. daphalis*)	*E. daphalis*	
V (*E. ausonia*)	*E. ausonia, E. simplonia, E. persica*	*E. ausonides*
VI (*E. crameri*)	*E. crameri, E. melanochloros, E. insularis*	

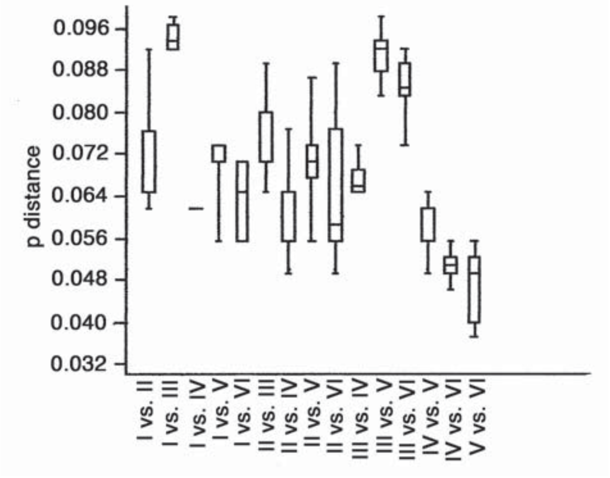

FIG. 3. Box plots of genetic distances within *Euchloe* species groups. I vs. II, I vs. III, I vs. IV, I vs. V, I vs. VI, II vs. III, II vs. IV, II vs. V, II vs. VI, III vs. IV, III vs V, III vs VI, IV vs. V, IV vs VI, V vs. VI.

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).

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

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, 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 cospecies. 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=

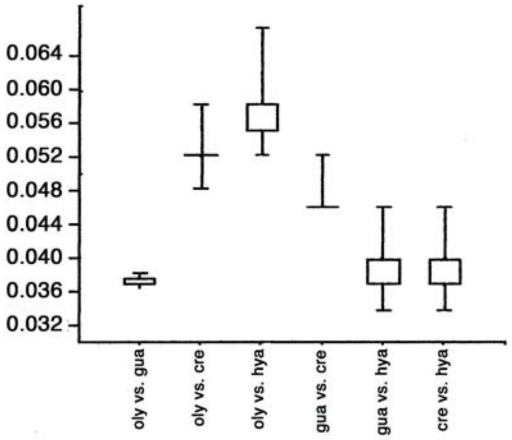

FIG. 4. Box plots of genetic distances of species within *Euchloe* species group II.

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

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

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

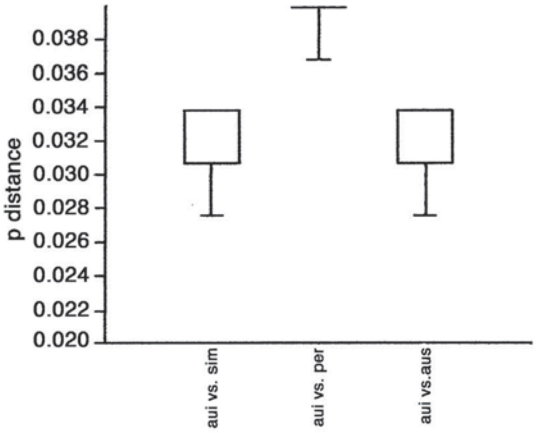

FIG. 5. Box plots of genetic distances of species within *Euchloe* species group V.

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
E. ausonides vs. *E. simplonia*	0.03916942	0.00129241
E. ausonides vs. *E. persica*	0.03256253	0.00191695
E. ausonides vs. *E. ausonia*	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 similiarities to *E. creusa*, leading to an occasionally proposed conspecificity of these two species (Talbot 1932-1935). Because of phenotypic similiarities 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, Knebelsberger & 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, Garfeld Co., Colorado (WB243). We refer this Colorado individual as closest to topotypical *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 topotypical *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. hyanthis*/*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 *Descurainea 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 *Descurainea 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 decribed 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* (Brassicaeae) 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 probabaly the result of secondary contact through their use of the weedy larval host *Descurainea 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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MOROCCAN SOURCE AREAS OF THE PAINTED LADY BUTTERFLY *VANESSA CARDUI* (NYMPHALIDAE: NYMPHALINAE) MIGRATING INTO EUROPE IN SPRING

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ABSTRACT. Each spring, *Vanessa cardui* (Linnaeus) migrates into Europe, sometimes in huge numbers. Although it is widely accepted that migrants come from North Africa, there is currently a dearth of information on the source areas they originate from. However, in a recent study, a strong association between *V. cardui* arriving in spring in Catalonia (NE Spain) and synoptic-scale winds originating in North Africa was found, which allowed us to predict the most likely location for the source areas in the Maghreb. In March 2009 we organized an expedition to confirm *in situ* the occurrence of immature stages and/or emerging adults at one of the hypothetical breeding areas in Morocco. Immatures were found at virtually all the sampled sites, with the maximum abundance being recorded in the Souss valley. Breeding habitats with plenty of thistles and mallows were sufficiently common to permit populations of great density to build up. We found a massive emergence site of 1.8 ha with an estimated population of 170,000 larvae, of which 155,000 had successfully pupated, the rest having been parasitised by the wasp *Cotesia vanessae*. Bird predation of adults was also recorded at this site. Our observations, together with the typical patchy distribution of the ruderal habitats used for breeding, suggest that source populations most frequently originate in small habitat pockets with favourable conditions that allow for high patch occupancy and levels of oviposition.

Additional key words: hostplants, parasitoids, predation, population outbreak, Morocco

Undoubtedly, *Vanessa cardui* (Linnaeus) stands out as one of the best known and most popular butterfly migrants in the world. Its spectacular migrations have been recorded for at least the last two centuries, both in Europe and North America (see Williams 1930 for an excellent and comprehensive review of early data). Mass movements across extensive regions and continents have been analysed by several authors in an attempt to synthesise and understand different aspects related to this migratory process, as for example seasonality, flight paths, orientation behaviour and the causes underlying dramatic fluctuations in abundance. In North America, the works of Abbot (1951), Tilden (1962), Williams (1970), Myres (1985), Giuliani & Shields (1997a) and Vandenbosch (2003), and in Europe those of Williams (1951), Pollard (1982), Hansen (1997), Stefanescu (1997), Pollard *et al*. (1998), Stefanescu *et al*. (2007) and Nesbit *et al*. (2009) have all addressed these topics.

Despite all this research, some crucial aspects regarding this migratory system have remained elusive until now. For example, there is a dearth of information on the source areas where the migrants recorded in spring, particularly in Europe, originate from. However, the enormous numbers involved in northward migrations in 'outbreak' years means that there must be some areas where breeding occurs on a vast scale in spring. A few such areas have been localised in Sonora (Mexico), Baja California and California, and Abbot (1951) and, especially, Giuliani & Shields (1995, 1997a,b, 1998) have provided accurate and fascinating descriptions of huge concentrations of larvae and massive emergences of adults in April, May and early June. Breeding grounds consist of desert or semi-desert and chaparral areas, characterised by a short plant-growth season highly dependent on scant and irregular rainfall (Abbot 1951). Moreover, the observations made in consecutive years in Inyo County, California (Giuliani & Shields 1995, 1997a,b, 1998), strongly suggest that butterflies use regular migratory paths and the same areas to breed, although the numbers occurring will fluctuate enormously in terms of resource availability (linked to climatic conditions; cf. Myres 1985; Vandenbosch 2003).

Although it is widely accepted that *V. cardui* migrating into Europe are of African origin (e.g. Pollard *et al*. 1998), there are in fact very few observations of mass breeding in Africa. Skertchly (1879) and Egli (1950; quoted by Johnson 1969, p. 42) localised two such areas in Sudan (in March) and near Algiers, respectively. In both cases, thousands of butterflies were seen to emerge from pupae in the early morning and then take off within an hour or so and head towards the sea. More recently, Owen (1987, 1989) reported vast numbers of larvae in Lanzarote (Canary Islands) in late February and in Madeira in April, and suggested that these islands could act as source areas for European migrants recorded in spring. Other potential sources can be traced from the detailed account provided by Williams (1930) of remarkable concentrations of larvae and/or adults showing ovipositing behaviour in several areas of north Africa (e.g. Egypt).

It is most likely, however, that the sources of spring migrants vary according to the European region under consideration. For instance, observations in some Eastern Mediterranean islands and Turkey seem to indicate that migrants recorded in Eastern Europe mainly originate in the Arabian Peninsula (John 2001), where directional flights of *V. cardui* are a common sight (Wiltshire 1940; Larsen 1976, 1982; Pittaway 1981). Indeed, all the available data strongly suggest that European spring migrants originate from the band of desert crossing North Africa and the Arabian Peninsula in a wide latitudinal zone roughly distributed south of 35º N (Emmet & Heath 1989; Shields 1992).

Much more detailed information comes from a recent study by Stefanescu *et al*. (2007), who analysed data from the Catalan Butterfly Monitoring Scheme (CBMS) for a period of over 10 years and found a strong association between *V. cardui* arriving in spring in Catalonia (NE Spain) and synoptic-scale winds originating in North Africa. This led these authors to suggest that significant butterfly migration was aided by African winds and occurred at several hundred metres above ground level. Meteorological trajectory analyses were then undertaken to identify probable source areas of migrants. According to a source-receptor model, migrants arriving in Catalonia in April–May mainly originate from three different areas in the Maghreb, located at a distance of 900–1,600 km: the Moroccan Anti-Atlas (and to a lesser extent in the equivalent area south of the Saharan Atlas in Algeria), the low plain of north-eastern Algeria stretching away across central Tunisia and up to the coast, and the coastal area of Tripolitania in Libya. If most migration is windborne and occurs at high altitudes butterflies would only need 20-36 hours to complete the journey with the help of tailwinds (Stefanescu *et al*. 2007). As the arrival of migratory waves in Catalonia follows a regular pattern and is concentrated in April and early May, this study provided, for the first time, testable predictions of the location and timing of source areas for *V. cardui* migrating into northern Spain in spring.

With this background information, in March 2009 we organized an expedition to one of the hypothetical breeding areas in Morocco to confirm *in situ* the occurrence of immature stages and/or emerging adults. In this article we describe the findings of the expedition and provide a full account of the biological observations that we gathered at one mass breeding site in western Morocco. For the first time, we present precise estimations of population density and mortality factors (viz. parasitism) at one source area of *V. cardui* in the African continent.

MATERIALS AND METHODS

Study species. The Painted Lady, *Vanessa cardui*, is one of the most widely distributed butterflies in the world, being absent only from tropical rain forests and parts of South America (Shields 1992). This cosmopolitan distribution is a consequence of its strong migratory behaviour coupled with the polyphagy of its larvae, two ecological traits that allow the species to thrive in virtually any kind of habitat.

Migratory phenology in the Palaearctic. In the Palaearctic, each spring the species arrives in Europe from northern Africa, spreading northwards from the Mediterranean in at least two successive generations (Williams 1970; Emmet & Heath 1989; Pollard *et al*. 1998). This poleward migration allows *V. cardui* to exploit resources that become available in spring and summer in the temperate zone, and to escape from the inhospitable environment of southern regions as the season advances.

In southern Europe, migratory waves are mainly recorded in April, May and early June (Stefanescu 1997; Pollard *et al*. 1998; Stefanescu *et al*. 2007). Migrants breed on a diversity of plants (see below) and give rise to a local generation at the start of summer. Newly emerged butterflies leave breeding areas quickly as they encounter the hot dry conditions typical of the Mediterranean summer and migrate northward in search of both nectar and egg-laying resources (Pollard *et al*. 1998). Adults arriving in Britain and Northern Europe in early summer are the progeny of the butterflies breeding in southern Europe; the few individuals regularly recorded there in the spring are thought to arrive directly from North Africa (Asher *et al*. 2001). Although adults emerging subsequently in northern Europe are forced to head for lower latitudes to escape increasingly unfavourable colder conditions at the end of the northern summer and early autumn, very few observations of southward movements exist (cf. Pollard *et al*. 1998). The lack of evidence of these return flights may be related to butterflies' use of alternative as yet unknown migratory routes: laboratory-reared autumn-generation butterflies flying in the UK displayed a highly significant mean orientation towards the SSW, consistent with a return migration to overwintering sites (Nesbit *et al*. 2009). Moreover, sudden appearances of huge numbers of worn adults in the Maghreb, the Canary Islands and the northern edge of the Sahel in September–October strongly support the existence of a return flight (C. Stefanescu & F. Páramo unpubl. data).

Larval hostplants and biological cycle. *V. cardui* is extremely polyphagous and many hostplants belonging to a number of plant families are known to be used in the wild (e.g. Ackery 1988; Whitebread 1988). However, in western Europe oviposition occurs mostly on thistles (many species in the tribe Cardueae, family Asteraceae) and common mallow, *Malva sylvestris* (Linnaeus) (e.g. Stefanescu 1997). Eggs are laid singly on the upper side of the leaves of the hostplant, each female potentially laying as many as 700 eggs (mean±SD: 507±57 eggs; Hammad & Raafat 1972). On hatching, larvae make a characteristic silken tent on the hostplant leaf. As it grows, the tent becomes very visible and may consist of the edges of one or more host leaves woven together. Excrements accumulate inside the tent, which remains highly conspicuous long after the larva has departed to pupate elsewhere.

V. cardui is a continuously–brooded species in which migration is used as an alternative to diapause as a means of surviving climatic extremes (Wiltshire 1949; Larsen, 1976). In laboratory-reared butterflies, Hammad & Raafat (1972) estimated a developmental time of ca. five days for the egg, 25 days for the five larval instars and 14 days for the pupa. The completion of the whole cycle in north-east Spain takes six to eight weeks, depending on temperatures (C. Stefanescu pers. obs.).

Natural enemies. Larvae and pupae are known to be attacked by several parasitoids (Shaw *et al*. 2009). Amongst those most frequently recorded is *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae: Microgastrinae), a gregarious larval parasitoid that parasitizes early instar larvae without killing them until they reach their fourth or, more usually, fifth instar. At that point, the larvae of the parasitoid erupt from the host to pupate in individual cocoons aggregated within a highly conspicuous mass of flocculent white silk. Cocoon masses are typically found close by the dead host larva. *Thyrateles camelinus* (Wesmael) (Hymenoptera: Ichneumonidae: Ichneumoninae) and several tachinids (Diptera: Tachinidae: Exoristinae) have also been reported as frequent larva-pupal parasitoids in the European (Linnaeus) range of *V. cardui*, as has the generalist *Pteromalus puparum* (Hymenoptera: Pteromalidae), which often parasitizes the pupae.

Data on predation of the adult stage are much scarcer. However, there are various observations of insectivorous birds (e.g. Spotted Flycatcher *Muscicapa striata* (Pallas) and European Bee-eater *Merops apiaster* (Linnaeus)) preying upon *V. cardui*, especially during migration (see Stefanescu & Julià 2002, for details).

Study area and sampling. The expedition was initially focussed on central western Morocco, at approximately latitudes 29º34'–31º15' N and longitudes 5º10'–8º10' W, one of the areas predicted as a source area for migrants arriving in north-eastern Spain in spring (see Stefanescu *et al*. 2007). We eventually extended our sampling area westwards to include the Souss valley. According to the timing of spring migration, we expected to find larvae in various developmental stages at the end of March and in early April (see above). Our data was complemented by additional observations made by several colleagues who had visited Morocco in February, March and April.

From 24 to 30 March 2009 we traveled across this region, performing detailed searches for eggs and larvae at different sites (Table 1). We chose those areas offering potential breeding grounds for *V. cardui* based on the presence of suitable hostplants (e.g. patches of thistles or mallows along road banks or in date plantations). We also carried out searches on other potential hostplants (e.g. *Echium* spp., *Forsskaolea tenacissima* (Linnaeus), *Plantago lanceolata* (Linnaeus), etc.) that have been recorded as hostplants in Morocco and the Canary Islands (C. Stefanescu pers. obs.). At each sampling site we spent a variable amount of time

TABLE 1. Locations surveyed for *Vanessa cardui* immatures on central western Morocco in March 2009. Abundance of immatures follows a qualitative scale, from 0 (no egg, larva or pupa found) to 5 (>1,000 immatures). MDV gives the value representing the mean developmental stage of the breeding population, from 0 (egg) to 7 (freshly emerged adult). Also indicated is the presence (1) or absence (0) of the parasitoid *Cotesia vanessae* in samples of wild larvae reared in the laboratory.

Study site	Longitude (ºW)	Latitude (ºN)	Altitude (m)	Date	Immature abundance	MDV	Presence of *C. vanessae*
P3	-7.54	31.54	931	24-Mar-09	0	-	-
P4	-7.38	31.27	2149	24-Mar-09	0	-	-
P6	-6.58	31.03	1164	25-Mar-09	0	-	-
P7	-6.32	31.13	1298	25-Mar-09	0	-	-
P8	-5.82	31.37	1452	25-Mar-09	0	-	-
P9	-5.34	31.45	1151	26-Mar-09	2	4.3	1
P10	-5.34	31.44	1132	26-Mar-09	2	5.5	1
P11	-5.20	31.14	896	26-Mar-09	2	3.7	1
P12	-5.27	30.99	885	26-Mar-09	0	-	-
P13	-5.52	30.79	848	26-Mar-09	2	2.5	1
P14	-5.58	30.79	868	26-Mar-09	3	3.9	1
P15	-6.56	30.66	1039	27-Mar-09	3	4.7	1
P16	-6.90	30.53	1447	27-Mar-09	2	3.3	0
P17	-6.98	30.47	1062	27-Mar-09	1	2	0
P18	-6.92	30.43	995	27-Mar-09	1	2	0
P19	-6.93	30.43	1008	27-Mar-09	1	2	0
P20	-7.91	30.52	1015	27-Mar-09	3	4.5	1
P21	-8.18	30.62	655	28-Mar-09	3	6.9	0
P22	-8.75	30.49	287	28-Mar-09	2	6.4	1
P23	-8.79	30.49	254	28-Mar-09	2	5.8	1
P24	-8.85	30.49	255	28-Mar-09	5	6	1
P26	-8.90	30.43	204	29-Mar-09	3	6.6	1
P27	-9.07	30.06	780	29-Mar-09	2	5	1
P28	-9.03	29.99	1069	29-Mar-09	2	4.1	1
P29	-9.00	29.89	1213	29-Mar-09	2	3	0
P30	-9.15	30.07	564	30-Mar-09	3	6.8	1
P31	-9.25	30.17	209	30-Mar-09	4	6.5	1
P32	-8.86	31.28	659	30-Mar-09	2	2.8	0
P33	-8.67	31.58	334	30-Mar-09	-	-	-
P34	-8.58	31.58	361	30-Mar-09	3	4	1

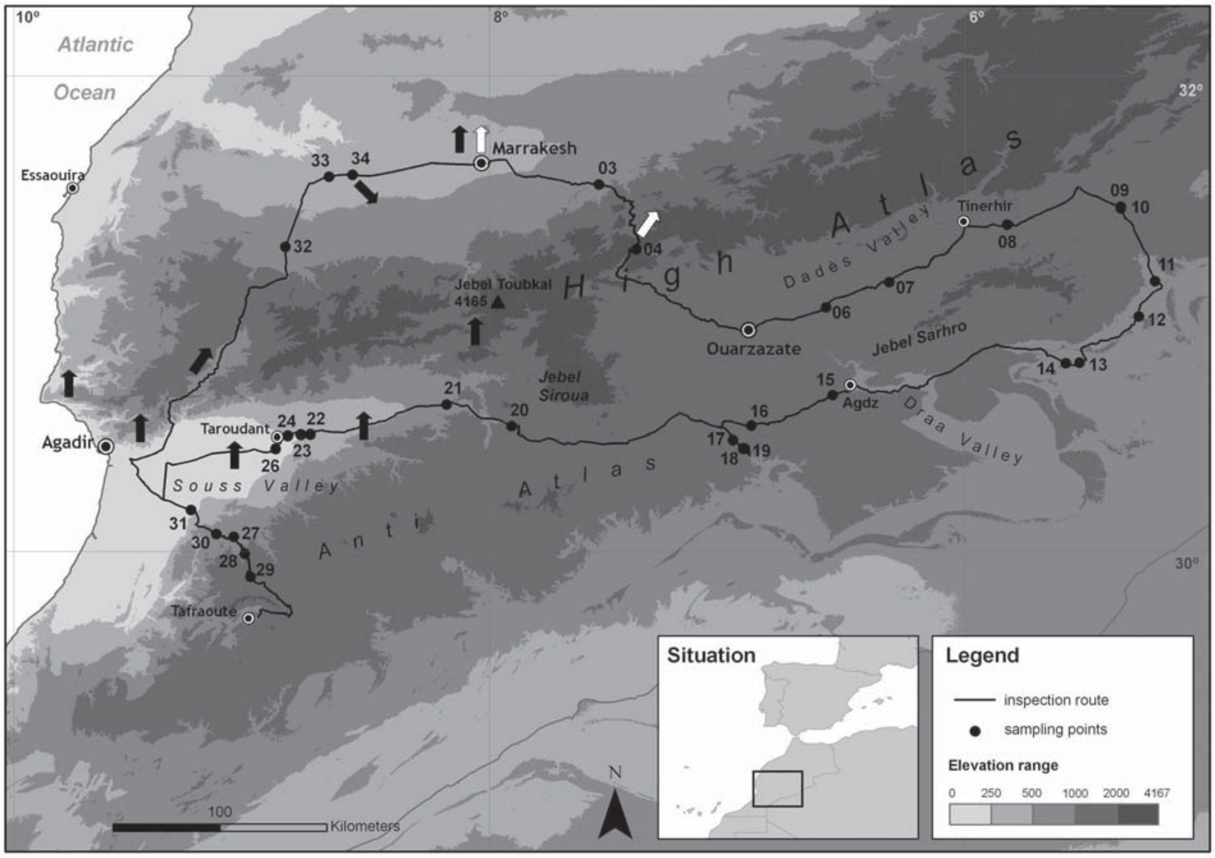

FIG. 1. Area surveyed in Morocco, with indication of sampling sites for immatures and adult migratory flights (black arrows: migrations of freshly emerged adults; white arrows: migrations of worn adults).

(between 15–60 min) that was proportional to the number of potential hostplants present. Although the resulting data was not strictly comparable, we believe that we spent enough time at each site to provide an accurate estimate of the breeding population, as expressed in the following qualitative scale: 0 = no immatures found (egg, larva or pupa), 1 = 1 immature, 2 = 2–10 immatures, 3 = 11–100 immatures, 4 = 101–1,000 immatures, 5 = >1,000 immatures. The presence of adults, their wing wear and behaviour were also recorded. Freshly emerged adults can be easily distinguished from immigrants by their bright colours and perfect wing condition.

At the sampling sites, each larval nest found was inspected to determine larval instar according to cephalic size. Large empty nests with accumulated frass were considered to correspond to final-instar larvae that had left to pupate elsewhere. We then calculated, for each sample, a value representing the mean developmental stage of the breeding population according to the scale: 0 = egg, 1–5 = larval instars, 6 = empty nest corresponding to the last larval instar or pupa; 7 = freshly emerged adults.

At site P24 (see Results) we localised an exceptional and perfectly delimited small area of 1.8 ha where pupae were hanging from the low vegetation in enormous densities and adult emergence was taking place. To estimate population density at this site we carried out a belt transect survey, consisting of pupal counts along 10 random replicated belt transects of 10 × 1 m. Starting at the centre of the patch, we used random numbers to determine the position and orientation of each of the 10 belt transects.

Parasitoids attacking the immature stages were also investigated. Wild caterpillars of any instar were collected whenever found at field sites and reared in the laboratory until they produced either parasitoids or adult butterflies. Cocoon masses of *Cotesia vanessae* found directly on hostplants were also used to record its presence in a breeding population. In addition, the incidence of *C. vanessae* was estimated at site P24 by counting the cocoon masses found along the belt transects established for pupal counts. A sample of 50 wild pupae was also collected at this site to investigate pupal parasitoids.

Results

Distribution of immatures. During the trip, we searched for immatures of *V. cardui* at 29 sites in an area of ca. 30,000 km^2 (Table 1; Fig. 1). Immatures were found at 23 sites, only being absent from the north-easternmost area, that is, the High Atlas and the high plateau situated between Ourzazate and the valley of Dadès. Past Tinerhir, immatures started to appear on a variety of hostplants, becoming commoner as we moved towards lowland areas near the Atlantic coast. This is clearly shown by the strong tendency of immatures to increase their abundance with decreasing altitude ($r = -0.65$, $P < 0.001$) and as we moved westwards ($r = -0.45$, $P = 0.014$). This increase in abundance was paralleled by an increase in the mean developmental stage of the breeding population, which showed the same trends as altitude ($r = -0.64$, $P = 0.001$) and longitude ($r = -0.44$, $P = 0.037$).

Hostplant use. Larvae were recorded on a variety of hostplants belonging to the families Asteraceae, Urticaceae, Boraginaceae, Malvaceae and Arecaceae (Table 2; Fig. 2). In general, thistles (tribe Cardueae) were the most commonly used hostplants, particularly *Onopordon* spp. (up to three different species) growing in habitats such as road banks and fallow land near cultivated areas and villages. Other hostplants commonly recorded were *Echium* spp. and *Forsskaolea tenacissima* (mostly in the Anti Atlas), and common mallow (especially near the coast). Moreover, at site P24, where we found massive numbers of larvae, an unidentified species of *Centaurea*, as well as other thistles and common mallow, were being used. Finally, a subsequent visit to site P3 in April 12 produced a surprising record of a 4th instar larva feeding on *Chamaerops humilis* (Linnaeus), a palm belonging to the family Arecaceae.

Parasitoids. *Cotesia vanessa*e was recorded at 16 of the 23 sites (70%) visited, from where we collected immature stages. It was widely distributed throughout the region, from the western lowland areas of the Souss valley to the eastern high plateau situated between Tinerhir and Agdz. Its apparent absence from sites P16–19, P29 and P32 was probably an artefact of the very small number of larvae collected for rearing at these sites (only 1–5 larvae).

Apart from *C. vanessa*e, the only recorded larval parasitoids were the solitary wasp *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae: Microgastrinae), reared from a single larva collected at site P20, and one unidentified Ichneumonidae Campopleginae and one unidentified Braconidae Microgastrinae, reared from two larvae collected at P15. On the other hand, several ichneumonid and chalcidoidea wasps were recorded as pseudohyperparasitoids of *C. vanessae* cocoons (cf. Shaw *et al*. 2009). Details of this parasitoid complex will be presented elsewhere (C. Stefanescu, M.R. Shaw & R.R. Askew in prep.).

In total, 17 out of 50 pupae collected at site P24 turned out to be parasitized by *Pteromalus puparum*. No larva-pupal parasitoids (e.g. either ichneumonid wasps or tachinid flies) were recorded from this sample. Neither did we record any dead pupae in the transect counts exhibiting the characteristic proteinaceous strands left by tachinid larvae when vacating their host pupa (Baumgart *et al*. 2003).

The population outbreak at site P24. Population densities were especially high in the Souss valley, the vast flat area extending around *oued* Souss immediately south of Agadir (Fig. 1). In the dry riverbed of the *oued* there were large concentrations of thistles, on which larvae from later instars and empty nests were very common. High densities were also recorded on thistles and *Echium* spp. growing on the margins of wheat fields and Argan, *Argania spinosa* (L.) Skeels, woodland, but also in dry sandy areas with sparse vegetation (e.g. with bushes of the Asclepiadaceae *Calotropis procera* (Aiton)). However, the highest density was found in the outskirts of the city of Taroudant (site P24, Fig. 1). Attracted by an unusual concentration of butterflies flying low over the ground, we located an emergence area consisting of a small patch of waste land of 1.8 ha, perfectly delimited by the main road, a river bank, and several buildings and crops, where there were huge numbers of fresh adults and pupae hanging in their thousands from the herbs and bushes (Fig. 2).

Pupal density in the patch was estimated at 85.8±27.8 pupae/10 m^2 (95% confidence interval), which gives a total number of 154,440±50,109 pupae for the whole patch (Table 3). Pupae were found on all types of vegetation, but were especially abundant on bushes of *Launaea arborescens*, a spiny Asteraceae that was likely selected as a pupation site due to the mechanical protection it affords against predators. In some bushes we counted up to 200–300 pupae (Fig. 2). Butterflies had already emerged from most of the pupae (94% of the pupae were empty). Of the remaining 6%, 1.5% of the pupae bore characteristic signs that could be attributed to invertebrate predation (e.g. lateral holes in the pupal case; see Frank 1967), 1.7% were parasitized by *Pteromalus puparum* (as estimated from our rearing sample), and 2.8% were healthy (Table 3).

Cocoons of *C. vanessae* (each one corresponding to a larva that had been parasitized) were also very common among the vegetation. From the transect counts, we estimated a density of 9.5±6.8 cocoon masses/10 m^2,

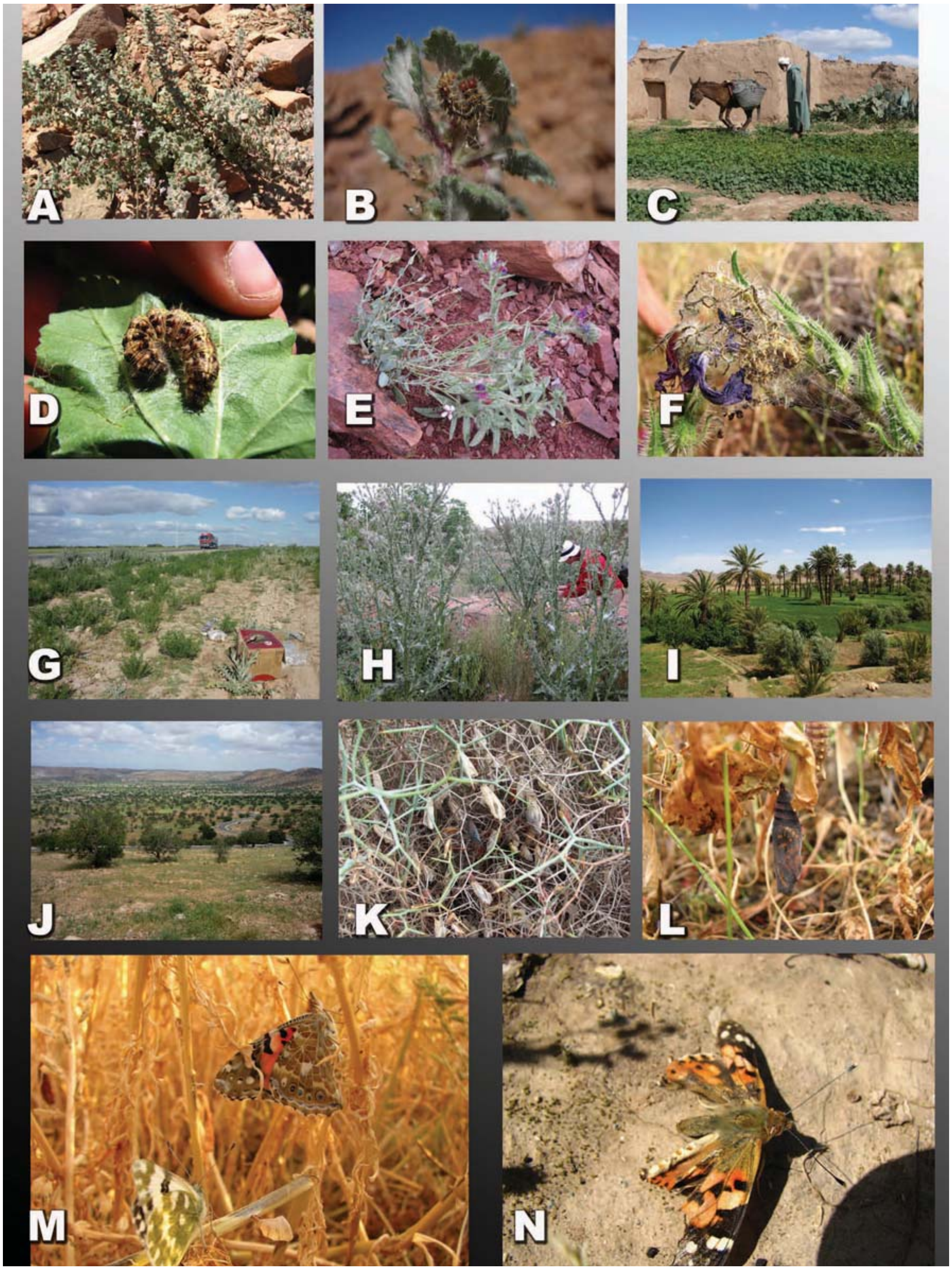

FIG. 2. Main hostplants of *Vanessa cardui* and explored landscapes in Morocco, March 2009. **A,** General aspect of *Forsskaolea tenacissima*; **B,** and plant with a 5th instar larva; **C,** patch of *Malva sylvestris* in a village; **D,** and plant with a 5th instar larva; **E,** *Echium* spp.; **F**, and plant with a 5th instar larva; **G,** road bank plenty of *Onopordon* sp.; **H,** and plants bearing larval nests; **I,** site P11, palm plantation in the Jebel Sarhro near Alnif; **J,** the Souss valley; **K–L,** concentration of pupae in the outbreak population at site P24; **M,** roosting of *V. cardui* and *Pontia daplidice* at site P24; **N,** a crippled adult at site P24, as a result of a heavy downpour at the time of emergence.

TABLE 2. Hostplants of *Vanessa cardui* recorded during the expedition to central western Morocco in March 2009.

Hostplants	Sites
Malvaceae	
Malva sylvestris	P11, P20, P24, P30, P32
Urticaceae	
Forsskaolea tenacissima	P17, P18, P19, P30
Boraginaceae	
Echium spp.	P16, P21, P27, P29, P30
Asteraceae	
Centaurea sp.	P24
Echinops spinosus bovei	P10, P13, P28
Onopordon spp.	P9, P10, P11, P13, P14, P15, P21, P22, P23, P24, P26, P28, P31, P34
Unidentified thistle	P16
Arecaceae	
Chamaerops humilis	P3*

* observation in a later visit, on 12 April 2009

which gives a total number of 15,120±12,188 cocoons for the whole breeding patch. The total population can therefore be estimated as consisting of 169,560±59,984 larvae of *V. cardui* (i.e. pupae plus cocoon masses), of which ca. 10% were parasitized by *C. vanessae* (Table 3).

Observations of adult behavior were obtained on two consecutive days, on the afternoon of 28 and the morning of 29 March. There was no sign of sexual behaviour (i.e. no males showed territorial or courtship behaviour; cf. Brown & Alcock 1991). Instead, butterflies spent most of their time looking for nectar sources. We recorded many butterflies nectaring on *Eucalyptus* sp. and, to a lesser extent, on *Nerium oleander* (Linnaeus) around the breeding patch. Other nectar sources included *Asphodelus fistulosus* (Linnaeus), *Peganum harmala* (Linnaeus), *Reseda alba* (Linnaeus) and the few larval hostplants that had not been completely consumed by the larvae (e.g. *Onopordon* sp., *Centaurea* sp. and *Echium* sp.). On the morning of March 29 we also saw numerous interacting pairs behaving in the manner described by Giuliani & Shields (1995): butterflies on the ground rose up to join butterflies flying above them, the two interacting butterflies then continuing flying together for a certain distance before resettling again or departing northward.

Butterfly emergence was also commonly recorded both in the afternoon and in the morning. In the afternoon of March 28 there were a couple of heavy downpours that lasted for several minutes. As a consequence, some of the emerging butterflies were unable to extend their wings successfully and were found dead on the ground the following day (Fig. 2). By late afternoon we observed many butterflies roosting in the patch itself. They mainly selected high dry twigs of *Reseda alba*, which was the most abundant plant in the area. It was not unusual to find groups of two or three Painted Ladies roosting together on the same plant, sometimes side by side with *Pontia daplidice* (Linnaeus) (Pieridae), the second most common butterfly at the site (Fig. 2).

By the time butterflies started to look for a roosting site, barn swallows, *Hirundo rustica* (Linnaeus), from a colony in a nearby building were attracted by the butterfly swarm. The birds started to hunt the butterflies, mainly chasing them while in flight but sometimes also by picking them off the vegetation. We recorded at least ten birds (including one Red-rumped Swallow, *Cecropis daurica* (Laxmann)) successfully capturing butterflies and taking them to their nests to feed their chicks.

Migratory flights. A migratory wave was detected on the afternoon of 24 March near Col du Tichka (2,250 m), at site P4. Worn adults were seen flying at 0.5–1 m above the ground in ENE direction, in singles or in pairs at an estimated density of 45 butterflies/15 min (as visible to a recorder on the ground). Several old females engaged in searching behaviour at P3 a couple of hours before were probably also part of the same migratory wave (see Fig. 1).

Except for two butterflies found at sites P7 and P10, adults were not detected again until the Souss valley. On

Table 3. Estimations of population density and mortality factors in the outbreak population of *Vanessa cardui* occurring at site P24 on the outskirts of Taroudant.

	Mean	± 95% CI
Density of pupae/10m²		
Already emerged	80.3	25.5
Not emerged yet	4.2	2.9
Dead with signs of predation	1.3	0.9
Total density	85.8	27.8
Density of cocoon masses of Cotesia vanessae/10m²	9.5	6.8
Number of pupae in the patch[1]		
Already emerged	144,540	45,865
Not emerged yet	7,560	5,143
Dead with signs of predation	2,340	1,612
Total number	154,440	50,109
Number of cocoon masses of *Cotesia vanessae* in the patch[1]	15,120	12,188
Total number of larvae in the patch[2]	169,560	59,984

[1]Patch area of 1.8 ha

[2]Number of pupae plus number of larvae parasitized by *Cotesia vanessae*

the other hand, from site P21 westwards, fresh adults were commonly recorded all along the route. Although at some sites only a few butterflies exhibited migratory behaviour (see the previous section), in the mornings and early afternoons of 28, 29 and 30 March we recorded several spectacular mass movements along the main roads between Taroudant and Agadir, Aït–Baha and Agadir, and Agadir and Chichaoua (Fig. 1). Many thousands (if not millions) of newly emerged butterflies were flying in a northward direction, 0.5–2 m above the ground. Many butterflies were knocked down by vehicles when crossing roads and on several occasions we saw house sparrows, *Passer domesticus* (Linnaeus), waiting on road banks to eat the butterflies killed by cars. Interestingly, all the migratory flights coincided with southerly winds, except for one abnormal southward movement (recorded at P34 at 1630 h), when the wind had also veered towards the south (Fig. 1). Fresh adults migrating northwards were also recorded in Marrakech and at a high mountain pass at 3,000 m near the summit of Toubkal during the first week of April (G. Muñoa pers. com.).

Other northward flights consisting of worn individuals were reported by ornithologists working at a bird-ringing station near Merzouga in the second week of March (M. Anton pers. com.). In contrast with the migratory flights recorded in the Souss valley, movements of worn individuals in early March (and also in the High Atlas on 24 March) may correspond to populations originating further south (e.g. in the Canary Islands). In other words, they may involve the progenitors of the butterflies that later emerged in our studied source areas.

Discussion

Our expedition to Morocco was organized to test in situ for the presence of high numbers of immature *V. cardui* in one of the source areas predicted by Stefanescu *et al.* (2007) of the migrants arriving in north-east Spain in April–May. Our predictions were essentially correct, as we did find immatures at virtually all the sampled sites except from the north-easternmost explored region and the central High Atlas, at altitudes of over 1,000 m, which lie on the edge of the predicted source region. The maximum abundance was found in the Souss valley, slightly more to the west than predicted by the model, although it must be stressed that our modelling approach should be regarded more as a useful tool for approximating the most likely source areas than a method for determining their exact locations.

It is also important to note that spring migrations in north-east Spain typically last for a period of nearly two and a half months, from early April to mid-June (see Fig. 3 in Stefanescu *et al*. 2007). Thus, the exact location of the source areas probably varies over time and the first migrants arriving in Spain are predictably those that develop in warmer areas earlier in the season. By late March, for instance, the abundance of immatures in the source region increased towards the west and at lower altitudes, that is, in those areas that fulfilled the right climatic conditions for larval and pupal development. Similarly, the mean stage of development of the breeding populations increased along the same variables, so that in the Souss valley butterflies were emerging. This coastal plain is characterised by its mild climate, with average minimum temperatures for January (the coldest month) ranging between 3ºC and 7ºC, in comparison with a range of 0ºC to 3ºC in the neighbouring mountain and inland areas (Sauvage 1963). As the season advances, it is very likely that other more northern and higher areas become favourable for breeding and therefore act as the main sources of migrants later in the spring. A clear example is provided by site P3 on the northern slope of the central High Atlas, where on March 24 we found no immatures but recorded several egg-laying females. A visit to the same site on 12 April, revealed the presence of many mid-aged larvae, from which adults would emerge by late April or early May. This example shows how the breeding area progressed northwards over the course of a period of three weeks.

Field work also allowed us to confirm that breeding habitats were suitable and common enough in the region to permit population densities to build up into the high numbers that are periodically recorded in Europe. Because *V. cardui* is a highly opportunistic species that uses a diversity of hostplants growing in ruderal areas (e.g. derelict land, road banks, field margins, etc.; see Fig. 2), there is no shortage of hostplants or habitats for reproduction. In this respect, the use of *F. tenacissima* in the Anti Atlas (confirming previous unpublished observations) is remarkable, especially because this plant can be very abundant, offering an alternative to the plants typically used in more ruderal habitats. Likewise, the unusual record of a larva feeding on *C. humilis* provides further evidence of the extremely polyphagous nature of *V. cardui* (Ackery 1988). Another crucial aspect of the field work was that we were able to confirm that hostplant quality was optimal in early spring, thus allowing larvae to exploit a highly temporal resource that deteriorates rapidly as the growing season advances in this semi-desert region (e.g. Pedgley *et al*. 1995).

Our observations, together with the typical patchy distribution of the ruderal habitats used for breeding, lead us to suggest that source populations in this Moroccan region do not stem from large continuous areas, but most frequently originate in small pockets hosting the most favourable conditions. Only under certain circumstances, that is when abundant larval resources coincide with abundant nectar sources and lead to higher patch occupancy and levels of oviposition (Janz 2005), will outbreak populations occur. An example of such a situation was provided by the single small patch of waste land near Taroudant of little over 1.5 ha, where more than 150,000 larvae had completed their development. Countless comparable sites occur all over the Souss valley, but also along many roads and near human settlements elsewhere, making the whole region a potential source area for the butterflies colonising Europe every year.

The finding of the massive emergence site together with the observation of vast numbers (probably several millions) of fresh butterflies migrating nearby, clearly indicates that outbreak populations originating in the Souss valley immediately depart to colonize regions situated to the north. This pattern agrees well with earlier reports of mass exodus soon after emergence (Skertchly 1879; Egli 1950; Giuliani & Shields 1995). Interestingly, we also frequently noted that pairs formed as individuals on the ground rose up to interact with those flying above them, before departing in a northward direction. These pairs with one butterfly pursuing another may last for the whole migratory journey, as they are a very common sight in *V. cardui* mass movements (e.g. Myres 1985; C. Stefanescu pers. obs.). Giuliani & Shields (1995) even suggested that this kind of behaviour could trigger the start of a migratory wave, a possibility that deserves further investigation.

If, as posited by Stefanescu *et al*. (2007), long-distance migration of *V. cardui* is associated with synoptic wind masses and occurs mostly at high elevation, we would expect the butterflies that departed from the Souss valley on 28–30 March to arrive in north-east Spain in the first week of April after a non-stop flight of only a couple of days. This prediction agrees well with data from the CBMS in 2009, which indicates that the first noticeable wave of migrants arrived in Catalonia around 4–5 April. On the contrary, if migration were restricted to the flight boundary layer close to the ground and was essentially non-wind assisted, butterflies would have arrived in Catalonia no earlier than 20 April at the earliest (taking into account a total distance of 1,500 km, a flight speed of 15 km/h and an average of five hours of migratory flight per day; cf. Abbot 1951). This is, in fact, a highly optimistic estimation, since bad weather would likely stop migration for long periods and energetic demands would lead to stops of various days for refuelling (e.g. Davis & Garland 2004). In this respect, it is interesting to note the negative effect of the southward wind that we recorded at site P34, which seemed to reverse the direction of the migratory movement. Therefore, although considerable uncertainty remains regarding the migratory process (e.g. the actual altitude at which butterflies fly), there is now strong evidence pointing to the selection of favourable tailwinds by migratory *V. cardui* (see also Myres 1985). This strategy, which minimizes energy consumption and travelling time and would be expected to evolve in an organism whose life history is shaped by long-distance migration, has indeed been confirmed in some other related species (e.g. *Vanessa atalanta* (Linnaeus) (Nymphalidae: Nymphalinae) and *Danaus plexippus* (Linnaeus) (Nymphalidae: Danainae); Gibo & Pallett 1979; Mikkola 2003; Brower *et al*. 2006; Brattström *et al*. 2008).

Finally, as far as we know, the present work is the first to provide a quantification of the population size and the impact of parasitoids in an outbreak area (but see the interesting descriptions by Giuliani & Shields (1995, 1997a,b, 1998), for outbreak populations in California).

Population density was assessed at 94,200±33,324.4 larvae/ha, which gives an idea of the huge number of butterflies that can be produced in such a vast region at the right phenological time. Likewise, we estimated that 8.9% of the larvae died as a result of the impact of the main parasitoid, *C. vanessa*e, while 1.7% of the pupae were attacked by *P. puparum*. Although the impact is moderate, the parasitoid populations will build up to enormous numbers on this single butterfly generation: taking into account that each butterfly larva produced 38.9±25.4 wasps (±SE; n = 28), a population of 588,637 *C. vanessae* can be estimated to have emerged from this single patch (without pseudohyperparasitism). This means that the impact of *C. vanessae* on a hypothetical second butterfly generation occurring in the same area would be extremely high. Therefore, our data give further support to Owen's (1987) hypothesis that migration in *V. cardui* is at least partly adaptive to reduce parasitoid load.

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OVERWINTERING CLUSTERS OF THE MONARCH BUTTERFLY COINCIDE WITH THE LEAST HAZARDOUS VERTICAL TEMPERATURES IN THE OYAMEL FOREST

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ABSTRACT. During winter, monarch butterflies form dense colonies in oyamel fir forests on high mountains in central Mexico, where the forest canopy serves both as a blanket, moderating temperature, and an umbrella, shielding the butterflies from rain. In this study we investigated the vertical dimension of the butterflies' use of the oyamel forest: we predicted that clusters form at the heights above ground that provide the greatest protection from freezing. By suspending temperature recorders at eight heights, from ground level up to 22m, we established two vertical transects in the forest. We set one transect in a densely forested area and the other in a thinned area, and we recorded hourly temperatures from Jan 13 through Feb 5, 2006. Intermediate heights in the forest, from 10 to 15m above ground, remained the warmest during the cold night and early morning. We also determined that this temperature distribution matched available records of the vertical distribution of butterflies roosting in branch and trunk clusters. The vertical temperature profiles became uniform during mid-day. Temperature extremes were moderated more during clear than during cloudy periods and more in the denser than in the thinned forest. Our results illustrate how the monarch butterfly is behaviorally adapted to the three-dimensional complexity of microclimate in the oyamel forest. A denser forest provides better protection for the overwintering survival of monarch butterflies than a thinned forest. This research bolsters our other microclimatic studies, all of which point to the urgency of prohibiting logging within the oyamel forest used as wintering habitat by monarch butterflies.

Additional key words: *Danaus plexippus*; conservation; Monarch Butterfly Biosphere Reserve; forest management; microclimate; trunk and bough clustering behavior; vertical temperature distribution; negative effects of forest thinning

Fall migrant monarch butterflies (*Danaus plexippus* L., Lepidoptera, Danainae) overwinter in the oyamel (*Abies religiosa* H.B.K., Pinaceae) forest ecosystem on 12 mountain ranges in the Transverse Neovolcanic Belt in central Mexico (Slayback *et al*. 2007; Slayback & Brower 2007). The current archipelago-like distribution of the oyamel ecosystem is a remnant of a much wider forest that retreated up onto the taller volcanic mountains as the environment warmed at the end of the Pleistocene (Manzanilla 1974; Rzedowski 1978; Snook 1993; review in Brower 1995). In this forest, from November through March the butterflies form colonies at elevations of 2,700 to 3,300 m, with extremely dense clusters on both the branches and trunks. Although the butterfly area is south of the Tropic of Cancer, because of its high elevation it is subjected to freezing temperatures on cold nights during much of the winter (Calvert & Brower 1986). Moreover, because the N–S running Sierra Madre mountain systems north of the volcanic highlands do not block northern polar air incursions (Hill 1969), the overwintering area can be strongly affected by cold fronts that interact with Pacific Ocean moisture and generate winter storms with rain, hail, snow, and rime ice (Lauer 1973; Garcia 1997).

Given that the monarch belongs to the tropical butterfly subfamily Danainae and has only moderate freeze resistance (Larsen & Lee 1994; Anderson & Brower 1996), it is a seeming paradox that the eastern North American population migrates to and spends the winter at such high elevations. The reason the butterflies do so is that the cool temperature and moisture inside the oyamel forest maintain the butterflies in a state of reproductive diapause (James, 1993) and allow them to conserve their lipid energy reserves that fuel their five month wintering period and their April remigration to the Gulf Coast states (Masters *et al*. 1988; Brower *et al*. 2006).

Anderson & Brower (1996) determined that 50% of monarchs die at -8ºC if they are dry, whereas if they are wet, 50% die at -4ºC, 80% are killed at -5ºC, and 100% are killed at -7ºC. The lethal combination for the overwintering butterflies is to be wetted by a winter storm and then subjected to freezing temperatures caused by the intense radiant heat loss when the sky clears. The effects of three killer storms on the butterflies have been documented by Calvert *et al.* (1983) for January 1981, by Brower *et al.* (2009) for February 1992, and by Brower *et al*. (2004) for January 2002. The 2002 storm killed an estimated 80% of all overwintering monarchs in Mexico.

An intact oyamel canopy moderates the microclimate within the forest in three ways. The canopy operates as a blanket that helps to hold heat inside the forest (Calvert *et al*. 1982, 1984, 1986; Anderson & Brower 1996). From 5–7 Jan 2008, for example, the minimum temperature beneath the forest canopy was +3.3º C, whereas in a nearby clearing it was –3.2ºC, i.e., 6.5º C colder (Brower *et al*. 2008b; see also Brower *et al*. 2009, Fig. 5). The canopy also serves as an umbrella sheltering the butterflies from rain (Anderson & Brower 1996) and from heavy dew formation. Several of our field studies have documented the presence of heavy dew and severe frosting in open areas ("llanos") and their absence within forests immediately adjacent to overwintering colonies (Calvert & Brower 1981; Calvert *et al*. 1982, 1986; Calvert & Cohen 1983; Alonso-Meija *et al*. 1992, 1993). Finally, oyamel tree trunks retain heat during the night, maintaining the butterflies' temperatures when they rest on the trunks (Brower *et al*. 2009). Thus, the oyamel forest contributes blanket, umbrella, and hot-water bottle effects, microclimatic factors that can be critically important during extreme freezes that follow winter storms.

Monarch butterflies do not distribute themselves evenly at all heights within the oyamel forest. In initial descriptions of the Sierra Chincua overwintering colony, Urquhart & Urquhart (1976), Brower (1977), and Brower *et al*. (1977) noted that the oyamel tree crowns were devoid of monarchs. This crown avoidance was confirmed during extensive aerial reconnaissance of the butterfly colonies (Slayback *et al*. 2007; see Fig. 1 in Brower *et al*. 2008b). At the lowest forest stratum, Calvert *et al*. (1982) and Calvert & Brower (1986) observed that mid-winter branch and trunk clusters rarely occur close to the ground.

Geiger (1950) and Geiger *et al.*'s (2003) summaries of forest microclimate led us to embrace a hypothesis that the butterflies avoid both the tree tops and the ground to reduce their probability of freezing. As Geiger pointed out, the tops of forest trees are colder at night than the branches beneath the canopy because tree tops lose heat through radiation to the open sky. Geiger *et al.* (2003) also noted that tree tops are subject to heavy dew condensation and rime ice deposition. We witnessed the latter during storms in January 1981 and 2002 (Calvert & Brower 1986; Brower *et al*. 2004). Finally, nighttime temperatures near the ground are often colder than at mid-forest levels due to radiant heat loss through openings in the canopy, cold air drainage (especially in mountainous terrains), and loss to the soil, which can serve as a heat sink.

The current study, spurred by our growing awareness of the spatial and temporal complexity of microclimates within oyamel forests, addresses the relationship of the butterflies' clustering behavior to the forest's vertical temperature profile. We first quantify the vertical

FIG. 1. Monarchs that have been dislodged from their clusters on the tree boughs and trunks are subject to lower and possibly lethal freezing temperatures. By shivering and crawling upwards as on this oyamel fir trunk, they are able to reestablish their clusters. Sierra Chincua colony, 11 Jan 1979 (photo, L.P. Brower.)

distribution of the trunk and bough clusters on the oyamel trees. We next compare temperatures recorded in an open area with those recorded beneath the forest canopy to demonstrate how the forest moderates the temperatures during mid-winter. We then compare the forest temperature moderation over 24 hours on clear versus cloudy days. Finally, we present the vertical temperature profiles within the oyamel forest at different times of day, for both clear and cloudy conditions, and for two trees differing in their degree of canopy cover.

We ask six questions: (1) What is the vertical distribution of monarchs? (2) How does air temperature vary with height within the forest? (3) Is vertical variation in temperature different during clear and cloudy periods? (4) How does the vertical temperature profile change over 24 hours? (5) Is the vertical temperature profile different in a closed versus an open forest? (6) Could the vertical distribution of the monarch clusters provide thermal benefits for the butterflies?

Answers to these questions amplify our knowledge of the microclimatic consequences for monarchs because of their association with the oyamel forests. They also have implications for how oyamel forests should be managed for the long-term conservation of the monarch butterfly.

MATERIALS AND METHODS

Measuring the vertical distributions of monarch clusters on oyamel trees. On 1 Mar 1979, W. Calvert and W. Zuchowski used a Spiegel Relaskop (Forestry Suppliers, Jackson, MS) to measure the vertical distributions of monarch trunk clusters on ten oyamels and the heights of nine of the ten trees (Table 1). These data were gathered in the Sierra Chincua 4 colony, located in Arroyo Zapatero, as described in Calvert & Brower (1986). On 5–6 Feb 2008 we measured the vertical distribution of branch clusters on 18 oyamel trees in the Arroyo Hondo colony, also on the Sierra Chincua, as described in Brower *et al*. (2008b). For the 2008 measurements, we used a stick method as follows: we first marked the 2 m height on a tree trunk; then, from approximately 30 m away, we held a short stick at arm's length that by eye matched the 2 m height on the tree; finally, we estimated the number of multiples of the 2 m length required to reach the cluster bottom, the cluster top, and the tree top. This method (Ontario Woodlot Assoc. 2003) provides estimates with an accuracy of approximately ±10%. Both the 1979 and 2008 measurements were taken in colonies that had not been affected by winter storms. The vertical extents of trunk and branch clusters generally coincide except after storms or other disturbances when the dislodged butterflies crawl back up the trunks as shown in Fig. 1.

The vertical transect study area. We conducted our vertical transect study in an oyamel forest on a ridge behind Chincua Station located in the Ejido los Remedios (Fig. 2). As described in Brower *et al*. (2009), the forest is within the elevational range of the known monarch overwintering areas on the Sierra Chincua and is 2–4 km ESE of where butterfly colonies have formed every year since the first Chincua colony was discovered in 1975. Even though monarch colonies have never been reported in this specific location, the forest characteristics (species composition, tree density, size distribution, slope, elevation, and southern exposure) are similar to the areas where monarchs do form colonies on the Sierra Chincua massif. The study area includes one of the sites used in Brower *et al*. (2009).

TABLE 1. Vertical distribution of monarch clusters on oyamel trees on the Sierra Chincua. Data shown are mean ± s.d. and (range).

	1979 trunk clusters	2008 branch clusters	diff
Clusters			
top of clusters	15.7 ± 3.0 m (10.0 to 19.8)	15.4 ± 2.4 m (11.0 to 20.7)	n.s.
bottom of clusters	7.2 ± 1.1 m (4.8 to 8.9)	5.9 ± 2.5 m (1.5 to 10. 4)	n.s.
vertical extent	8.5 ± 2.9 m (3.5 to 12.0)	9.5 ± 2.9 m (5.5 to 17.1)	n.s.
Trees with these clusters			
height	26.6 ± 4.8 m (15.5 to 30.5)	20 to 30 m **	
dbh	37.1 ± 14.5 cm (20.3 to 66.2)	14.9 ± 4.4 cm (6.8 to 20.8)	p<.001
Sample size	10 *	18	

*N = 9 for tree height measureiments

**estimated

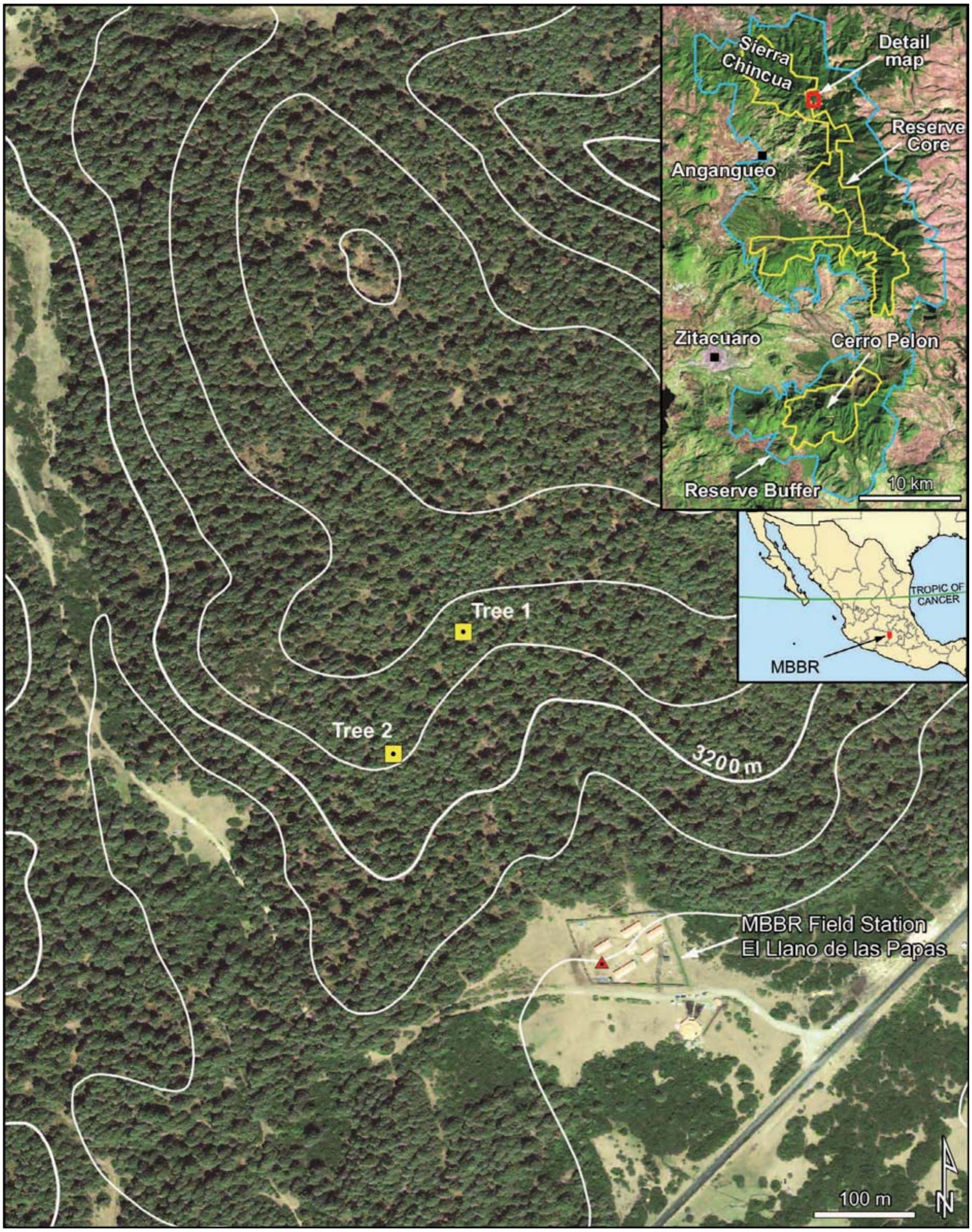

FIG. 2. The main map shows the location of our weather station as a red triangle, the Monarch Butterfly Biosphere Reserve (MBBR) Field Station (on el Llano de las Papas), the locations of Trees 1 and 2, and elevation contours at 20 m intervals, on top of a GeoEye-1 satellite image (© GeoEye) made on 11 April 2009. The distance from the weather station to Tree 1 is 350 m and to Tree 2 is 290 m. Tree 1 and 2 are 140 m apart. The down-slope facing aspects were 150º for Tree 1 and 160º for Tree 2. The inset map shows the location of this site within the MBBR and the location of the Sierra Chincua on top of a Landsat 7 satellite image made on 16 Jan 2003.

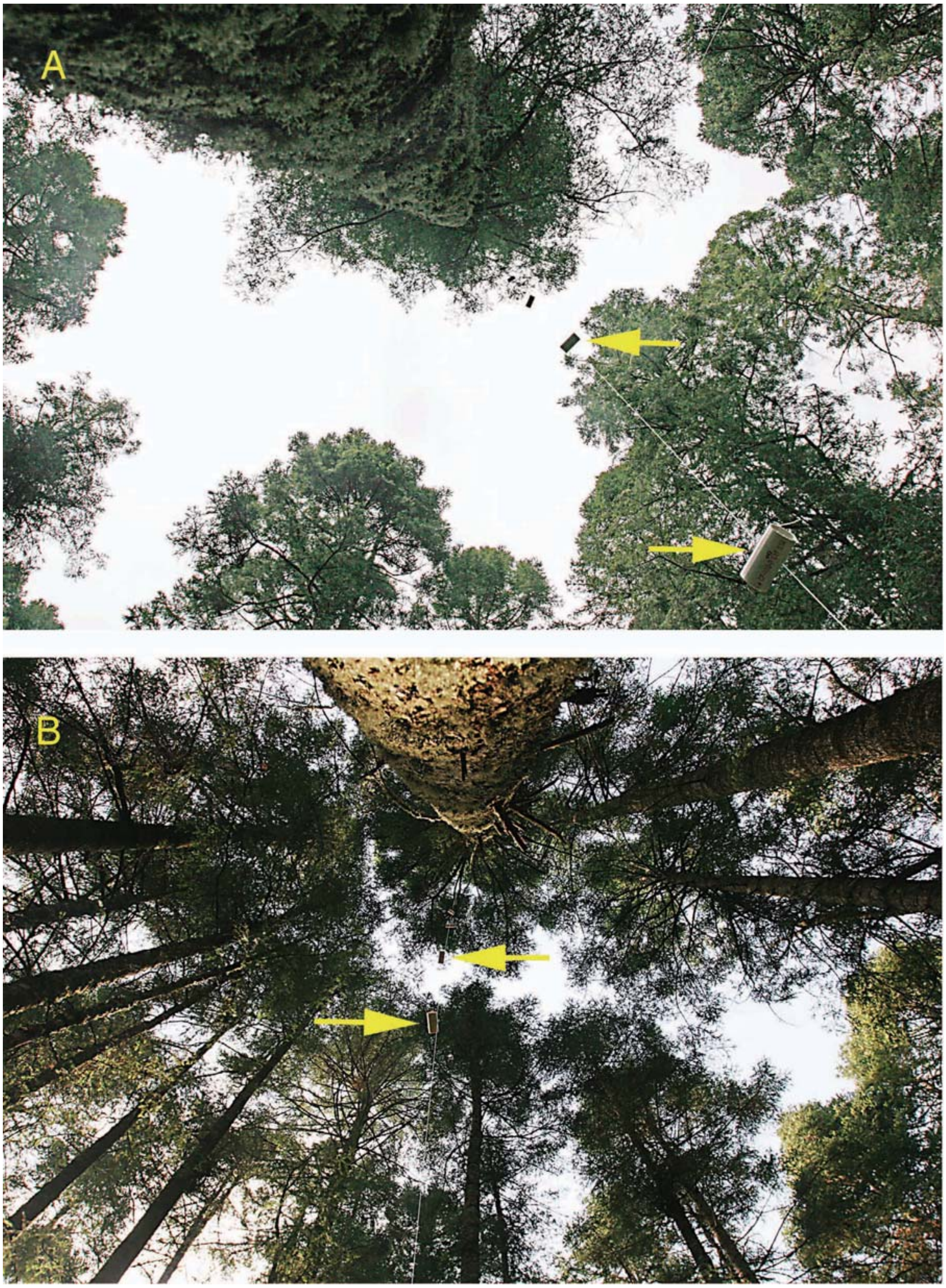

FIG. 3. The experimental trees. (A) view of the vertical transect at Tree 1; (B) same, Tree 2. Four of the eight cylinders in which the Thermochrons were mounted are visible in A, while two are visible in B; two cylinders in each photo are marked by arrows. The canopy above Tree 1 is 34% open to the sky, and Tree 2 is 13% open. (Photos, L. P. Brower, from the same facing angles as the transects.)

Small-scale timber removal has taken place in this site periodically, and all trees are relatively young, similar to those in the Chincua colony when studied during the 2007–2008 overwintering season (Brower *et al*. 2009).

Two trees were selected within the forest that differed in the immediately surrounding forest cover. The images in Fig. 3 were taken from the ground looking straight up at each tree's canopy with a Canon D-20 camera and an EF-S 17–40 mm lens set at 17 mm. Adobe Photoshop pixel analysis (Hein 2006) indicated that 34% of the open sky showed above Tree 1 and 13% above Tree 2; that is, Tree 1 was in a relatively open area of the forest whereas Tree 2 was in a more closed area. Both trees were approximately 40 cm in diameter and 30 m tall. The slopes at Trees 1 and 2, measured with a clinometer, were both approximately 15 degrees, and their aspects were 140º and 150º, respectively. For reasons of security and equipment, our data acquisition was limited to two trees.

We used a Garmin GPS MAP60CSx unit to locate the positions of the two oyamel trees and plotted them on an orthorectified, pan-sharpened GeoEye-1 satellite image (© GeoEye) with 0.50 m resolution, taken on 11 April 2009. The location of our weather station and the two oyamel fir trees are shown in Fig. 2. The coordinates for the weather station were: 19º 39' 41.9" N and 100º 16' 6.2"W, at an elevation of 3160 m. The tree coordinates were: Tree 1: 19º39' 52" N and 100º16' 10'' W; Tree 2: 19º39' 48" N and 100º16' 13'' W.

Setting the vertical transects. The vertical line and cylinders for Tree 1 were set on 11 Jan 2006 with the line facing 340º; those for Tree 2 were set on 12 Jan 2006 with the line facing 255º. Both Fig. 3 A & B were taken on 12 Jan 2006, an overcast day. We used a Big Shot slingshot (Sherrill Arborist Supply, Greensboro, N.C.) to throw a nylon string over an upper bough on each oyamel. For each height, one temperature data logger (see below) was mounted on a lateral wall inside a white plastic PVC cylinder (12.5 cm long, inside diameter 5.2 cm, outside diameter 6.0 cm). The cylinders were suspended horizontally from nylon strings tied through small holes bored 1.25 cm from each end. The cylinder strings were attached to the suspended vertical line, which was pulled up so that the cylinders were suspended at eight heights (0, 3, 6, 9, 13, 16, 19, and 22 m above the ground). The cylinders were free to rotate. The temperature recorders were shielded from direct sunlight by the PVC cylinder and by the forest canopy. The main string was secured near the base of the tree so that the transect hung 0.5 to 1 m away from the tree trunk (Fig. 3A & B).

Temperature measurements. Each hour the weather station (WeatherHawk, Model 232, Logan, UT) recorded the previous hour's average, minimum, and maximum temperatures, with an accuracy of 0.01ºC. Vertical transect temperatures were measured with iButton Thermochrons (Model DS1921G, Maxim Integrated Products, Dallas Semiconductor). These small (1.6 cm by 0.7 cm) digital recorders measure temperature in 0.5ºC increments, with a range of -40º to 85ºC and a rated accuracy of ±1.0ºC (Dallas Semiconductor); we have previously determined that under our experimental conditions their accuracy is better than ±0.5ºC (Brower *et al*. 2008b). We programmed the Thermochrons to record one instantaneous reading each hour. They were in place by mid-afternoon on 12 Jan 2006 and were retrieved on 6 Feb 2006. We used the data for 24 days, from 13 Jan through 5 Feb 2006. For a comparison of temperature profiles during different weather conditions, we selected five cloudy days (Jan 25, 26, 28, 29, and 31) and five clear days (Jan 13, 18, 19, and Feb 4, 5) for analysis of mid-morning, mid-day, and afternoon temperature profiles. Similar choices were made for nighttime comparisons (19:00 through 07:00 hrs) under cloudy conditions on Jan 24–25, 25–26, 26–27, 27–28, 28–29) and under clear conditions (Jan 13–14, 18–19, 19–20, Feb 3–4, 4–5). The difference between the cloudy and clear days (Duchon & O'Malley 1999) is illustrated by comparing the highest recorded solar output for each day (recorded hourly); the averages of the daily high readings (mean ± 95% C.I.) were 519.2 ± 56.0 watts/sq-meter for the five cloudy days and 814.8 ± 22.2 watts/sq-meter for the five clear days (t-test, $p < 0.001$). The only precipitation recorded during the 24-day study was on cloudy days, 6 mm on Jan 26 and 9 mm on Jan 27.

Analysis. All data were analyzed using SPSS versions 16 and 17 (SPSS, 2008). We analyzed patterns in the vertical profiles using regression analysis, both linear and polynomial, with tree incorporated as a fixed effect and day as a random effect in a mixed effects model. All regressions used height and height squared as explanatory variables. The daily temperature ranges of the two trees were compared by paired t-tests, with a strict Bonferroni correction (Walsh, 2004) applied to the comparisons at all heights.

To examine temporal autocorrelation of the data, we developed a correlogram based on time lags $j = 1$ to 9 hr (Diggle 1990). The results revealed significant autocorrelation for time lags up to four hours but negligible effects for longer lags. Thus, measurements close in time on the same day are not independent, while records from different days provide independent measures. To ensure correctness of the patterns we're reporting, we assessed the validity of these patterns by comparing them to the same analyses using only those data that were separated by five or more hours.

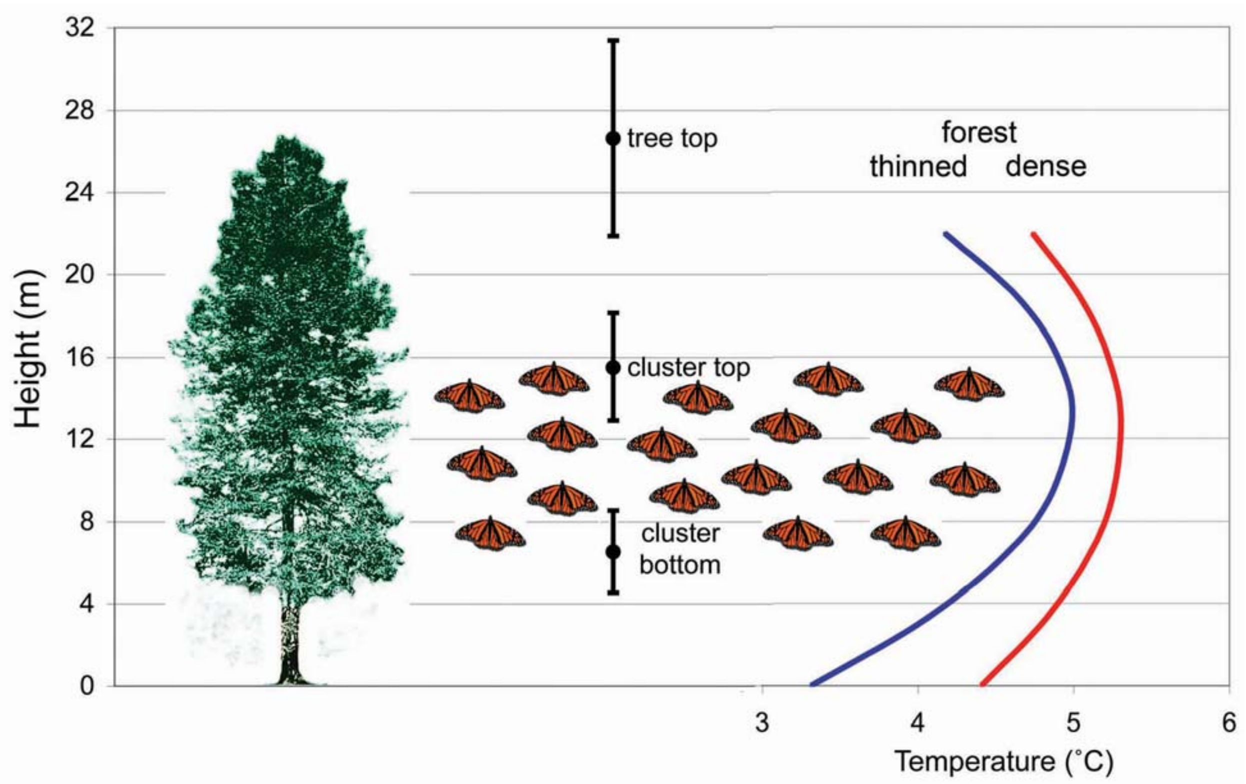

FIG. 4. The relationship of nighttime vertical temperatures (right) and monarch cluster heights (center) on an oyamel fir of average height (27 m, left). The black height bars are mean m above the ground ± 1 s.d. (data from Table 1). The blue and red lines show the fitted nighttime vertical temperature profiles from measurements made from the ground to 22 m on two oyamels; the blue line is from Tree 1 in the thinned forest, while the red line is from Tree 2 in the denser forest (data from Fig. 7; regressions given in the text). The height of the lowest branches varies. The clusters averaged 6.4 m above the ground and extended on average upwards to 15.5 m, which is more than 10 m below the canopy (Table 1), with most monarchs concentrated at middle levels. The mid section of both trees is about 1–2° warmer than both the ground and the upper canopy temperatures. The data thus indicate that the butterflies behaviorally select the safest microclimate by avoiding the ground and the treetop, where they would be subjected to more severe cold temperatures, and (in the treetop) to wetting by dew. The vertical temperature profile for the tree in the thinned forest is up to 1°C colder at all heights. The tree image is based on Earle (2009).

RESULTS

Vertical distribution of the trunk and bough clusters on oyamels. The trunk clusters in 1979 extended, on average, between 7.2 m and 15.7 m above the forest floor, with an average vertical extent of 8.5 m (Table 1). The average tree height was 26.6 m, that is, 11 m above the top of the clusters. The branch clusters in 2008 showed a similar pattern: the clusters were between 5.9 m and 15.4 m above the ground, with an average vertical extent of 9.5 m (Table 1). Although we did not measure tree heights in 2008, we estimated them to be between 20 and 30 m; thus, the canopy extended 5 to 15 m above the topmost clusters. Fig. 4 summarizes these vertical distributions of the clusters on a 27 m oyamel.

Moderation of daily temperature extremes by the forest canopy. The moderating effect of the canopy on temperatures is evident from a comparison of the ambient temperatures at the 3m height at our two study trees with those at the nearby weather station (Fig. 5). The temperatures inside the forest beneath the canopy are always cooler during the day and almost always warmer at night than they are in the open area. Through the 24 days, the temperature range at the weather station was 23.8°C (from –4.9° to +18.9°C), while the temperature range for the two forest trees was 15.6°C (from –0.3° to +15.3°C). Thus, the temperature varied in the open area 8.2°C more than beneath the forest canopy, and freezing temperatures occurred only once in the forest compared to most nights in the open.

Comparison of the temperature patterns during cloudy versus clear conditions. The temperature regimes on cloudy and clear days at the 3 m height under the forest canopy and in the nearby open area reveal an interaction between the effects of canopy and cloud cover (Fig. 6A and B). Each hourly temperature inside the forest is based on the average of Trees 1 and

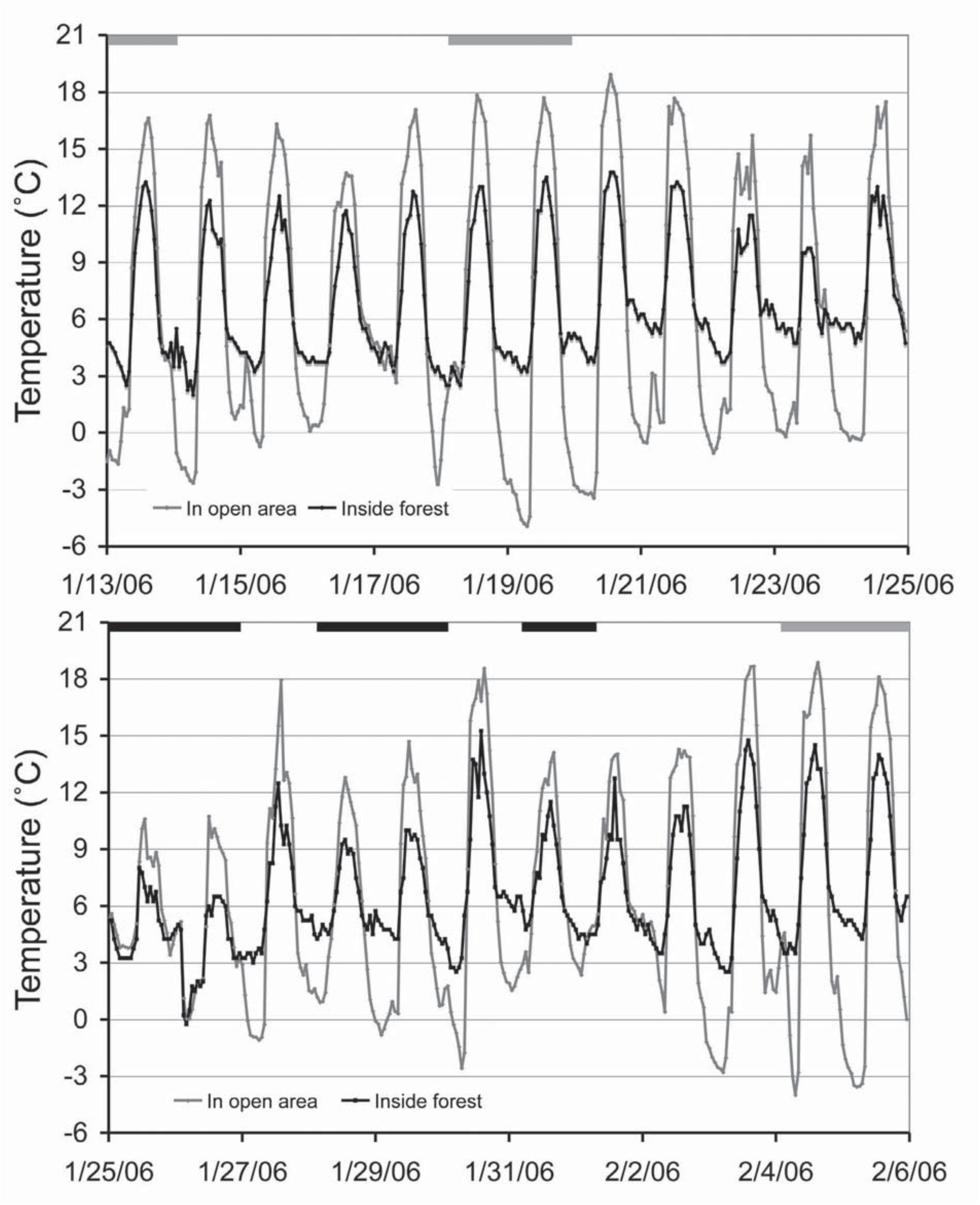

FIG. 5. Hourly temperatures from 13 Jan to 5 Feb 2006. The temperatures in the open area (gray line) are from the WeatherHawk weather station located in the open area on El Llano las Papas (Fig. 2); the inside-forest readings (black line) are the ambient temperatures at the 3m height beneath the nearby oyamel forest, averaged for Tree 1 and Tree 2. Marked by the labelled tick marks, each day begins at 0:00. Dark bars along the top designate the five cloudy periods analyzed and the gray bars designate the five clear periods. The data show that the forest substantially moderates the microclimate: freezing temperatures occur almost nightly in the open but rarely beneath the canopy, and the temperatures inside the forest are always cooler during the day than in the open area and almost always warmer at night.

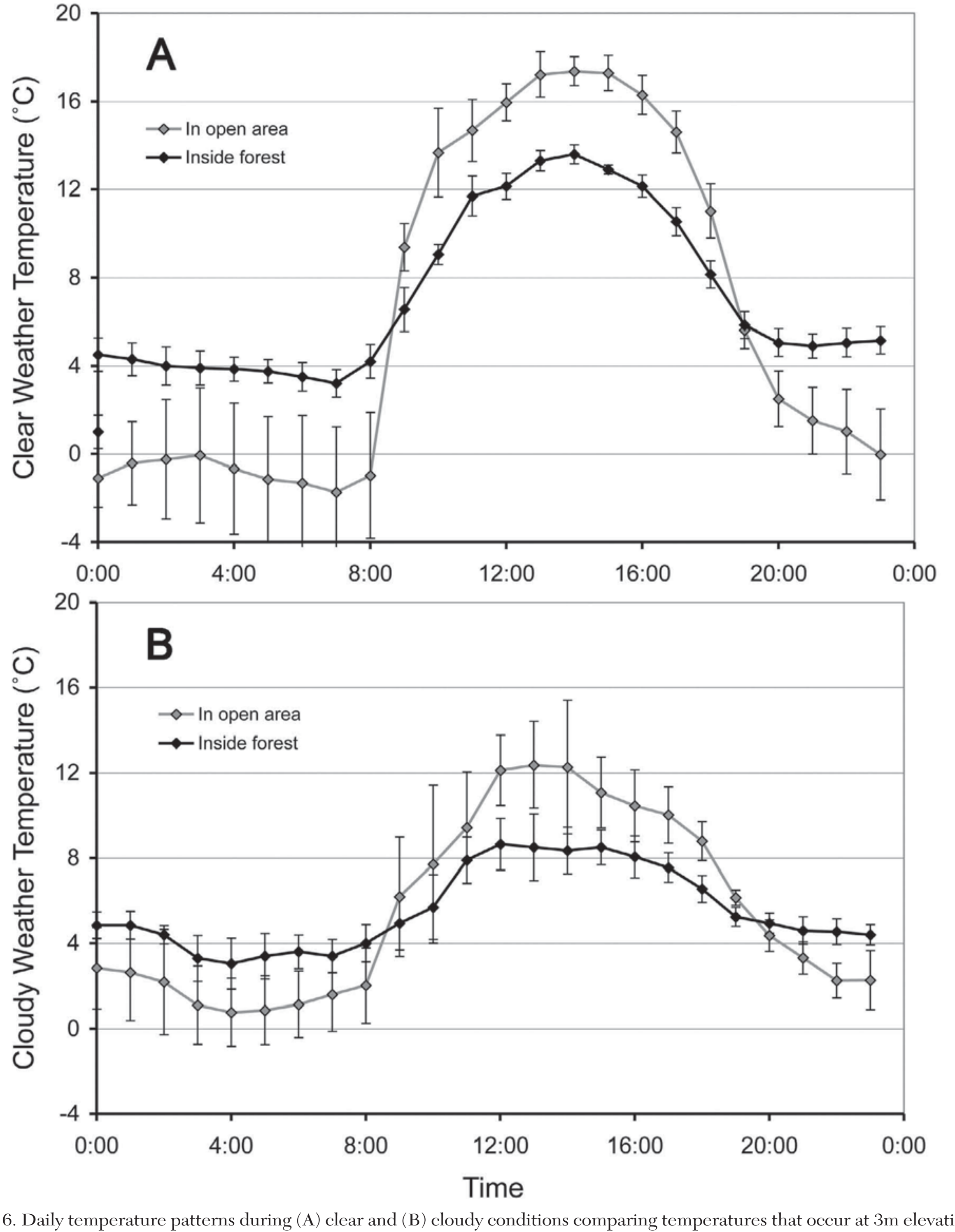

FIG. 6. Daily temperature patterns during (A) clear and (B) cloudy conditions comparing temperatures that occur at 3m elevation inside the forest (black line) with temperatures in the nearby open area (gray line) as recorded at the weather station. Each hourly temperature inside the forest is based on the average of Trees 1 and 2 for five clear days (Jan 13, Jan 18–19, Feb 4–5) or five cloudy days (Jan 25–27, Jan 27–29, Jan 31). The error bars show 95% C.I. Under all conditions, the open area is warmer during the day and colder at night. The data illustrate that: (1) the freezing risk is highest during nighttime (from 19:00 to 7:00); (2) during both weather conditions the canopy holds heat inside the forest during the nighttime and reduces the probability of the butterflies' freezing; and (3) clouds have little effect on forest temperatures at night but they substantially reduce daytime temperatures in both the forest and the open area.

2 for five cloudy days or for five clear days. The open area temperatures are based on the same 5 days from the weather station.

Under clear conditions, for all hours from 10:00 through 17:00 the daytime forest temperature was significantly cooler, by 3–4ºC, than in the open. The nighttime forest temperature was significantly warmer, by 4–5ºC, between 21:00 and 08:00. In contrast, under cloudy conditions the difference between the forest and open sites was reduced, especially at night. The daytime temperature rise was smaller both in the open and in the forest; the difference between the two sites each hour was 1–4ºC, and significant during only four of the hours. On cloudy nights the difference between the forest and open sites was reduced to 2–3ºC, and was significantly different for only two nighttime hours.

These results illustrate that clouds (1) reduce the radiant energy loss at night in open areas, but (2) have little effect inside the forest. The data also indicate that the forest (3) maintains cooler daytime temperatures during both clear and cloudy daytimes and (4) also maintains warmer temperatures at night irrespective of cloud cover. Moreover, (5) the moderating effect of the canopy is greater under clear conditions than under cloud cover. Most importantly, the blanket effect of the forest during the night reduces the risk of the butterflies' freezing and during the day reduces the temperatures and therefore also the rate at which the butterflies burn lipids.

Vertical temperature profiles during the nighttime. During the night and early morning, from 19:00 through 07:00 hrs, the intermediate heights were warmer than the ground and the top of the forest for both vertical transects (Fig. 7). At each height and for each transect, the average temperature was based on 312 measurements (13 records per night for 24 days). A polynomial model yielded the best fit for each tree, and the regressions were significant (Tree 1, $F=228.58$, $p<0.001$; Tree 2, $F=68.96$, $P<0.001$). The regressions for the two trees were also significantly different ($t=15.424$, $p<0.001$). For Tree 1, $\text{Temp} = -0.010\ \text{Ht}^2 + 0.259\ \text{Ht} + 3.311$; for Tree 2: $\text{Temp} = -0.006\ \text{Ht}^2 + 0.147\ \text{Ht} + 4.405$. Equivalent results obtained when using only separated data points per night to avoid autocorrelation (three records per night, at 20:00, 01:00, and 06:00 hrs, for 24 days): Tree 1, $\text{Temp} = -0.010\ \text{Ht}^2 + 0.260\ \text{Ht} + 3.338$; for Tree 2: $\text{Temp} = -0.006\ \text{Ht}^2 + 0.151\ \text{Ht} + 4.443$; statistics in Table 2). The warmest heights given by the regressions were at 13.0 m for Tree 1 and at 12.2 m for Tree 2; based on the actual measurements, the warmest temperatures were from 6 to 16 m for Tree 1 and from 13 to 16 m for Tree 2. The regressions indicate that the ground was 1.7ºC colder than the warmest intermediate heights for Tree 1 and 0.9ºC colder for Tree 2, and that the temperature at 22m was 0.8ºC colder than the warmest intermediate height for Tree 1 and 0.6ºC colder for Tree 2. Both regressions were significant, as were the curvilinear

TABLE 2. Regression statistics for analyses of the nighttime temperature profiles measured at eight heights on two oyamel trees (2496 measurements for each when all data are analyzed; 576 measurements for separated, independent temperatures). For the analysis by temperature differences, the temperature at the 3 m height was subtracted from the temperature at each of the seven other heights. The regression statistics show the fit of the polynomial (curvilinear) model to the temperature data, while the quadratic coefficients show the significance of the curvilinear component. These statistical results confirm that nighttime temperatures are significantly warmer at intermediate heights.

	Tree 1	Tree 2
Analysis by actual temperatures		
regression	F = 228.58; p < 0.001	F = 68.96; p < 0.001
quadratic coefficient	t = 17.367; p < 0.001	t = 10.579; p < 0.001
R square	0.155	0.052
Analysis by separated temperatures		
regression	F = 55.498; p < 0.001	F = 19.624; p < 0.001
quadratic coefficient	t = 8.545; p < 0.001	t = 5.696; p < 0.001
R square	0.159	0.061
Analysis by temperature differences		
regression	F = 1584.25; p < 0.001	F = 521.25; p < 0.001
quadratic coefficient	t = 45.721; p < 0.001	t = 29.084; p < 0.001
R square	0.560	0.295

(quadratic) components of the regressions (Table 2). The temperatures were consistently lower for Tree 1, which had 34% of the canopy opened to the sky, compared to Tree 2 which had only 13% of the canopy opened to the sky (Fig. 2A and B). The curvilinear regressions of nighttime temperatures for Tree 1 and Tree 2 are summarized diagrammatically in Fig. 4 and demonstrate that the vertical distribution of monarch clusters matches the safest part of the vertical temperature profiles in the oyamel forests.

Similar patterns resulted when we ran a separate analysis of the vertical temperature profiles based on the temperature differences between the actual reading at each height and the temperature measured at the 3 m height. We ran this analysis to exclude the influence of some days being warmer than others (treating time as a random effect), and therefore to reduce the variance in day-to-day measurements. As a result, the R square values were much higher, as were the F and t statistics (Table 2). The regression equations based on the temperature differences are identical to the regression equations based on the actual temperatures, except for the axis intercepts (–0.729 for Tree 1 and –0.786 for Tree 2). Thus, the curvature of each line (the dependence of temperature on height above the ground) and the interpretation of the results were identical whether the regressions were based on actual temperatures or temperature differences.

We examined the influence of cloud cover on the nighttime temperature profiles. Our initial analyses showed that, when we separated the data for clear and cloudy nights, a polynomial regression provided a better fit than a linear regression for temperatures of both trees under both clear and cloudy conditions. This result was similar to that with all data combined (Fig. 7). Comparisons of the polynomial model to the linear model gave these measures of fit: Tree 1 clear weather, R square (polynomial) =0.656 versus R square (linear) =0.266; Tree 1 cloudy 0.326 versus 0.041; Tree 2 clear 0.412 versus 0.118; Tree 2 cloudy 0.168 versus 0.002). From the regressions, intermediate heights (12 to 14m) of Tree 1 were 3.0° warmer than the ground during clear nights but only 1.0° warmer on cloudy nights. For Tree 2, in the denser part of the forest, the differences were less, at 1.3° for clear nights but only 0.6° for cloudy nights. From the analyses for Tree 1, we found greater curvature (a greater difference between temperatures at intermediate heights and those at the top and bottom of the transect) on clear nights than on cloudy nights ($t=2.971$, $p=0.003$). For Tree 2, the differences during clear and cloudy conditions were reduced and not quite significant (greater curvature on clear nights, $t=1.754$, $p=0.080$).

Nighttime temperatures may also be viewed separately for cloudy and clear nights (Fig. 8). The same vertical pattern is apparent as in Fig. 7, with Tree 1 being colder than Tree 2 but with the cloudy nights having lower temperatures.

Temperature profiles during mid-morning hours. During morning hours (0900 and 1000; Fig. 9) of clear days, the temperature increased significantly from the ground towards the canopy (regression, effect of height: $t=3.890$, $p<0.001$ for Tree 1; $t=1.991$, $p=0.048$ for Tree 2). The averages over all heights were 8.7±0.5°C for Tree 1 and 9.0±0.5°C for Tree 2. In contrast, on cloudy days the vertical temperature profile was remarkably uniform (regression, effect of height: $t=0.277$, n.s. for Tree 1; $t=0.312$, n.s. for Tree 2) and averaged 5.3±0.5°C for both trees. This average was less than 1°C warmer than the nighttime average (Figs. 7, 8). The differences between clear and cloudy days were significant (Tree 1: $t=12.198$, $p<0.001$; Tree 2: $t=11.627$, $p<0.001$), with the average temperature being more than 3° cooler on cloudy days than on clear days.

Temperature profiles during the mid-day hours. By mid-day (12:00 and 13:00) the nighttime profile was fully erased on both clear and cloudy days, and temperatures were uniform throughout the vertical profiles (Fig. 10). There was no effect of height on temperature for either transect (Tree 1: $t=0.007$, n.s.; Tree 2: $t=0.309$, n.s.). The clear days, however, were significantly warmer than cloudy days (Tree 1: $t=11.279$, $p<0.001$; Tree 2: $t=12.015$, $p<0.001$). For Tree 1, mid-day temperatures averaged 12.7° on clear days and 8.5° on cloudy days, while for Tree 2, the averages were 12.3° and 7.9°.

Temperature profiles during the afternoon hours. On clear and cloudy days, the afternoon hours (from 1500 to 1700) continued to produce a mostly uniform vertical temperature profile (Fig. 11) as overall temperatures began to decrease. Similar to mid-day, there was no effect of height on temperature for either transect under cloudy conditions (Tree 1: $t=0.153$, n.s.; Tree 2: $t=0.216$, n.s.), and despite some variability in temperatures, there was no consistent effect of height during clear conditions either (Tree 1: $t=0.883$, n.s.; Tree 2: $t=1.137$, n.s.). On Tree 1, we suspect that the 19 m Thermochron received direct insolation on clear days, producing a temperature spike at that height. As was true during morning and at mid-day, temperatures were significantly higher on clear than on cloudy days (Tree 1: $t=16.684$, $p<0.001$; Tree 2: $t=23.410$, $p<0.001$); the averages were 12.7° on clear days and 8.1° on cloudy days for Tree 1 and 11.8° on clear days and 7.8° on cloudy days for Tree 2.

Daily pattern. Comparison of Figs. 7-10 shows that,

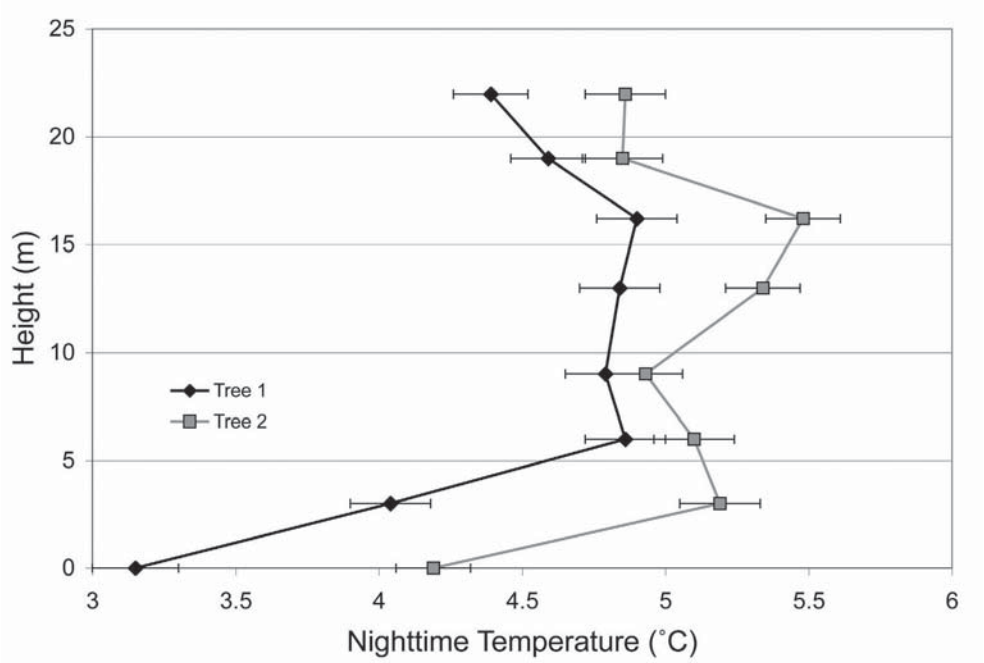

FIG. 7. Average nighttime (19:00 to 07:00 hrs) temperature measurements at eight heights above the ground for Tree 1 (black line) and Tree 2 (gray line) over 24 days (13 Jan through 5 Feb 2006). Error bars show 95% C.I.; statistics are in Table 2. These results, in combination with Table 1, indicate that the vertical distribution of monarch clusters matches the safest part of the vertical temperature profiles in the oyamel forests. At night Tree 2 averages 0.55 degrees warmer than Tree 1, which is more exposed. The curvilinear regression lines are displayed along with the monarch cluster height preferences in Fig. 4.

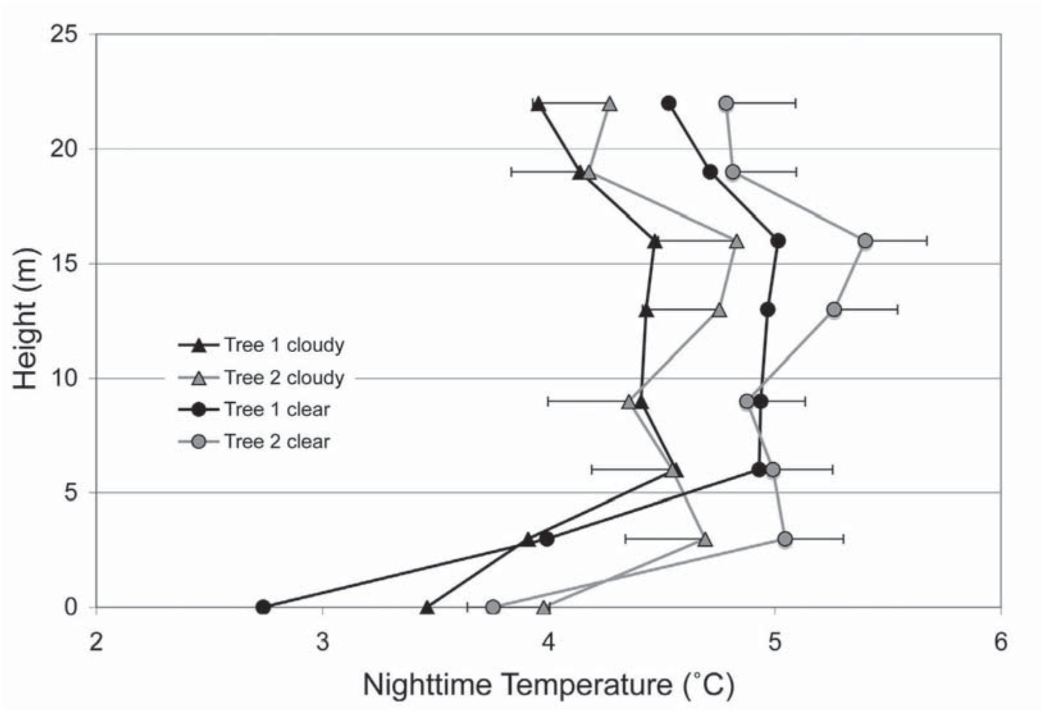

FIG. 8. Average nighttime (19:00 to 07:00 hrs) temperature measurements at eight heights above the ground for Tree 1 and Tree 2 during five cloudy nights and five clear nights. Error bars show 95% C.I. Fig. 7, in contrast, shows the overall comparison of Tree 1 and Tree 2 during all nights.

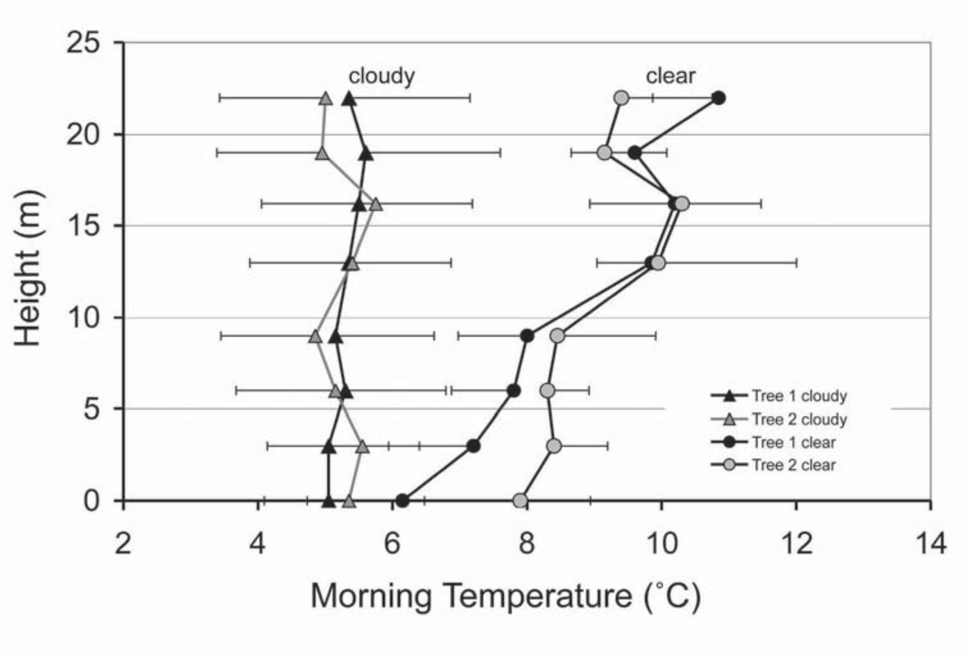

FIG. 9. Vertical temperature profiles during mornings (0900 and 1000 hr) for both Tree 1 and Tree 2, with a comparison of cloudy and clear days. Each data point is an average calculated from 10 measurements during five cloudy days and five clear days. Error bars represent 95% C.I.

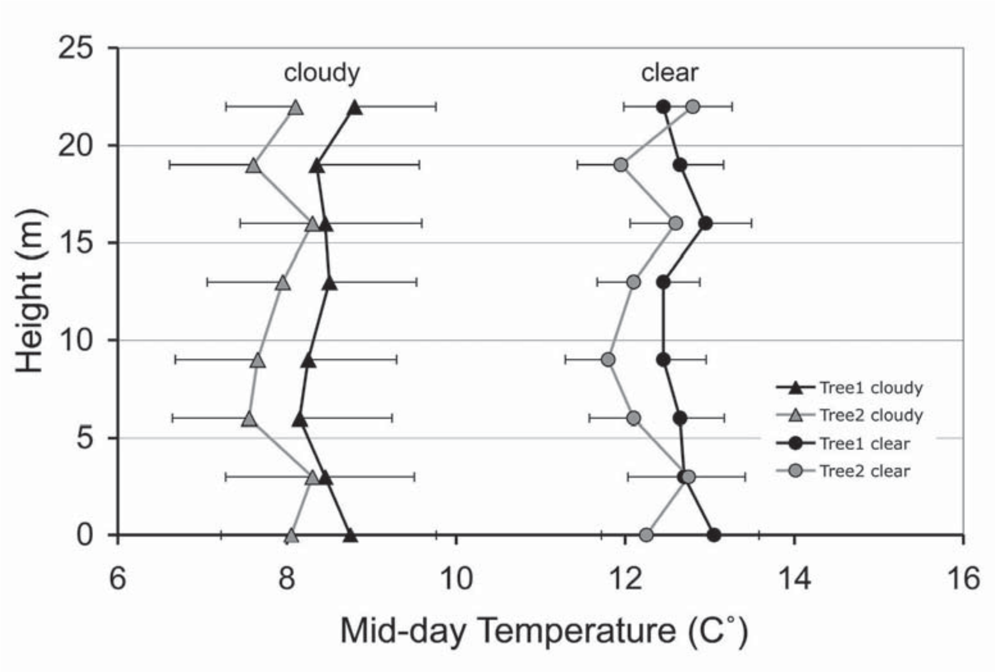

FIG 10. Vertical temperature profiles at mid-day (1200 and 1300 hrs). The temperature profiles became vertically uniform by mid-day on both cloudy and clear days, and clear days were uniformly about 4º warmer than cloudy days at all heights. Each average was calculated from 10 data points (2 hours per day over 5 days that were either cloudy or clear); error bars represent 95% C.I.

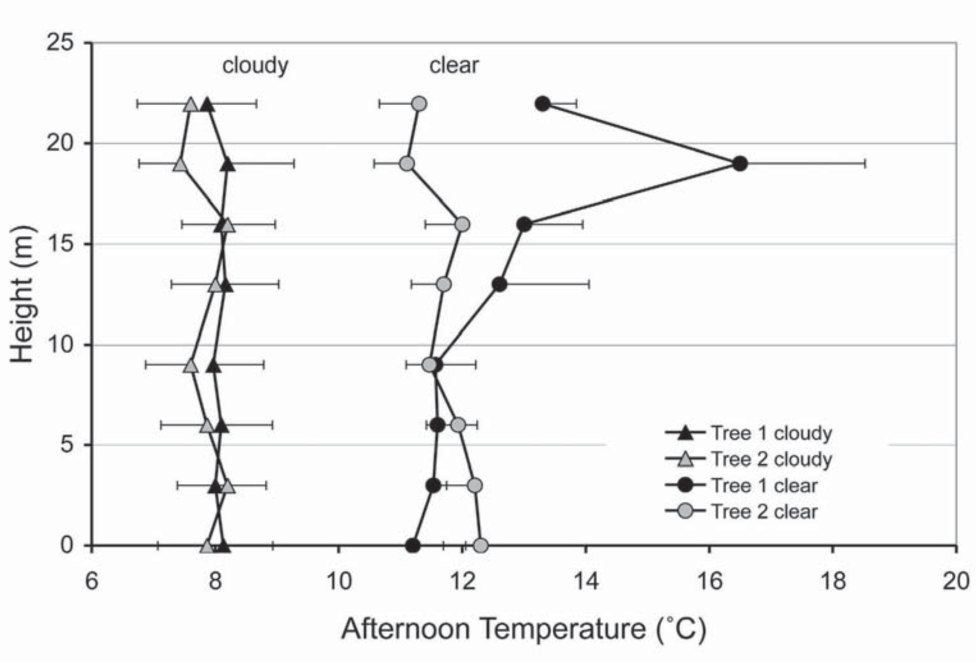

FIG. 11. Vertical temperature profiles for afternoon (1500, 1600, 1700 hr) temperatures for both transects, showing a comparison of cloudy and clear days. These afternoon temperature profiles were nearly the same as mid-day (Fig. 10) and were uniform and nearly identical for the two trees on cloudy days. On clear days, they were both about 4ºC warmer, with no vertical differences in Tree 2. The spike in temperature for Tree 1 at the 19 m level resulted from its receiving direct insolation in the late afternoon. Each average is calculated from 15 measurements (3 hours across 5 days); error bars represent 95% C.I.

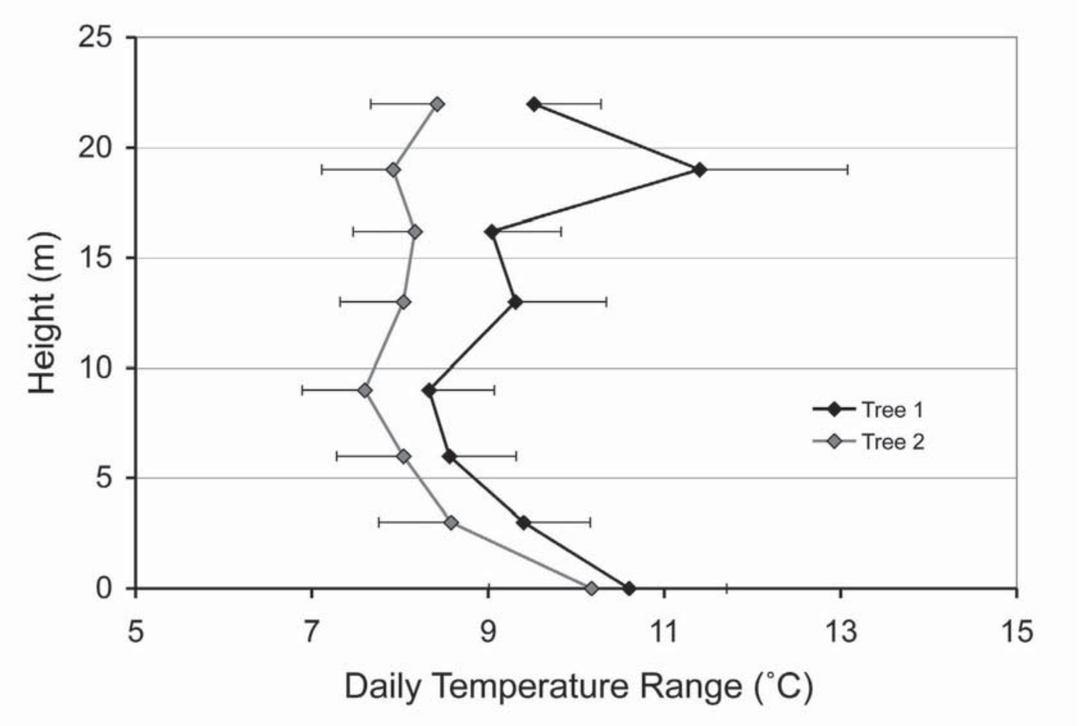

FIG. 12. The average daily range in temperatures at each height along the vertical transects of Tree 1 (black line) and Tree 2 (gray line). Tree 1, in the more open area of the forest, varies more in temperature each day than does Tree 2. The temperature spike for Tree 1 at 19 m was caused by late afternoon sunlight directly warming the Thermochron. Error bars represent 95% C.I.

during both clear and cloudy weather, the curvilinear nighttime profile (Fig. 7) becomes uniform as the day advances (Figs. 9–11), and the temperatures at all heights are about 4° warmer on clear days than on cloudy days (Figs. 9–11).

Effect of forest density. The vertical temperatures within the forest were further moderated by the extent of tree cover (Fig. 12). Each point in the graph shows the average daily range (daily maximum minus the daily minimum temperatures) for each tree at each height over the 24 days of the study. The daily range in temperature for Tree 1, in a more open part of the forest, averaged 9.6±2.7ºC, whereas the daily range for Tree 2, in denser forest, averaged 8.2±2.0ºC. The overall difference is significant (paired t-test, $t=9.509$, $df=183$, $p<0.001$), and the differences at each height except the ground are also significant (paired t-tests with strict Bonferroni correction [Walsh 2004], from $t=3.990$ to $t=6.633$, $df=22$, $p \leq 0.001$ for each of the seven heights above the ground; n.s. at ground level). These results illustrate that opening of the forest canopy increases the daily temperature range at all heights in the forest, a result that has conservation implications.

DISCUSSION AND CONCLUSION

Why do monarchs cluster at intermediate heights? The data presented in this paper establish that monarch butterflies form clusters on the oyamel boughs and trunks at intermediate heights beneath the forest canopy (Fig. 4) and that these heights coincide with the warmest nighttime temperatures in the forest (Fig. 7). Clustering at these slightly warmer intermediate levels during severe cold events may be critical when the temperature drops below –3ºC, the temperature at which the freezing risk for wet butterflies increases dramatically (Anderson & Brower 1996). We interpret these data as indicating an adaptive behavior that reduces the probability of freezing mortality in the oyamel overwintering forests.

A behavioral response to the warmest parts of conifer forests is not unique to monarchs. For example, Wachob (1996a, b) reported that mountain chickadees seek out the warmest microclimates in a high elevation coniferous forest for nesting and foraging. The clusters may moderate their own immediate microclimate (Brower *et al*. 2008b), in part by blocking wind and reducing convection. Furthermore, there is evidence that trunk clusters attain greater thermal protection than do branch clusters (Brower *et al*. 2009).

Exceptions to the typical vertical cluster distribution have occurred after storms that dislodge millions of butterflies (Brower *et al*. 2002) and also after the clusters have been disrupted by wind or by logging. Monarch butterflies are unable to fly below a thoracic temperature of approximately 13–15ºC (Masters *et al*. 1988; Alonso-Meija *et al*.1993), but they are capable of crawling when their thoracic temperatures are as low as 4ºC (Alonso-Meija *et al*. 1993). The daytime temperatures we measured beneath the forest were usually high enough (Fig. 6) to enable the butterflies to crawl back up onto the tree trunks (Fig. 1) and eventually reestablish their clusters at intermediate heights (Brower & Calvert unpubl. obs.).

Opportunities for butterflies to remain quiescent or fly during the day. From morning through afternoon, on both clear and cloudy days (Figs. 9–11), the temperatures at all heights in the forest were as much as 10ºC below flight threshold, except when the sun hit the highest branches (Fig. 11). Under these conditions, butterflies were able to remain quiescent and minimize consumption of their lipid reserves. In contrast, in the nearby open area (Fig. 6), the flight threshold was exceeded for four or more hours on most clear days, from at least 12:00 to 16:00.

Despite the energetic benefits of remaining cool and inactive, however, on sunny days, large numbers of butterflies leave their clusters and fly to water. We hypothesize that they must drink periodically to maintain their water balance. Although butterflies shaded by the forest cannot bask, at ambient temperatures greater than 6ºC they can elevate their thoracic temperature by shivering (Alonso-Meija *et al*. 1993). When ambient temperatures are 1–3° below flight threshold, shivering butterflies can reach flight threshold (Masters *et al*. 1988). On clear days, therefore, between approximately 11:00 and 16:00, the butterflies clustering at all levels within the forest could shiver, reach flight threshold, and fly to water or perform other necessary behaviors. Those that bask in direct sunlight could rapidly raise their thoracic temperatures (Masters *et al*. 1988) and fly either back into shaded areas or engage in other active behaviors. In contrast, on many cloudy days the butterflies are not able to reach flight threshold and therefore cannot leave the colony.

Effect of canopy openings on the vertical temperature profile. Previous experiments have demonstrated that for butterflies stranded at ground level, denser forests provide greater protection against freezing than do more open forests (Calvert *et al*. 1982, 1983, 1984; Calvert & Brower 1986; Brower *et al*. 2004). Comparison of the vertical transects of Tree 1 and Tree 2 (Fig. 3A & B) demonstrates that this pattern holds true above the forest floor, as well: even a slight opening in the canopy increases the daily temperature range at all heights within the forest. During the dangerous nighttime period, the temperatures beneath the more exposed tree were colder at all heights by at least 0.5º (Fig. 7). Furthermore, the variation of the daily temperature measured at all heights was 0.5º to 4º C greater beneath the more open canopy (Fig. 12). These slight temperature differences can be critical when the ambient temperature beneath the forest canopy drops below –3ºC (Anderson & Brower 1996). More generally, our results are consistent with the findings of Chen *et al.* (1999), who determined that openings in a Douglas-fir forest (*Pseudotsuga menziesii* [Mirb.] Franco) in Washington impaired the ability of the canopy to buffer the understory radiant energy exchange.

Implications for lipid conservation. An additional challenge that confronts monarchs during the overwintering season is conserving the lipid reserves upon which they depend for their winter survival and their spring migration back into the southern U.S. breeding habitat in late March and early April (Brower *et al*. 2006). Our vertical temperature data show that the temperature beneath the forest canopy during the daytime at all heights and on both clear and cloudy days was several degrees cooler than in the nearby open area (Fig. 6). Thus, the forest canopy provides substantial moderation of temperature extremes that results in the dual benefits of freezing protection during the night and lipid conservation during the day. The uniform vertical temperature profile during the daytime indicates that the butterflies' resting height does not affect their lipid use, as long as the butterflies avoid the highest canopy layer.

Future Research. The lowest temperature recorded at a weather station in an oyamel forest area (in the Valle de Mexico) was –11ºC (Madrigal-Sánchez 1967). The lowest temperatures recorded so far in an open area in the Sierra Chincua has been –8ºC (Alonso-Meija *et al*. 1992), and inside a colony, where whole branch clusters of wetted monarchs froze in situ, the low was estimated to have been -4.4ºC (Brower *et al*. 2004). Measuring the vertical temperature distribution beneath canopies during severe cold periods is imperative. We predict that the intermediate heights beneath the closed oyamel canopy remain the safest areas within which to cluster.

Collecting data from additional vertical transects, including relative humidity as well as temperature, and extending the measurements to the tops of the oyamels, would provide a more complete understanding of how the butterflies respond to the complex vertical microclimate. We predict that the microclimate of the exposed upper canopy will resemble that of the open field area by being exposed to heavy dew and frost and by being dangerously colder at night and warmer during the day.

An important variable that we have not addressed is wind, which can substantially alter the microclimatic profile in coniferous forests (Raynor 1971; Gustavsson *et al*. 1998). Based on occasional direct field observations (Brower unpubl.) as well as the aftereffects of the January 2002 storm (Brower *et al*. 2004), there can be little doubt that forest thinning increases the negative impacts of wind, both on cluster architecture and in disrupting the vertical microclimate profile.

Following the vertical microclimate profile and relating it to colony architecture from colony formation in November through the winter and into the spring may provide an adaptive explanation of why the colonies move down arroyos in February and March (Calvert & Brower 1986). Are the butterflies tracking a shifting optimal microclimatic profile as the dry season advances?

On the south side of Cerro Pelon (located in the southernmost part of the Reserve, map in Slayback *et al*. 2007), monarchs regularly form colonies in mixed forest stands of oyamel, Mexican cedar (*Cupressus lusitanica* Miller, Cupressaceae), and smooth bark Mexican pine (*Pinus pseudostrobus* Lindl., Pinaceae). In contrast to the pines and the cedars, the oyamel branch architecture allows monarchs to form very large bag-like clusters that provide them with a temperature advantage (Brower *et al*. 2008b). We predict that the vertical temperature profiles beneath both cedars and pines will be found to provide a suboptimal microclimate compared to that beneath oyamels.

Implications for conservation of the oyamel forests. The vertical temperature profiles illustrate the three-dimensional complexity of the microclimate within the oyamel forest and add to an understanding of the importance of dense, undisturbed forest in protecting overwintering monarch butterflies. The fact that the temperature in the transect of the less dense forest was lower at night and had a wider daily range at all heights means that dense forest provides greater temperature protection during winter cold events.

Observations dating back to 1977 have led us to conclude that the oyamel fir is almost certainly the preferred tree species on which monarchs cluster (Soto-Nunez & Garcia 1993; Calvert 2004; Brower unpubl. field notes) and has likely been so since pre-historical times. This conclusion is supported by fragmentary reports of the original forests in this region (*e.g.*, Leopold 1950). Based on observations made around 1938, Loock (1950, p. 32) stated: "between the altitudes of 9,500 and 11,000 feet, there is another clearly marked zone which is occupied by *Abies religiosa*. This magnificent tree occurs in pure stands of dense forest, reaching heights of up to 150 or more feet with diameters of up to 5 or 6 feet." Such large trees in the primordial overwintering forests would have provided additional temperature buffering against freezing for the butterflies that clustered on their trunks (Brower *et al*. 2009). While we may never know what the original climax oyamel forest was like, it is instructive to consider how its original forest architecture may have affected the butterflies' clustering behavior and winter survival. One approach to this question would be to compare and contrast the three dimensional complexity and the effects of disturbances in current oyamel forests with those in old growth Douglas-fir forests in the Cascades in Washington (Spies & Franklin 1991; Ishii *et al*. 2004; Nadkarni *et al*. 2004; Parker *et al*. 2004). How might the structure of such forests, including trees of variable heights and ages and the occurrence of forest gaps, snags, and complex spacing, have provided the microclimate to which monarch butterflies originally adapted?

If the oyamel forest is the safest overwintering environment, why do the butterflies also cluster on cedars and pines? The most likely explanation is that the monarchs are returning to overwintering areas that were formerly oyamel climax forests (Soto-Nunez & Garcia 1993). The current mixed forests are almost certainly successional, resulting from repeated logging and break-away forest fires that are ignited to clear land for agriculture (Loock 1950; Leopold 1950; Perry 1991; Snook 1993; Brower & Missrie 1998).

Firmly establishing that oyamels provide the optimal overwintering microclimate is important for managing both the core and buffer zones in the Monarch Butterfly Biosphere Reserve. Extensive illegal logging has occurred in both zones, and limited logging is allowed in the buffer zone. Reforestation policy should be based on a scientific assessment of the original stand compositions on the known overwintering massifs. It is possible that the best policy within the Reserve is to restore pure oyamel stands at higher elevations and mixed oyamel/pine/cedar forests at lower elevations.

This paper provides evidence that small openings in the forest canopy cause a lessening in the temperature buffering provided by the oyamels at mid-heights of the forest, i.e., those heights at which the butterflies form their clusters. The greatest threat to maintaining these forests as safe overwintering havens is the illegal logging that has accelerated in recent years (Ramirez *et al*. 2003, 2005; Anon. 2004, 2008; Brower *et al*. 2008a, 2009; Honey-Roses 2009a, b). The accumulating microclimatic information underscores the necessity of stopping logging and overwhelmingly supports our assertion that the key to winter survival of the butterflies is the microclimate protection provided by the intact coniferous forest canopy, principally by the oyamel fir.

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A NEW SPECIES OF *EUCLEMENSIA* (COSMOPTERIGIDAE) FROM THE UNITED STATES

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ABSTRACT. A new species of *Euclemensia* (Cosmopterigidae) is described from Louisiana. The imago and genitalia of the male and female are illustrated, and diagnoses are provided to differentiate the new species from the four other described species of the genus.

Additional key words: Louisiana, Kermesidae, scale parasitoid, disjunct species.

Despite the extremely high diversity of Lepidoptera and their level of importance in biotic communities, our knowledge of the Lepidoptera fauna in North America is quite depauperate, especially for microlepidoptera. This lack of knowledge of our native species is combined with the need to provide baseline inventories for specialized and restricted communities. Descriptions of new species in these unique habitats are needed to facilitate conservation efforts by land management personnel as well as to provide new information for those studying evolutionary history and biogeography.

A survey of moths on Barksdale Air Force Base was conducted during 1996. During this survey male and female specimens of a new species of *Euclemensia* were collected in a prairie habitat with scattered shrubs and *Quercus stellata* Wangenheim (Fig. 1). Collections in similar prairies, glades and other habitats in southeastern U.S. since 1996, as listed in a database of sampled habitats (Brown & Lee 2010), have not yielded additional specimens. This species is not present in the major collections holding microlepidoptera, including the U.S. National Museum of Natural History, the Canadian National Collection, the Essig Museum at Berkeley, CA, and the private collection of Vernon Brou in Louisiana. Although only a male and female specimen of this new species have been collected, this species is described to document a fifth species of this genus and to document the fauna of the prairie habitat on Barksdale Air Force Base.

Euclemensia Grote (Cosmopterigidae) is an enigmatic genus among the Lepidoptera because its four known species have been described from disjunct regions of the world, and only *E. bassettella* (Clemens), the type species, is encountered frequently. The lack of diversification in this genus is combined with the unusual larval habit of being parasitoids of scale insects.

Two species of *Euclemensia* are known to occur in America north of Mexico, *E. bassettella* in eastern United States and Canada and *E. schwarziella* (Busck) in Arizona, both of which have been illustrated by Hodges (1978). *Euclemensia caminopa* (Meyrick) was described from St. Augustine, Trinidad based on a single female that was illustrated by Becker (1999); no other specimens of this species are known. *Euclemensia woodiella* (Curtis) has not been seen since its original collection near Manchester, England in 1829, and it is considered to be extinct; it has been redescribed and illustrated by Koster & Sinev (2003).

Larvae of both *E. bassettella* and *E. schwarziella* are parasitoids of scale insects, specifically *Kermes* spp. and *Allokermes kingii* (Cockerell) (Hemiptera: Kermesidae)

Fig. 1. Type locality of *Euclemensia barksdaleella* n. sp. Lee and Brown at Barksdale A.F.B., with Quercus stellata and in foreground.Fig. 2. Adult photo of *Euclemensia barksdaleella* n. sp. Lee and Brown. Scale bar: 2 mm.

(Olsen 1919; Hollinger & Parks 1919; Hodges 1978; Turner & Buss 2005). Some references to *Kermes* Boitard as hosts may be inaccurate because *Kermes* has been restricted to four western species and one eastern species (Bullington & Kosztarab 1985). Ten species previously assigned to *Kermes* and two additional new species have been assigned to *Allokermes* Bullington and Kosztarab, and these include six species occurring in the eastern United States (Bullington & Kosztarab 1985).

MATERIALS AND METHODS

Specimens were collected with a 15-watt blacklight box trap, as illustrated by MacGown (2006), with calcium cyanide A dust used as a fumigant. Dissection and slide mounting methods for genitalia followed Clarke (1941), except that the preparations were stained in eosin and mounted in Euparol. A Leica MZ 125 Stereo-Microscope and Olympus Compound Microscope were used for examining specimens and slide mounts at magnifications ranging from 10–100x and 100-400x, respectively. Terminology for genitalia follows Koster & Sinev (2003), including use of brachia for dorsal projections of the tegumen, as defined by Hodges (1978) for Cosmopterigidae, and valvella for the sclerotized plates surrounding the phallus. Measurements were made with an ocular micrometer in the stereo-microscope at magnifications of 40x for the forewing, and 100x for the male genitalia. Lengths of male genital structures were measured for the right brachium (left in Fig. 3) along the medial margin, for the tegumen along the lateral margin (its greatest length), for the right valva along the costal margin from base to apex, and for the valvella from the ventral base to dorsal apex. The photograph of the imago was made with a Leica stereoscope with Image Pro Plus 5.1 program for autoformatting.

Euclemensia barksdalensis **n. sp.** Lee and Brown (Figs. 2–4)

Description. Adult (Fig. 2): Wing length 3.0 (1 female)–4.0 mm (1 male). Head and thorax metallic dark gray. Antenna dark brown with four white segments near apex. Labial palpus smoothly scaled, yellowish white, second segment slightly longer than third segment, with a few dark brown scales on the apex of third segment. Legs dark brown; hind tibiae with two silvery white annulations at bases of silvery white spurs. Forewing dark brown, with a postbasal band of yellowish orange scales, extending to base between two dark brown basal spots. Fringe dark brown. Hindwing dark brown. Abdomen dark brown, with band consisting of 6–7 irregular rows of dentate microtrichia on anterior margins of terga IV-VII.

Male genitalia (Fig. 3): Brachia 0.84 length of lateral margin of tegumen, 0.60 length of costal margin of valva, and 0.55 length of valvella; valva simple of nearly uniform width from base to apex, apical and ventroapical margins setose on inner surface, scaled and setose on outer surface; valvella developed as pair of lateral plates extending from base of valva to slightly beyond bases of brachia, subequal in length with valva; phallus cylindrical, with a large cornutus on vesica; vinculum broad, without saccus.

Female genitalia (Fig. 4): Apophyses anteriores 0.57x length of apophyses posteriores; ostium bursae in membrane between seventh and eighth abdominal sterna with antrum encircled by sclerotized band; ductus bursae short, connecting with corpus bursae and smaller appendix bursae, both having dentate microtrichia detectable with compound microscope, signum absent.

Diagnosis. This new species can be differentiated from other *Euclemensia* by the dark brown forewing with a postbasal yellowish orange band. The forewings of *Euclemensia bassettella* and *E. caminopa* are black or indigo blue with crimson red or reddish orange markings, respectively. Forewings of *E. woodiella* and *E. schwarziella* are dark brown with extensive yellow to orange markings beyond the postbasal area. The two known specimens of *E. barksdalensis* have shorter forewings (3–4 mm) than most *E. bassettella*, although the latter can range in size from 3 mm to 6 mm. Of the 161 specimens of *E. bassettella* that were examined, including six specimens from Barksdale A.F.B., only slight variations in wing pattern or color were detected, and none of these variations were close to the distinctive color and pattern of *E. barksdalensis*. The male and female genitalia of *E. barksdalensis* and *E. bassettella* are very similar in shape, and these two species are examples of different species that cannot be reliably differentiated by male genitalia. Males of both species have a single cornutus in the phallus, in contrast to two cornuti in *E. schwarziella*. The relative lengths of the brachia to the tegumen, valva, and valvella are similar between *E. barksdalensis* and *E. bassettella*. The only difference between the two species in male genitalia is the presence of more setae on the medioapical region of the valva in *E. bassettella*, but this may be a variable character. The female genitalia of *E. barksdalensis* differs from that of *E. bassettella* in having a completely sclerotized ring encircling the antrum in contrast to having a sclerotized dorsal plate on the antrum in the latter species, based on dissections of three specimens from Mississippi and Louisiana. The ventral portion of the antrum of *E. bassettella* is unsclerotized. In addition, the dorsal area of the eighth tergite is membranous in *E. barksdalensis*, whereas it is weakly sclerotized in *E. bassettella*.

Holotype. [♂] Bossier Parish, LA., Barksdale A.F.B., 32°29'29"N 93°35'07"W, 11 April 1996, D.M. Pollock (Genitalia slide MEM No. 1028). Deposited in the US National Museum of Natural History.

Paratype. 1[♀ Bossier Parish, LA., Barksdale A.F.B., 32°29'29"N 93°35'07"W, 11 April 1996, D.M. Pollock (Genitalia slide MEM No. 1029). Deposited in the Mississippi Entomological Museum.

Etymology. The name is derived from the type locality at Barksdale Air Force Base.

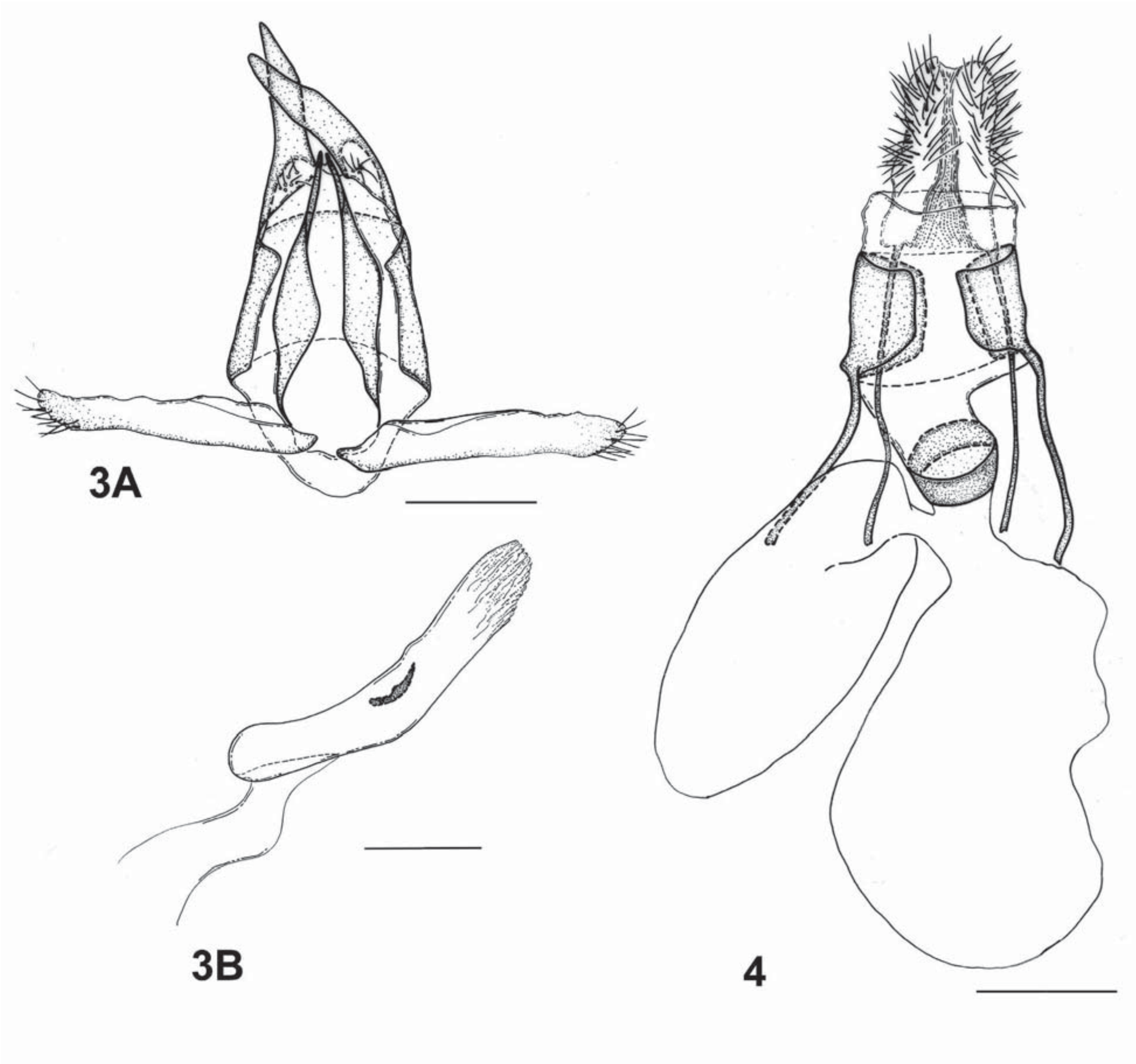

Fig. 3–4. **(3)** Male genitalia of *Euclemensia barksdaleella* n. sp. Lee and Brown, A. Tegumen, vinculum, and valva. B. Phallus. Scale bar: 0.2 mm. **(4)** Female genitalia of *Euclemensia barksdaleella* n. sp. Lee and Brown. Scale bar: 0.2 mm.

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REPRODUCTIVE BEHAVIOR OF *SPEYERIA DIANA* (NYMPHALIDAE) IN ARKANSAS

Additional key words: Diana fritillary, threatened butterfly, reproductive biology, mating behavior, great spangled fritillary

The Diana fritillary, *Speyeria diana* (Cramer 1775) (Nymphalidae), is considered a rare species across most of its range (Rudolph *et al*. 2006; Campbell *et al*. 2007). The North Carolina and Arkansas Heritage Programs currently list *S. diana* as an imperiled species of special concern (rank S2/S3) due to its rapid decline over the past two decades; it is also included on the Xerces Society Red List of Pollinator Insects (Vaughan & Shepard 2005; Howard & Legrand 2009). The conservation network, NatureServe, assigns *S. diana* a Global Status of G3/G4, which describes the species as very rare or local throughout its range, found locally in a restricted range (21 to 100 occurrences), and threatened throughout its range (NatureServe 2008). Because of its rapid disappearance across portions of its former distribution, *S. diana* may soon become a candidate for listing under the Endangered Species Act of the United States (Federal Register 1991, Vol.56, no. 225, pp. 58, 831). Currently, *S. diana* is not protected through any special conservation status, although it appears to be declining across certain portions of its distribution (Carlton & Nobles 1996; Moran & Baldridge 2002; Cech & Tudor 2005; Wells unpublished).

Our goal here is to describe the reproductive behavior of *S. diana* observed during two separate occasions in Mount Magazine State Park, Arkansas, in the summer of 2007. We are aware of only two published accounts of this species mating in the wild, one from Missouri in 1853 (Strecker 1900), and the other more recently from Bath County, Virginia (Cohen & Cohen 1991).

Bionomics. The Diana fritillary is univoltine, producing one generation per year. Adult males emerge and take flight in late May, typically several weeks before females (Allen 1997; Cech & Tudor 2005). Males patrol along the edge of forest habitat, and have an active and mobile lifestyle. While males begin to die off in late July, females persist somewhat cryptically into early October (Opler & Krizek 1984; Adams & Finkelstein 2006). Females are believed to be longer lived than the males, and are often found resting quietly in the cover of forest for much of the day, nectaring or ovipositing on the forest floor (Klots 1951; Spencer 2006).

In general, *S. diana* inhabits moist cove forests and deep woodland areas near streams. Adult Diana fritillaries are often found in open areas feeding on tall, high-quality nectar sources such as milkweeds, butterfly bushes or large fall composites (Moran & Baldridge 2002; Spencer 2006; Baltosser 2007; Ross 2008). Violets (Viola, Violaceae) are the only larval host plants used by *Speyeria*. Each female Diana fritillary can lay thousands of eggs singly on ground litter during the month of September in the vicinity of violets (Allen 1997; Cech & Tudor 2005). The hatched larvae immediately burrow deep into the leaf litter of the forest floor where they overwinter until the following spring.

Historical observations. According to natural historical accounts, the orange and black *S. diana* male was first noted when the type specimen of this species was initially described by the Dutch naturalist Pieter Cramer near Jamestown, Virginia (Cramer & Stoll 1777; Klots 1951). These coastal populations of *S. diana* have since been extirpated since the 1950s (Scott 1986; Howard & Legrand 2009). In August 1864, the prominent 19th century lepidopterist, William Henry Edwards, was the first to formally describe natural populations of the iridescent blue-green female *S. diana*, then *Argynnis diana*, in Kanawha, West Virginia (Edwards 1864). Holland (1883) captured and pinned what he believed at the time to be the "first specimen of the female *Argynnis diana* ever put upon an insect pin"

Fig. 1 *Speyeria diana*, the Diana fritillary, was observed mating on two occasions during summer 2007 in Mount Magazine, Logan County, Arkansas. The female (on left) is an iridescent blue and black, while the smaller male (on right) has bright orange and black coloration.

sometime between 1858 and 1861 in Salem, North Carolina. However, a note published by the renowned entomologist, Herman Strecker, dated 9 March 1900 describes what is likely the actual earliest described female *S. diana* held in the collection of a Baltimore collector and lepidopterist, J.P. Wild. In this note, Strecker described what was defined taxonomically at that time as a female of *Argynnis diana* captured in copula with a male in Missouri around 1853 (Strecker 1900). Upon inspection of the Strecker collection, most of which is now held at the Field Museum of Natural History in Chicago, Illinois; however, we were not able to find any copulating pair of Diana fritillaries. In fact, we found no *S. diana* specimens from Missouri in the entire Strecker collection. We welcome any knowledge of the whereabouts of the aforementioned Strecker specimens, as well as any other historical accounts of mating in *S diana*.

Field observations. We observed two copulating pairs of Diana fritillaries on 9 and 23 June 2007 in Mount Magazine State Park, Logan County, Arkansas (Fig. 1). Mount Magazine State Park is one of Arkansas' newest state parks, and is managed by Arkansas State Parks through a special partnership with the US Forest Service. The Park conserves 904 hectares of mountain habitat, including Arkansas' highest point at 840m, and is surrounded by more than 4,500 hectares of mixed, moist deciduous forest in Ozark National Forest. The US Forest Service designates 14 invertebrate species found in Mount Magazine as endemic, rare, sensitive or threatened, including *S. diana* (Ross 1998).

We observed *S. diana* copulation for a duration of 4hrs on 9 June 2007, and for 2hrs on 23 June 2007, between 1200 hrs and 1700hrs. In general, nymphalids are known to mate for durations of thirty minutes to over 5hrs (Brower *et al*. 1965; Miller & Clench 1968; Pliske & Eisner 1969). We observed dozens pairs of S. cybele mating in the same vicinity, and at the same time, as *S. diana*. The mating behaviors of *S. diana* appear to be highly similar to that of the widespread great spangled fritillary, *S. cybele*, lending observational support to hypotheses relating *S. diana* and *S. cybele* as probable sister species (Hammond 1978; Baltosser 2007; Dunsford 2009).

We witnessed carrying pair behavior in *S. diana* during copulation, which is rarely observed in nature. During both of our observations, the female Diana fritillary alighted, carrying the limp male high into the branches of the forest after several hours of copulation. The only other published description we have found of *S. diana* mating behavior was Cohen & Cohen (1991), who also reported the *S. diana* female carrying the male in Bath County, Virginia. The carrying pair behavior we observed in *S. diana* is consistent with that displayed by other butterflies in the genus *Speyeria*, where females frequently carry their male partners during mating (Shields & Emmel 1973).

The habitats where we encountered mating pairs of *S. diana* and *S. cybele* were open fields that contained a rich mix of high quality nectar plants bordered by dense forest. We noted the following plant species present where *S. diana* and *S. cybele* pairs were mating: coreopsis (*Coreopsis lanceolata*), purple coneflower (Echinacea purpurea), butterfly weed (*Asclepias tuberosa*), bee balm (*Monarda* spp.), yarrow (*Achillea millefolium*), ironweed (*Vernonia* spp.), thistle (*Cirsium* spp.), and goldenrod (*Solidago* spp.). During both of our observations, the female *S. diana* nectared on purple cone flower throughout copulation (Fig. 1), while the males did not ever feed. It has previously been suggested that *Speyeria* butterflies may confine their activities to particular types of nectar-producing flowers, specifically those that provide the highest quality of energy-packed sugars (Ross 2003; Rudolph *et al*. 2006). Future investigation that quantifies nectar quality across a wide variety of *S. diana* habitats would be very useful in examining relationships between *S. diana* reproductive behavior and floral composition.

While our field observations are limited in scope, they provide important insight into the copulation, mating time, and carrying pair behavior of a threatened North American butterfly that is rarely seen mating in nature. Future investigation should focus on quantifying specific habitat requirements for *S. diana*, including the documentation of larval and nectar plant associations across this species' entire distribution to better inform future conservation efforts.

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A NEW RECORD OF THE FRUIT PIERCING MOTH *ORAESIA EXCAVATA* (BUTLER) (EREBIDAE: CALPINAE: CALPINI) FOR HAWAII AND THE UNITED STATES

Additional key words: Asia, Pacific, peach, pear, orchard pest, Menispermaceae

In November 2009, an exotic fruit piercing moth was collected at an elevation of 1125 m in Kula, Maui, Hawaii by W. G. King, and the specimen was submitted with his insect collection for an introductory entomology course at the University of Hawaii. Soon thereafter, the species was independently collected by the authors and others from other localities on the islands of Maui, Kauai, Oahu, and Hawaii (C. Campora, B. Kumashiro, C. Jacobsen pers. comm.), and tentatively identified as *Oraesia excavata* (Butler) (Erebidae: Calpinae: Calpini), which was confirmed by M. Pogue (2010). To our knowledge, this is the first record of establishment of *O. excavata* outside of Asia, and certainly the first record of establishment in the USA. Widespread surveys have not taken place, so the full extent of the invasion within Hawaii remains unknown. However, the species has been collected from widely dispersed sites on the islands of Kauai, Oahu, Maui, and Hawaii, and eradication is not considered a possibility.

The large moth is quite distinctive, with a scalloped trailing edge of the forewings, unusual porrect palpi, and orange head and ventral surface of the body and legs (Fig. 1), which easily distinguish it from other species present in Hawaii. Antennae of males are pectinate, while those of females are simple. When resting with folded wings, the moth somewhat resembles a dead leaf with a scalloped dorsal edge, and the beaklike palpi are noticeable (Fig. 2).

Because we observed only a few eggs and larvae, and none were successfully reared completely from egg to adult, we are unsure of the number of instars, duration of life cycle, and extent of color variation. We successfully reared one field-caught caterpillar to adulthood (Fig. 2), confirming the association between caterpillars and adults. Six eggs were laid in the laboratory by a female caught by S. Montgomery *et al*. at Kokee, Kauai. These were roughly spherical (diameter about 0.80 mm), light brown with dark brown splotches, and loosely adhered to a substrate within the collecting container (Fig. 3). Newly hatched larvae (Fig. 4, body length 3.2 mm, head capsule width 0.45 mm) are uniformly grey with black tubercules and setae. Intermediate instars (Figs. 5 and 6) are very dark

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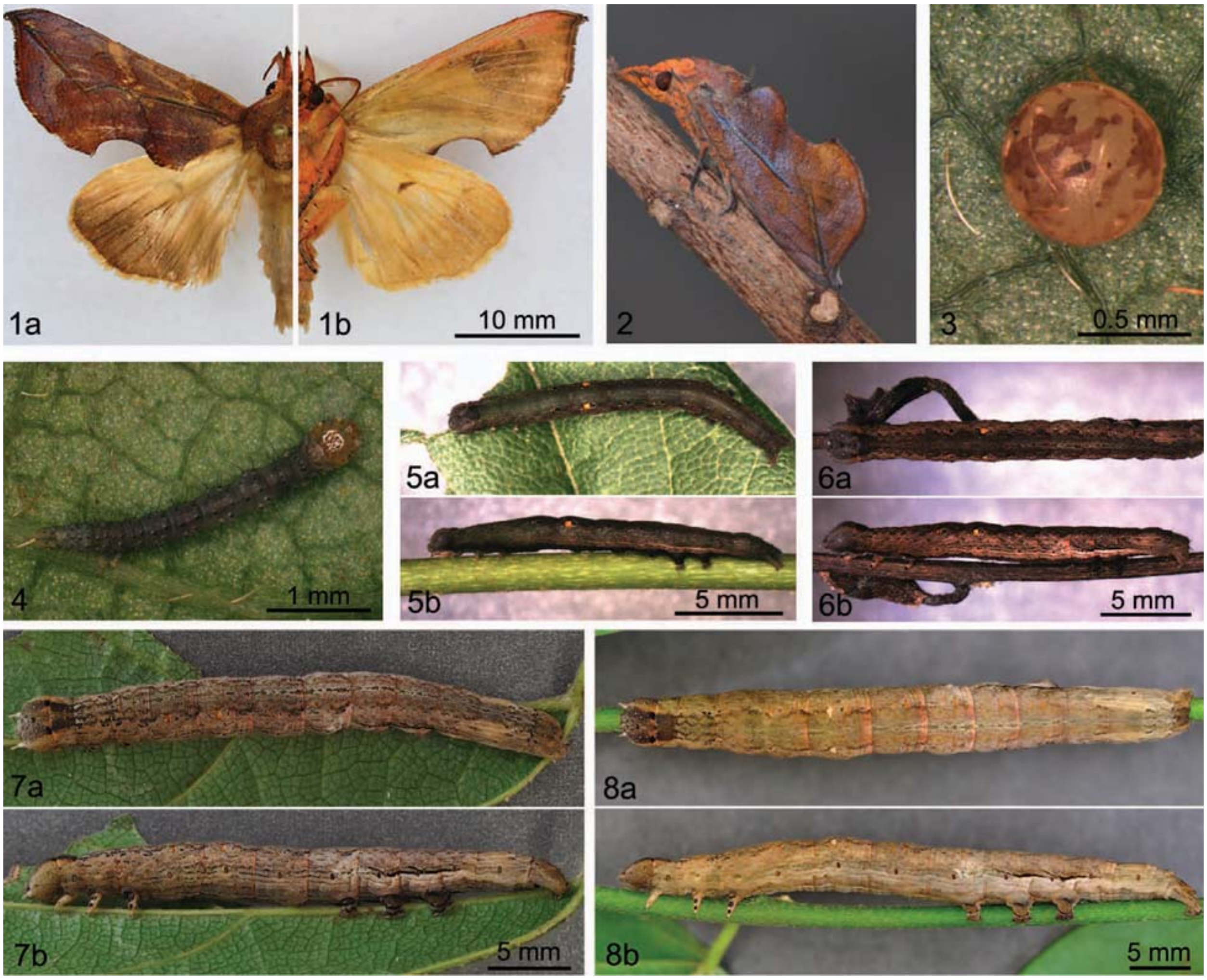
Originally described from Japan (Butler 1878), *O. excavata* is a major pest there as well as in other Asian regions, including China (Chen *et al*. 1992; Liu 2002), Korea (Lee *et al*. 1970), Taiwan (Taiwan Forestry Research Institute 2010), and Thailand (Pholboon 1965). It has been recorded as a pest on many soft fruit crops, including peaches, plums, pears, apples, grapes, citrus, mango, loquat and papaya (Liu 2002; Zhang 1994; H. Tsumuki pers. comm.). The adult uses its



FIGS. 1–8: **1)** *Oraesia excavata*, ♂. USA: HI, Oahu, Koolau Mtns. Waahila Ridge. 360m. 21.308N, 157.796W. UV light trap. 9 Jan 2010. W. Haines. **a,** Dorsal surface. **b,** Ventral surface. **2)** *O. excavata*, ♀, live moth showing resting position. USA, HI, Maui, Olinda, Hawea Pl., 890 m. 20.822N, 156.297W. reared ex: *Cocculus orbiculatus*. collected: 12 Feb 2010, pupated: 18 Mar 2010, emerged: 9 Apr 2010. F. and K. Starr. **3)** *O. excavata*, egg laid by female collected at Kokee, Kauai. **4)** *O. excavata*, first instar larva, newly hatched from egg laid by female collected at Kokee, Kauai. **5)** *O. excavata*, intermediate instar larva, USA, HI, Maui, Olinda, Hawea Pl., 890 m. 20.822N, 156.297W. ex: *Cocculus orbiculatus*. F. and K. Starr. Collected: 7 Mar 2010. Photographed: 17 Mar 2010. **a,** dorsal view. **b,** lateral view. **6)** *O. excavata*, intermediate instar larva, same individual as Fig. 5. Photographed: 22 Mar 2010. **a,** dorsal view. **b,** lateral view. **7)** *O. excavata*, late instar larva, same individual as Fig. 5. Photographed: 30 Mar 2010. **a,** dorsal view. **b,** lateral view. **8)** *O. excavata*, penultimate instar larva, from same locality as Fig. 5. Collected: 12 Feb 2010. Photographed: 17 Mar 2010. **a,** dorsal view. **b,** lateral view.

barbed proboscis to pierce ripe fruit and feed on juice, often causing rot to develop around the injury, and causing some fruits to fall prematurely (Hattori 1969). In some regions of Asia, this species is considered the most important moth pest of fruit crops (Lee *et al.* 1970; Chen *et al*. 1992). It is on quarantine lists for India (Plant Quarantine Organization of India 2003), Australia (Biosecurity Australia 2008), and New Zealand (Ministry of Agriculture and Forestry 1999) as an importation threat from countries where it occurs.

The genus *Oraesia* Gueneé is mostly pantropical, and includes about 25 species of fruit piercing moths (Zaspel & Branham 2008). Although there are many exceptions, calpine moths, including the genus *Oraesia*, predominantly specialize on the plant family Menispermaceae for oviposition (Fay 1996). Based on published records, menispermaceous plants are certainly preferred hosts for *O. excavata* (Pholboon 1965; Fujimura 1972; Ohmasa *et al*. 1991), but there are also some records of caterpillars feeding on other plant families, for instance *Lepisanthes rubiginosa* (Sapindaceae) (Pholboon 1965). In Japan, caterpillars of *O. excavata* feed primarily on *Cocculus orbiculatus* (L.) (Menispermaceae), a creeping vine that occurs outside of orchards (Ohmasa *et al*. 1991). This plant is also native to Hawaii, and is a local host for *O. excavata*. However, because *C. orbiculatus* is not a particularly common or abundant plant in Hawaii, it is possible that *O. excavata* may utilize other hosts as well. We collected *O. excavata* caterpillars on *C. orbiculatus* at two sites in Hawaii: upland mesic forest at Olinda, Maui (890 m), and dry coastal shrubland at Kaena Point on Oahu (73 m), suggesting that *O. excavata* tolerates a wide range of moisture, elevation, and temperature.

Many fruits grown in Hawaii, including papaya, mango, banana, orange, and guava, will likely be fed upon by adult *O. excavata*, but it remains to be seen whether damage will be economically significant. At this time, excessive fruit-piercing damage has not been reported by fruit growers in Hawaii. Another fruit piercing moth *Eucodima phalonia* (Clerck), has been established in Hawaii for 25 years (Heu *et al*. 1985), but is not considered a major pest there, despite being very problematic elsewhere in the Pacific (Fay 1996; Reddy *et al*. 2005). Its relative innocuousness in Hawaii may be due to high levels of attack by *Trichogramma* and other parasitoids (Heu *et al*. 1985). This may prove to be the case with *O. excavata* as well.

There has been considerable research towards the control and monitoring of *O. excavata* in Asia, including sex pheromones (Ohmasa *et al*. 1991), plant kairomone attractants (Miyazaki *et al*. 1972; Tian *et al*. 2008), chemical deterrents (Fujimura 1972; Tian *et al*. 2007), bagging of fruits (Fujimura 1972; Liu 2002), pesticides (Fujimura 1972; Liu *et al*. 2001), and light traps (Hattori 1969; Fujimura 1972; Liu *et al*. 2001; Liu 2002), but these have had only limited success. A female sex pheromone of *O. excavata* was identified and found to be very attractive to males (Ohmasa *et al*. 1991), but is not commercially available or currently used for monitoring or control in Japan (H. Tsumuki pers. comm.). The odor of ripe fruit is also attractive to the moths, and traps may be baited either with the fruit themselves, or a chemical mixture that simulates fruit odor (Tian *et al*. 2008).

Although *O. excavata* and *O. emarginata* are major pests in Asia, a risk assessment conducted by the New Zealand Ministry of Agriculture and Forestry (Tyson *et al*. 2009) concluded that the risk of importation of these species along with fruit was negligible, because larvae and eggs are not associated with fruit trees, while adults are nocturnal, highly mobile, and unlikely to remain on produce during the daytime when fruit is harvested. It is unknown from which country or by what pathway *O. excavata* was introduced to Hawaii, but it appears to have spread rapidly upon arrival, being independently collected or photographed on four islands within months of initial detection. It is possible that adults actively flew or were carried by winds among the different islands, but considering the apparently rapid spread between distant islands (greater than 115 km of open ocean between Oahu and Kauai), human-mediated transport seems likely. Since adult noctuiod moths often seek shelter in dark places during the day, it is possible that they could be transported in packing crates or containers, even if not associated with a specific product. The lack of a known invasion pathway may complicate inspection efforts for this pest.

Because of the importance of orchard and vine crops in the continental US, *O. excavata* could potentially become a pest if established there, and its geographic range in Asia is similar in climate to regions of North America, especially the Eastern US. One major factor which might limit establishment or population growth in North America is the occurrence of suitable larval host plants. There are only six species of Menispermaceae (5 native, 1 exotic) established in the continental US, and most of these are confined to the southeastern part of the country. (Rhodes 1997). West of the Rocky Mountains, there are no native or naturalized menispermaceous species in the continental US, other than *Cocculus diversifolius* de Candolle in the very southern parts of Arizona and Texas. However, east of the Rocky Mountains there are five species of Menispermaceae, including the widespread natives *Cocculus carolinus* (L.) and *Menispermum canadense* L.

(Rhodes 1997). Of course, it is also possible that *O. excavata* could establish or even thrive on a different plant family, since its degree of host-specificity has not been evaluated.

Given the potential threat of this species to fruit crops in the mainland United States, it would be worthwhile to study the biology and distribution of *O. excavata* in Hawaii. Monitoring methods could be developed in Hawaii and used for early detection or interception of *O. excavata* in the mainland United States.

Material examined (deposited at University of Hawaii Insect Museum, UHIM):

Adult moths: 1 ♂ , USA: HI, Maui, Kula, Upper Kimo Dr. 1125 m. 20.775N, 156.298W. 24 Nov 2009. G. King; 2 ♂, USA: HI, Maui, Kokomo, 2955 Kailiili Rd. 477m. 20.867N, 156.305W. At UV light. 17 – 19 Dec 2009. W. Haines; 2 ♂, USA: HI, Oahu, Koolau Mtns. Waahila Ridge. 360 m. 21.308N, 157.796W. UV light trap. 9 Jan 2010. W. Haines; 1 ♀, USA: HI, Oahu, Ewa, Aloun Farms citrus grove. Unbaited fruit fly trap. 58 m. 21.374N, 158.045W. 25 Jan 2010. L. Leblanc; 1 ♀, USA: HI, Kauai, Koke'e State Park. S. Montgomery, C. Campora, S. Lee; 1 ♀, USA, HI, Maui, Olinda, Hawea Pl., 890 m. 20.822N, 156.297W. reared ex: *Cocculus orbiculatus*. collected: 12 Feb 2010, pupated: 18 Mar 2010, emerged: 9 Apr 2010. F. and K. Starr; 1 ♂, USA: HI, Hawaii Island, Ka'u, Kahuku, Ocean View Estates, 550 m. 19.073N, 155.750W. 6 Dec 2010. S. Montgomery., 2 ♀, USA: HI, Hawaii Island, Ka'u, Kahuku, near Ocean View Estates, 550 m. 19.07N, 155.74W. 5 Jan 2011. S. Montgomery.

Larvae: 5 larvae, USA, HI, Maui, Olinda, Hawea Pl., 890 m. 20.822N, 156.297W. ex: *Cocculus orbiculatus*. 12 Feb – 16 Mar 2010. F. and K. Starr.; 5 larvae, USA, HI, North Oahu, Kaena Point, 73 m. 21.575N, 158.258W. ex: *Cocculus orbiculatus*. 15 May 2010. W. Haines.

Other existing material (photographs examined): 1 unknown sex, USA: HI, Oahu, Lualualei, Halona Valley, 500 m. 21.424N, 158.098W. 8 Dec 2009. C. Campora, S. Montgomery, S. Lee (specimen kept by S. Montgomery).; 1♂, USA: HI, Oahu, Nuuanu, on eaves of roof, 21 Dec 2009. G. Uchida. (Specimen at Hawaii Department of Agriculture); 1 unknown sex, USA: HI, Hawaii Island, Kawaihae, Honouli St., 20.062N, 155.839W, 95m, at residence, 7 Jul 2010 (photographed but not collected).

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BRITISH BUTTERFLIES: A HISTORY IN BOOKS. By David Dunbar. 176 pp. Hardbound; approx. 7 x 10 in; nearly 60 color and 20 halftone illustrations; ISBN: 978-0-7123-5096-9; £45.00 ($72.00 in US); The British Library, London. 2010. Available principally from the British Library (http://shop.bl.uk/mall/BritishLibrary/), Aurelian Books (http://www.aurelianbooks.com/index.php), and the University of Chicago Press (US orders; http://www.press.uchicago.edu/).

After visiting my home for the first time, a friend recently observed that the size of my library exceeds that of my Lepidoptera collection—the antithesis of most lepidopterists. As a young man I acquired an intense passion for books, particularly those that contain references to North American butterflies. This interest extends, to a lesser degree, to publications about the butterflies of Britain and Europe. Not only do our faunas share affinities, but the study of entomology in America is rooted in the Old World. My own historical research would not be possible without consulting the many works of British naturalists. Electronic publications and digital libraries are now commonplace, but many of us still sense a connection to books that cannot be conveyed through the glowing screens of computer monitors or handheld reading devices. If you share this bond, then *British Butterflies* is the book for you. A decade ago Michael Salmon's *Aurelian Legacy: British Butterflies and Their Collectors* became the quintessential reference on British lepidopterists. David Dunbar has done for British butterfly books what Salmon did for those who wrote them.

A lepidopterist since his youth and a London bookseller for nearly 30 years, Dunbar is intimately familiar with the topic. The stated purpose of this book is "not to repeat well-known and documented information on Lepidoptera, but rather to point the reader in the direction of source or background material that is more comprehensive." The small size of this book is misleading, as Dunbar adeptly covers the most relevant works published since the year 1634, mentioning nearly 600 titles in the process. He documents over four centuries of butterfly books in Britain, writing in a non-technical style that demonstrates his passion for the subject. It is astonishing that several hundred books have been published about British butterflies, despite the fact that the region is inhabited by fewer than 60 resident species.

Often lacking in historical treatments, Dunbar briefly examines the manufacture of books, including early methods of engraving and lithography, as well as advances (and budget-conscious shortcuts) in the binding process. Included are discussions on classification, scientific lists, the Linnaean system, and the importance of butterflies in art and romance. Dunbar also examines recent books that advocate practical fieldwork and the conservation of butterflies. His bookseller's perspective is evident in remarks about print runs and overstock pricing. Reaching beyond books, Dunbar includes sections on wall charts, as well as cigarette and trade cards that feature butterflies. To me, Dunbar's most valuable contribution is his overview of older works, from the 17th through the 19th centuries, arranged chronologically and by theme (e.g. science & systematics, collecting & fieldwork, and children's books). Many full color illustrations accompany the text.

I found very little to criticize about this book. It is unfortunate that the figures are too small to permit a full appreciation of the many illustrations that are reproduced, but I prefer the smaller format of this book to that of an unwieldy coffee table volume. Dunbar could have cited a couple of additional publications, such as Pamela Gilbert's *A Source Book for Biographical Literature on Entomologists* (2007). He mentions that the frontispiece included in early issues of *The Aurelian* by Moses Harris is dated 1780, but this date is actually associated with a self portrait of Harris that appeared in another book by Harris entitled *An Exposition of English Insects* (the frontispiece in the *Aurelian* is undated). In light of Dunbar's inclusion of cigarette and trade cards, it is somewhat surprising that he did not incorporate a section about postage stamps. This is especially true considering the popularity of philately and the fact that Britain has issued a number of stamps that feature butterflies, including a series in 2008 that showcased endangered species. A set of four butterfly stamps, issued by Britain in 1981, is currently offered for sale by Dunbar's own business, *Aurelian Books*. Considering the large amount of helpful information presented in this book, these issues are of minor importance.

Many of the books featured by Dunbar are very rare, but the majority of the titles published prior to 1900 are now available for viewing online through such websites as Internet Archive, Biodiversity Library, and Google Books. Although scanned images are valuable to researchers who lack access to the original publications, they rob us of the pleasure of holding the books in our hands, turning the pages, smelling the aromas of print and paper, and experiencing firsthand the splendor of their illustrations. Not only is British Butterflies a valuable compendium, it reminds us how easy it can be to reconnect with our past. All we have to do is open a book.

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MANUSCRIPT REVIEWERS FOR 2010 (VOLUME 64)

Manuscript reviewers are anonymous contributors to the scientific rigor, clarity, and quality of text and illustrations in the papers published by the Journal of the Lepidopterists' Society. The reviewers' input is invaluable and always welcomed by authors, editors and readers. We hope their careful work continues to allow the Journal to increase quality and readership. On behalf of all the authors and the editorial staff of the Journal, respectful acknowledgement is given to the reviewers for contributions published in Volume 64.

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