

Full Issue

Source: The Journal of the Lepidopterists' Society, 65(4)

Published By: The Lepidopterists' Society

URL: <https://doi.org/10.18473/lepi.v65i4.a13>

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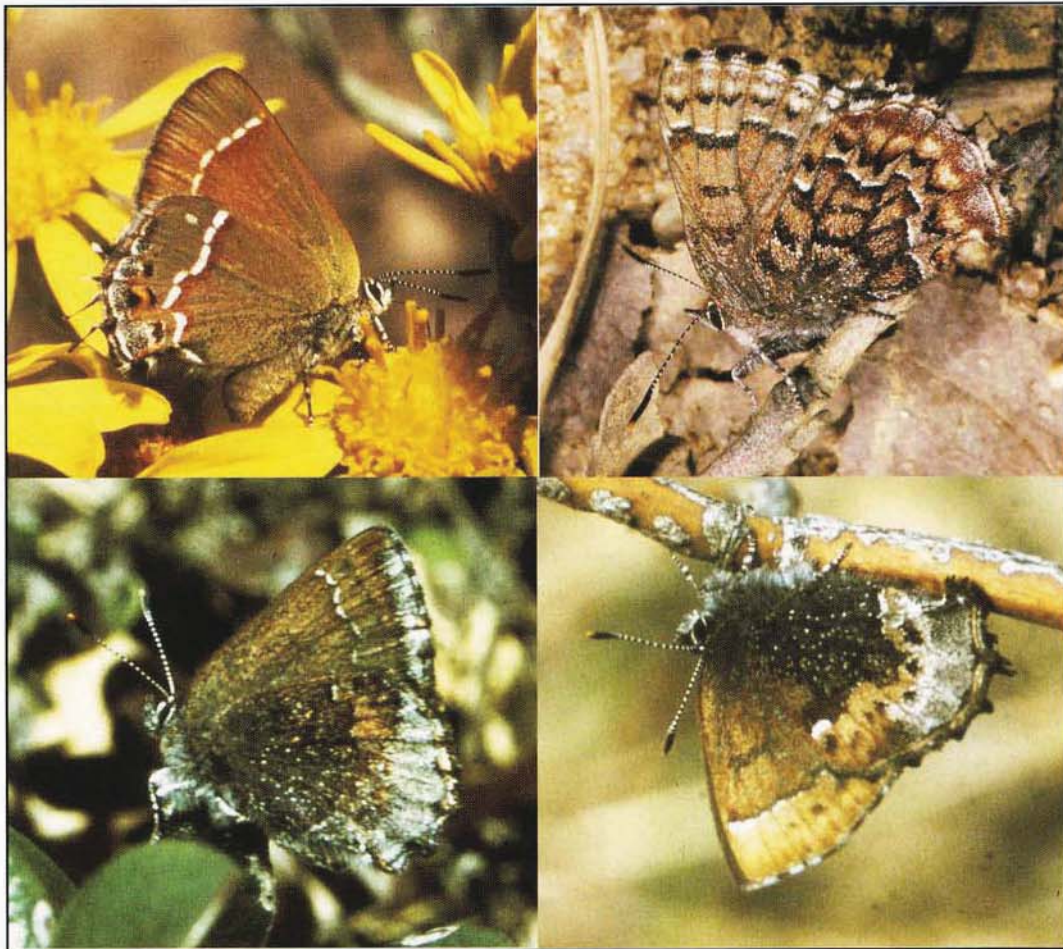
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Volume 65 Number 4
December 2, 2011
ISSN 0024-0966

Journal of the Lepidopterists' Society



Published quarterly by The Lepidopterists' Society

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Journal of The Lepidopterists' Society (ISSN 0024-0966) is published quarterly by The Lepidopterists' Society, % Los Angeles County Museum of Natural History, 900 Exposition Blvd., Los Angeles, CA 90007-4057. Periodicals postage paid at Los Angeles, CA and at additional mailing offices. POSTMASTER: Send address changes to The Lepidopterists' Society, % Natural History Museum, 900 Exposition Blvd., Los Angeles, CA 90007-4057.

Cover Illustration: Images from the study of leaf-mining Gracillariidae from *Schinus terebinthifolius*. Clockwise from top left: *Caloptilia schinusifolia* Davis and Wheeler, *Leurocephala schinusae* Davis and Mc Kay (photos by Patricia Gentili), leafmine of *L. schinusae* on *S. terebinthifolius* in Argentina, Cocoon of *L. schinusae* with last (5th) instar larva (photos by Fernando Mc Kay). See article starting on page 61.

JOURNAL OF THE LEPIDOPTERISTS' SOCIETY

Volume 64

2011

Number 4

Journal of the Lepidopterists' Society
65(4), 2011, 205–222

ALLOZYME-BASED PHYLOGENY OF NORTH AMERICAN *Callophrys* (S. L.) (LYCAENIDAE)

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Abstract. In allozyme generated trees, test populations of species of the North American *Callophrys* (sensu lato) (a group of hair-streak/elfin butterflies) clustered within the genera *Mitoura*, *Callophrys*, *Incisalia*, and *Loranthomitoura*. The pine-feeding species of *Incisalia* clustered weakly, but separately from non-pine feeding species of *Incisalia* (*Deciduphagus*). The trees present dissimilarities from recent taxonomic arrangements of *Mitoura* species and subspecies. Larval food plants (*Calocedrus*, *Juniperus*, and *Cupressus*), often used for distinguishing *Mitoura* taxa, do not necessarily follow the pattern of genetic relationships among populations. *Mitoura thornei* and *M. muiroi* probably deserve no greater than subspecies status under *M. loki* and *M. siva* respectively. *Mitoura gryneus* and *M. siva* populations, considered conspecific by some workers, do not display a gradual geoclineal blend zone and are probably best considered separate taxa. A putative population of *M. gryneus* from Arkansas may deserve species status.

Additional key words: *Mitoura*, *Incisalia*, *Loranthomitoura*, Cupressaceae, *Juniperus*, *Calocedrus*, *Cupressus*, *Chamaecyparis*, allozyme, hairstreaks

Clench (1961) redefined *Callophrys* Billberg as an omnibus genus comprised of six subgenera: *Callophrys*, *Cyanophrys* Clench, *Incisalia* Scudder, *Mitoura* Scudder, *Sandia* Clench & Ehrlich, and *Xamia* Clench. Johnson (1981) used the infratribe Callophryina within the lycaenid tribe Eumaeini to encompass all components of Clench's *Callophrys*, plus the Old World *Ahlbergia* Bryk, while recognizing generic rank for Clench's subgenera. Johnson (1992a) and Ballmer & Pratt (1992) added *Cisincisalia* and *Loranthomitoura*, respectively, to the list of callophryine genera. Johnson (1992b) further split *Incisalia* by recognizing *Deciduphagus* for those species that do not feed on Pinaceae. The higher taxa within Callophryina have been variously recognized as subgenera of *Callophrys* (e.g., Ziegler 1960; Clench 1961; Harris 1972; Howe 1975; Scott 1986; Opler & Warren 2002; Pelham 2008) or separate genera (e.g., dos Passos 1964; Miller &

Brown 1981; Pyle 1981; Bailowitz & Brock 1991; Bird et al. 1995). Robbins (2004) recognizes a *Callophrys* Section of the Eumaeini (equivalent to Johnson's original concept of Callophryina) containing the genera *Cyanophrys* and *Callophrys*, and assigns to the latter all taxa represented in this study without recognizing subgeneric divisions.

A mainstay for taxonomic distinctions in classical taxonomy has been differences in genital structure. The lack of consensus for taxonomic boundaries within *Callophrys* (s. l.) has been fueled by clusters of taxa with great similarity in genital structure, but with distinct differences in adult morphology, larval morphology, and ecology (especially host specificity). Similarly, the taxonomic boundaries of specific and infraspecific taxa within such clusters are often unsettled due to the absence of direct evidence of barriers to gene flow. Johnson (1976) employed both male and female genital

features to characterize *Mitoura* species, but subsequent workers have found such features to be too plastic to warrant such categorical use (Brown 1983; Ferris 1992; Layberry et al. 1998; Nice & Shapiro 2001). Alar characters, such as ground color and maculation, traditionally used to help distinguish taxonomic boundaries, are often similarly variable within populations. Thus, while Clench (1981) discriminated *C. millerorum* Clench from *C. spinetorum* on the basis of differences in wing maculation, Robbins (1990) synonymized the two, considering them as extremes of a continuum of variation within one *C. spinetorum* population. Warren (2005) also discusses variability in alar characters among some Oregon *Mitoura* populations.

The taxonomy of *Mitoura* has long been controversial. *Mitoura* species range throughout most of North America and are found nearly everywhere their cupressaceous food plants occur (Scott 1986; Opler & Wright 1999). Miller & Brown (1981) recognized ten *Mitoura* species north of Mexico: *gryneus* (Hübner), *hesseli* Rawson & Ziegler, *rosneri* K. Johnson, *barryi* K. Johnson, *byrnei* K. Johnson, *nelsoni* (Boisduval), *siva* (W. H. Edwards), *loki* (Skinner), *spinetorum* (Hewitson), and *johnsoni* (Skinner). Two of these species, *spinetorum* and *johnsoni*, were removed from *Mitoura* and placed into *Loranthomitoura* based upon food plant and autapomorphies (Ballmer & Pratt 1992). Johnson (1978), Tilden & Smith (1986), and Emmel et al. (1998) recognized a ninth species, *muiri* (Hy. Edwards), a taxon originally described as a subspecies of *M. nelsoni*. Lastly, Brown (1983) described a tenth species, *thornei*.

More recently, Guppy & Shepard (2001) treated the *Thuja* associated *M. byrnei* as a junior synonym of *M. rosneri* and Warren (2005) synonymized *M. barryi* with *M. plicataria* Johnson, originally described as a subspecies of *M. rosneri*. Warren (2005) further suggested that all *Thuja* associated populations in the Pacific Northwest might be referable to *M. rosneri*. Scott (1986) and Robbins (2004) recognized only two *Mitoura* species north of Mexico: *hesseli* and *gryneus*; their concept of *gryneus* subsumes all *Mitoura* taxa treated here other than *hesseli*. Opler & Wright (1999) recognized a similar taxonomic arrangement, but distinguished *muiri*, *nelsoni*, and *thornei* as separate from *gryneus*.

Two *Mitoura* taxa in the southern United States are of conservation concern due to their restricted ranges and habitat loss. *Mitoura gryneus sweadneri* F. H. Chermock is restricted to coastal stands of *Juniperus virginiana* L. var. *silicicola* (Small) J. Silba in Florida and has experienced recent habitat loss due to

urbanization (Emmel 1993). *Mitoura thornei* is confined to Otay Mountain, San Diego County, and adjacent lands in southern California where its larval host plant, *Cupressus forbesii* Jepson, occurs and is threatened by increased fire frequency (Brown 1993).

The complexity of *Mitoura* populations is epitomized by the situation in the San Bernardino Mountains of Southern California, where four morphologically and ecologically distinct and narrowly allopatric *Mitoura* populations occur: *loki*, *nelsoni*, *siva juniperaria* Comstock, and what some southern California lepidopterists informally refer to as a "high elevation *nelsoni*". Each is associated with a different larval host plant. The *M. s. juniperaria* and *M. loki* populations, respectively, occur in the northern and southern portions of the range, at low to moderate elevations (mostly 3–5000') in association with what may be different chemical races of *Juniperus californica* Carr. (Vasek 1966; Vasek & Scora 1967). Both are multiple brooded in most years. Typical *M. nelsoni*, on the other hand, is univoltine and occurs at intermediate elevations (4–6000') on the coastal facing slopes in association with *Calocedrus decurrens* (Torrey) Florin. The "high elevation *nelsoni*", also univoltine, occurs above 7000' in association with *J. occidentalis australis* Vasek and differs in appearance from typical *nelsoni* in absence of pinkish overlay found in freshly eclosed *nelsoni* specimens and generally browner ventral appearance, with occasional greenish cast (Ballmer & Pratt pers. obs.).

Ballmer & Pratt (1989, 1992) demonstrated that a suite of morphological characters of the immature stages could be used to characterize some subdivisions (as genera) of *Callophrys* (s. l.), as well as some species of *Mitoura* and *Loranthomitoura*. More recently, Nice & Shapiro (2001) showed that biochemical (allozyme) characters could yield direct evidence of gene flow (or lack of it) among some *Mitoura* populations. Here, we present allozyme-based evidence for the genetic distances and phylogenetic relationships among representatives of most taxonomic groups within North American *Callophrys* (s. l.), as well as populations of several putative *Mitoura* taxa. We use genetic distances (Nei 1972) and allozyme characters to construct a number of computer-generated phylogenetic trees, based on various analytical algorithms. We compare these trees with respect to recognized specific and generic concepts.

MATERIALS AND METHODS

Enzyme Analyses. Allozymes are enzymes that exhibit variability among individuals of a species due to genetic variability in the DNA subunits (alleles) that

TABLE 1. Enzymes used for analyses.

aconitase (ACO-1 & ACO-2)
adenylate kinase (AK)
alpha glycerophosphate dehydrogenase (α GPD)
aspartate amino transferase (AAT-1)
glucose-6-phosphate dehydrogenase (G6PD)
glucose phosphate isomerase (GPI)
isocitrate dehydrogenase (IDH-1 & IDH-2)
malic dehydrogenase (MDH-2)
NADP dependent malic enzyme (ME-1)
peptidase [2 loci: leucyl-glycine (PEP-1) and leucyl-glycyl-glycine (PEP-2) as substrates]
phosphoglucomutase (PGM)
superoxide dismutase (SOD-2)

express them. This enzyme variability is detected through electrophoretic separation of a homogenate, using an appropriate buffer and gel substrate. Individual fresh and/or frozen adult butterflies were homogenized in 50 μ l of buffer (0.005 M Tris-HCl pH 7.5), electrophoresed on 10% starch gels, stained for enzymes, and scored following the procedure of Pratt (1994). Homogenates were stored in microtiter plates at -70°C and electrophoresed with a citrate-aminopropyl-morpholine continuous system (pH 8.5) (Clayton & Tretiak 1972). Fifteen enzymes (Table 1) were stained with conventional histochemical stains provided as 12 different recipes in Shaw & Prasad (1970). Some isozymes, such as IDH-1 and IDH-2, stained with the same enzyme staining recipe.

Analyses of Allelic Variation. Allelic variations of 15 presumptive loci of 400 hairstreaks were scored by distance from the origin. Genetic distances, F statistics, and trees were determined by BIOSYS-1 (Swofford & Selander 1989). Sample sizes are shown in Table 2. The various measures of genetic distances calculated by BIOSYS-1 were Nei, unbiased Nei, minimum Nei, unbiased minimum Nei, Nei identities, unbiased Nei identities, Rogers, Modified Rogers, Prevosti, Cavalli-Sforza & Edwards chord, Cavalli-Sforza & Edwards arc, and Edwards "E". Trees were constructed from genetic distances and presence/absence of allelic characters using *Strymon melinus* as an outgroup with PAUP* (Swofford 1998).

UPGMA trees were constructed by the method of Sneath & Sokal (1973) through BIOSYS-1 and PAUP* using genetic distances and the unweighted pair-group method with arithmetic averaging algorithm. Distance Wagner trees (BIOSYS-1), using the multiple addition criterion algorithm of Swofford (1981), were produced with Rogers, Modified Rogers, Prevosti, Cavalli-Sforza

& Edwards chord, Cavalli-Sforza & Edwards arc, and Edwards "E" distances (Farris 1972). Only trees having the highest cophenetic correlation and low percent standard deviation for the various genetic distances were chosen for analysis. In such trees, the genetic distances between populations are closest to the actual branch lengths in the tree (Pratt & Wright 2002; Pratt et al. 2006). Edwards and Minimum Nei distances and presence/absence of allelic characters were also used to construct trees with PAUP* using neighbor-joining methodology. TreeView (Page 2009) was used to print high-resolution trees from the PAUP* generated trees.

RESULTS

The taxa in this study collectively displayed great genetic variability of 118 alleles for the 15 allozymes studied. Of the 118 allelic characters, 22 were common to all populations and 12 were found in only individual populations. All 15 loci were polymorphic at least in some taxa. The trees constructed by BIOSYS-1 and PAUP* analyses differed somewhat, yet conformed to commonly accepted concepts of subgroups within *Callophrys* (s. l.) (genera or subgenera, species and/or species groups). The trees suggest some novel hierarchical relationships.

Figure 1 illustrates a minimum Nei distance tree having the highest cophenetic correlation (0.917) and lowest percent standard deviation (17.656) among trees produced with Nei, minimum Nei, unbiased Nei, and unbiased minimum Nei distances, using the UPGMA algorithm. Although similar trees based on other genetic distances had higher cophenetic correlations and lower percent standard deviations, the tree in figure 1 is illustrated because Nei distances are often cited as measures of genetic distance between taxa (Ayala et al. 1974; Brittnacher et al. 1978; Gorman & Renzi 1979; Angevine & Brussard 1979; Berlocher & Bush 1982; Pashley 1982; Mensi et al. 1988; Pratt 1994; Paggi et al. 1998; Pratt & Wright 2002; Pratt et al. 2006) and are used for determining molecular clocks (Nei 1971; Maxson & Maxson 1979; Berlocher & Bush 1982; Mensi et al. 1988). A similar tree was constructed through PAUP* with minimum Nei distances and the neighbor-joining methodology (Fig. 2).

A tree with the highest cophenetic correlation (0.963) and second lowest percent standard deviation (6.303) was constructed using the multiple addition criterion algorithm of Swofford and Edwards "E" distances (Fig. 3). Three similar trees having a cophenetic correlation of 0.960 or greater were created with Cavalli-Sforza & Edwards chord, Cavalli-Sforza & Edwards arc, and Modified Rogers distances. In these analyses *I. eryphon* and *I. niphon* clustered together as a sister to the

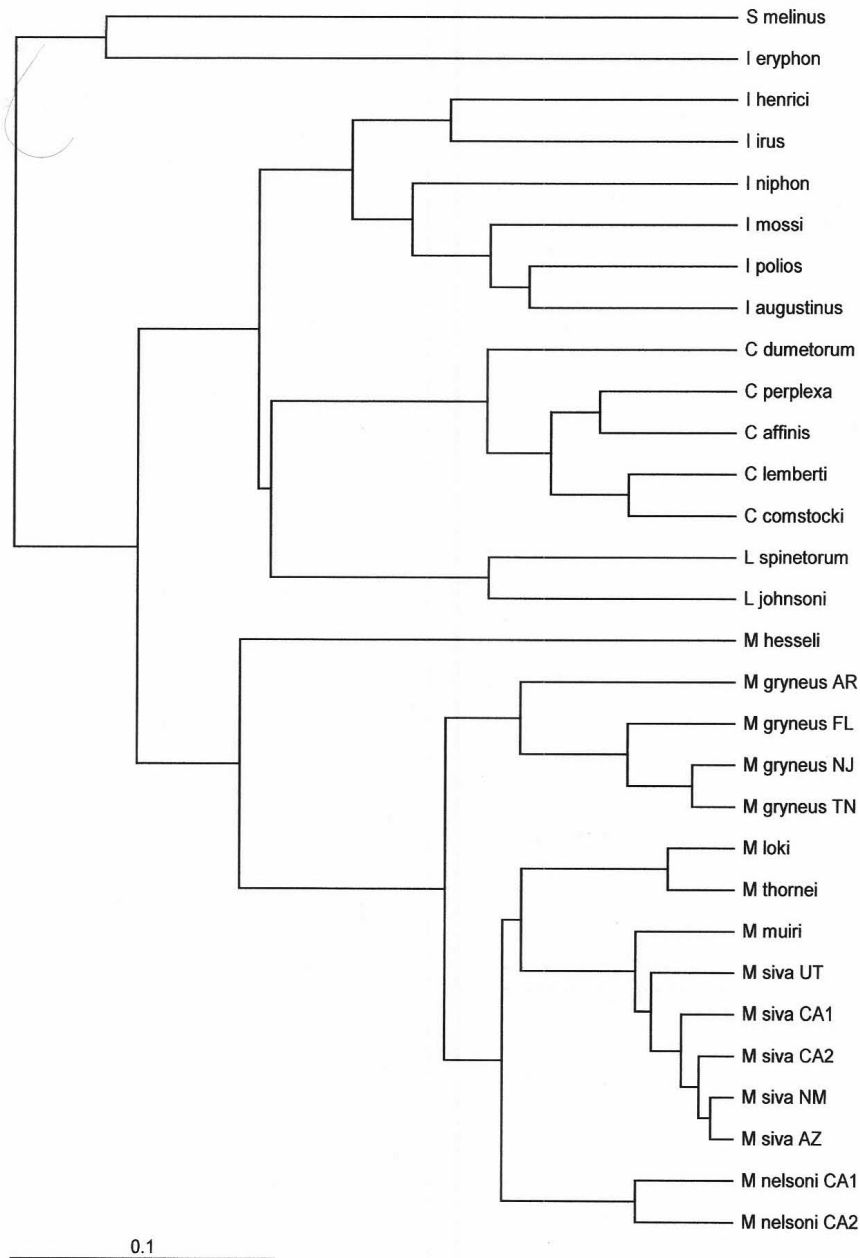


FIG. 1. Nei minimum distance tree using UPGMA algorithm of North American hairstreaks of *Callophrys* (s. l.). *M gryneus* FL, *M gryneus* AR, *M gryneus* NJ, and *M gryneus* TN are *Mitoura gryneus* from Florida, Arkansas, New Jersey, and Tennessee, respectively. *M siva* CA1, *M siva* CA2, *M siva* UT, *M siva* NM, and *M siva* AZ are *Mitoura siva* from La Panza (*California mansfieldi*), New York Mountains (California), Pintura (Utah), Albuquerque (New Mexico), and Rose Peak (Arizona), respectively. *M nelsoni* CA1 and *M nelsoni* CA2 are *Mitoura nelsoni* from Mountain Home (California) and Onyx Summit (California), respectively.

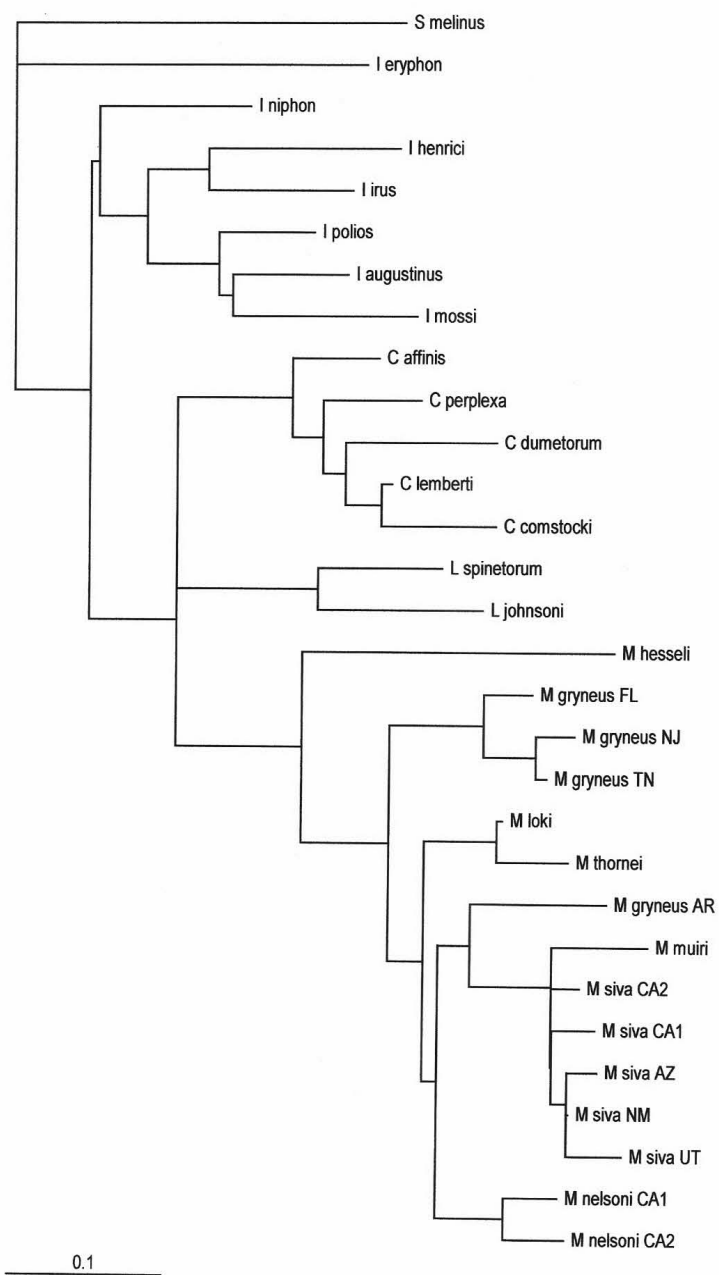


FIG. 2. Nei minimum distance tree using neighbor-joining methodology of North American hairstreaks of *Callophrys* (s. l.). *Strymon melinus* is used as an outgroup. The names of test populations are as in Fig. 1.

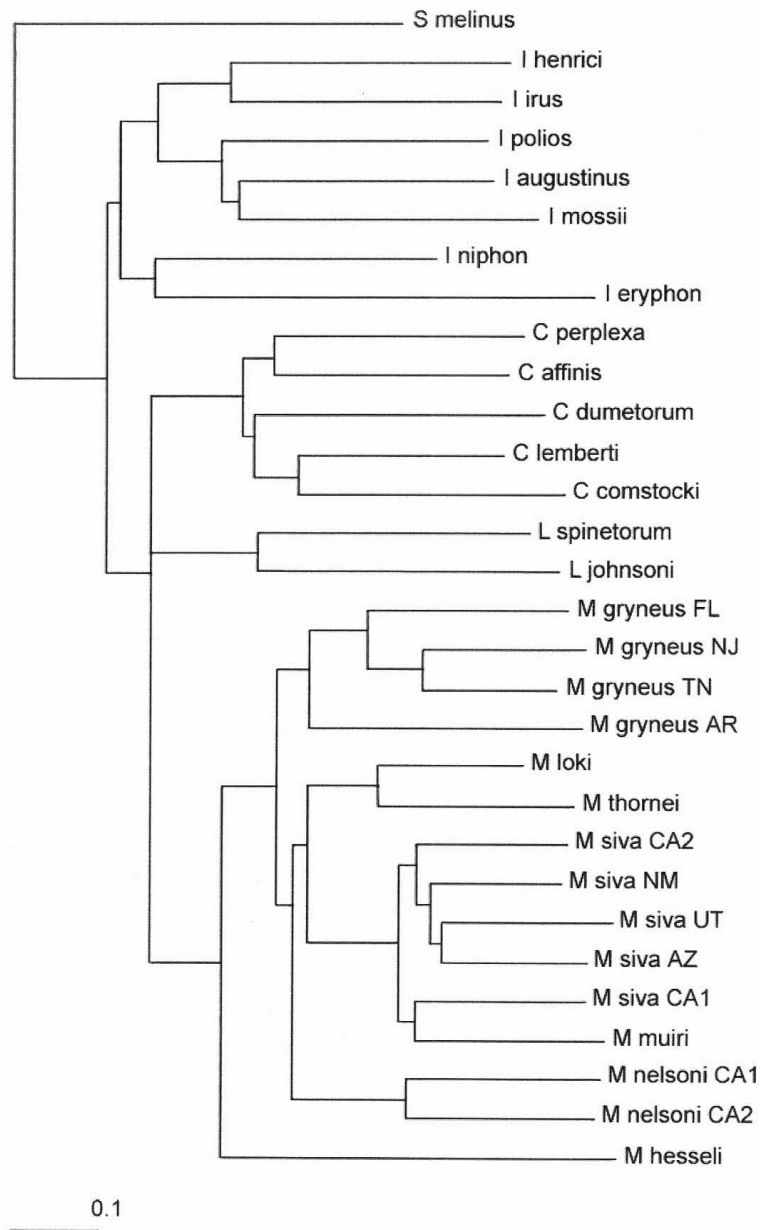


FIG. 3. Distance Wagner tree of North American hairstreaks of *Callophrys* (s. l.) group using the multiple addition criterion algorithm of Swofford and Edwards "E" distance. Total length of the tree is 8.792. The tree has a cophenetic correlation of 0.963 and percent standard deviation of 6.303. The names of test populations are the same as given in Fig. 1.

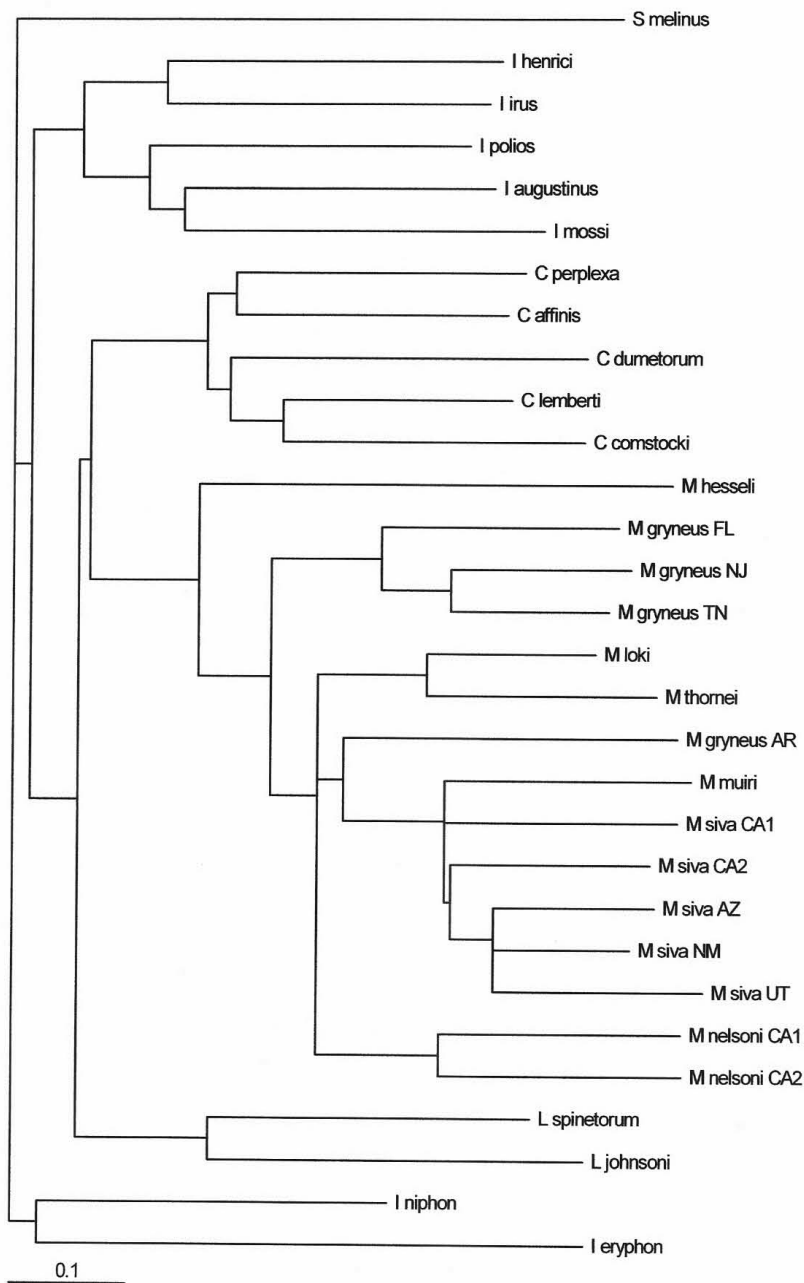


FIG. 4. Edwards distance tree using neighbor-joining methodology of North American hairstreaks of *Callophrys* (s. l.). *Strymon melinus* is used as an outgroup. The names of test populations are as in Fig. 1.

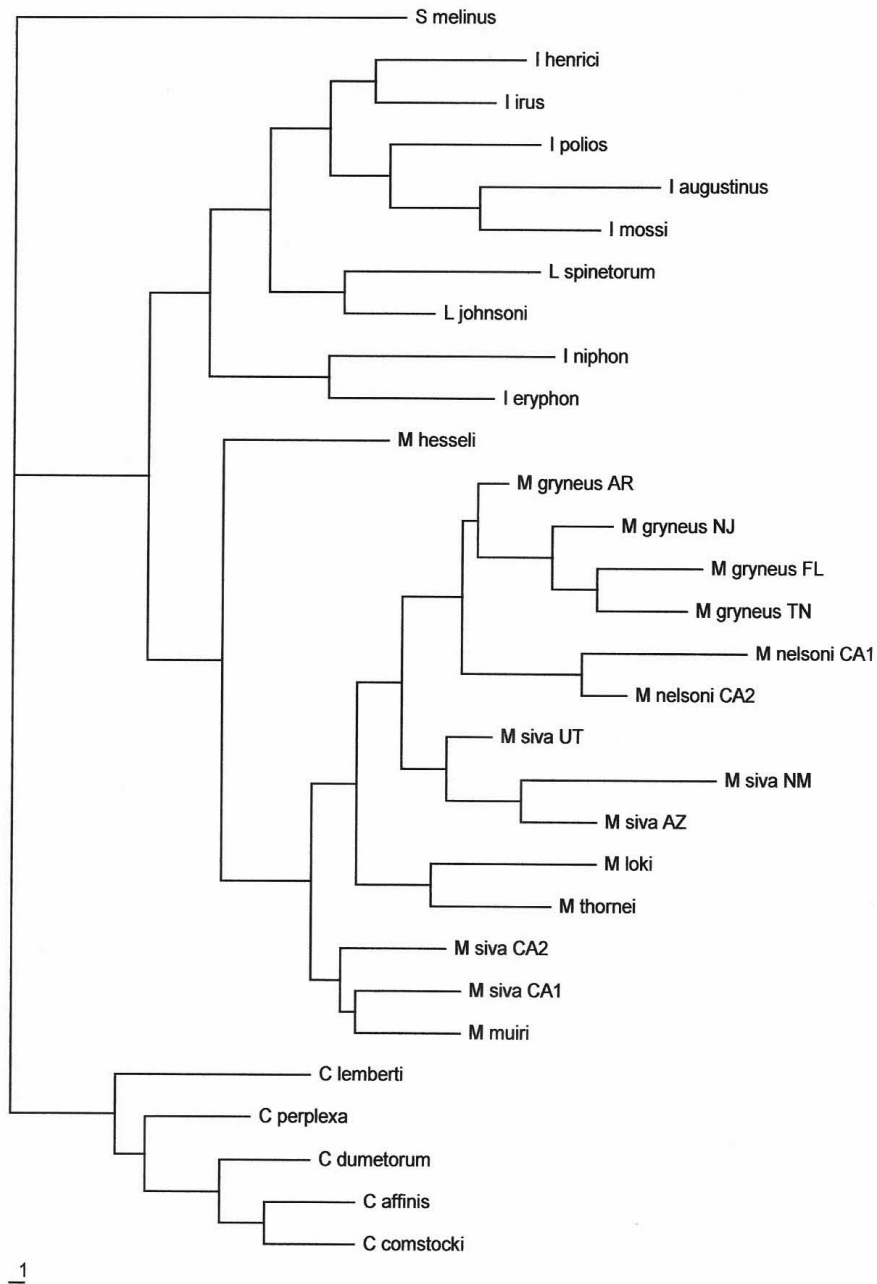


FIG. 5. Tree created using presence/absence of allelic characters and the neighbor-joining methodology of North American hair-streaks of *Callophrys* (s. l.). *Strymon melinus* is used as an outgroup. The names of test populations are as in Fig. 1.

Table 2. Sample sizes and locations of hairstreak populations used for enzyme analyses

<i>Genus</i>	<i>species/subspecies</i>	N	Location
<i>Strymon</i>	<i>melinus</i>	11	Albuquerque, NM
<i>Incisalia</i>	<i>augustinus</i>	10	Chatsworth, NJ
	<i>mossii</i>	12	Forest Falls, CA
	<i>polios</i>	11	Chatsworth, NJ
	<i>irus</i>	10	Mizpah, NJ
	<i>henrici</i>	34	Nanticoke, DE
	<i>niphon</i>	17	Chatsworth, NJ
	<i>eryphon</i>	4	Sugarloaf Mt., CA
	<i>Callophrys</i>	<i>affinis</i>	5
<i>perplexa</i>		10	Riverside Co., CA
<i>dumetorum</i>		5	Marina Dunes, CA
<i>lemberti</i>		6	Sierra Nevada, CA
<i>comstocki</i>		3	Providence Mts., CA
<i>Loranthomitoura</i>	<i>spinetorum</i>	13	Lassen County, CA
	<i>johnsoni</i>	2	Lassen County, CA
<i>Mitoura</i>	<i>hesseli</i>	19	Chatsworth, NJ
	<i>nelsoni nelsoni</i>	10	Mountain Home, CA1 (assoc. w/ <i>C. decurrens</i>)
	<i>nelsoni ssp.</i>	11	Onyx Summit, CA2 (assoc. w/ <i>J. occidentalis</i>)
	<i>muiiri</i>	32	Cuesta Ridge, CA
	<i>gryneus gryneus</i>	24	Vineland, NJ
	<i>gryneus gryneus</i>	11	Rome, TN
	<i>gryneus gryneus</i>	5	Magazine Mountain, AR
	<i>gryneus sweadneri</i>	6	Yankeetown, FL
	<i>loki loki</i>	27	Long Canyon, Riverside Co., CA
	<i>thornei</i>	17	Otay Mountain, CA
	<i>siva siva</i>	27	Albuquerque, NM
	<i>siva siva</i>	10	Rose Peak, AZ
	<i>siva siva</i>	11	Pintura, UT
	<i>siva mansfieldi</i>	25	La Panza, CA1
<i>siva siva</i>	12	New York Mts., CA2	

TABLE 3. Heterozygosities for 15 loci and food plant families

Species/Population	Heterozygosity		Food plant families
	Direct count	H-W expected*	
<i>Strymon melinus</i>	0.234 (0.058)	0.289 (0.069)	many families
<i>Incisalia augustinus</i>	0.238 (0.060)	0.230 (0.057)	Ericaceae, Rosaceae, Rhamnaceae, & others
<i>Incisalia mossii</i>	0.097 (0.051)	0.161 (0.062)	Crassulaceae
<i>Incisalia polios</i>	0.193 (0.041)	0.275 (0.063)	Ericaceae
<i>Incisalia irus</i>	0.184 (0.044)	0.193 (0.045)	Fabaceae
<i>Incisalia henrici</i>	0.164 (0.050)	0.160 (0.048)	Fabaceae, Rosaceae, Aquifoliaceae, & others
<i>Incisalia niphon</i>	0.273 (0.056)	0.277 (0.053)	Pinaceae
<i>Incisalia eryphon</i>	0.067 (0.030)	0.133 (0.054)	Pinaceae
<i>Callophrys affinis</i>	0.187 (0.072)	0.166 (0.063)	Polygonaceae & Rhamnaceae
<i>Callophrys perplexa</i>	0.107 (0.041)	0.106 (0.042)	Polygonaceae & Fabaceae
<i>Callophrys dumetorum</i>	0.153 (0.052)	0.138 (0.047)	Polygonaceae
<i>Callophrys lemberti</i>	0.211 (0.053)	0.285 (0.071)	Polygonaceae
<i>Callophrys comstocki</i>	0.133 (0.063)	0.133 (0.064)	Polygonaceae
<i>Loranthomitoura spinetorum</i>	0.086 (0.033)	0.191 (0.057)	Viscaceae
<i>Loranthomitoura johnsoni</i>	0.300 (0.095)	0.256 (0.074)	Viscaceae

TABLE 3. Continued

Species/Population	Heterozygosity		Food plant families
	Direct count	H-W expected*	
<i>Mitoura heseli</i>	0.257 (0.086)	0.198 (0.059)	Cupressaceae
<i>Mitoura nelsoni nelsoni</i>	0.349 (0.090)	0.425 (0.084)	Cupressaceae
<i>Mitoura nelsoni</i> (high elev.)	0.319 (0.074)	0.333 (0.054)	Cupressaceae
<i>Mitoura muiri</i>	0.137 (0.050)	0.176 (0.064)	Cupressaceae
<i>Mitoura gryneus</i> (NJ)	0.119 (0.058)	0.122 (0.061)	Cupressaceae
<i>Mitoura gryneus</i> (TN)	0.113 (0.044)	0.125 (0.050)	Cupressaceae
<i>Mitoura gryneus</i> (AR)	0.213 (0.072)	0.190 (0.063)	Cupressaceae
<i>Mitoura gryneus</i> (FL)	0.178 (0.044)	0.183 (0.045)	Cupressaceae
<i>Mitoura loki loki</i>	0.249 (0.050)	0.296 (0.066)	Cupressaceae
<i>Mitoura thornei</i>	0.176 (0.044)	0.190 (0.047)	Cupressaceae
<i>Mitoura siva</i> (NM)	0.251 (0.052)	0.269 (0.059)	Cupressaceae
<i>Mitoura siva</i> (AZ)	0.200 (0.051)	0.217 (0.058)	Cupressaceae
<i>Mitoura siva</i> (UT)	0.193 (0.057)	0.195 (0.053)	Cupressaceae
<i>Mitoura siva</i> (CA2)	0.263 (0.064)	0.264 (0.059)	Cupressaceae
<i>Mitoura siva</i> (CA1)	0.215 (0.059)	0.219 (0.060)	Cupressaceae

* Unbiased Hardy-Weinberg equilibrium estimate (see Nei, 1978)

remaining *Incisalia* populations (subgenus *Deciduphagus*). But using Edwards distances and neighbor-joining methodology, PAUP* constructed a tree in which *I. eryphon* and *I. niphon* clustered together as a sister to all other callophryine taxa (Figure 4). The *Loranthomitoura* species (*johnsoni* and *spinetorum*) clustered separately from all *Mitoura* populations in all BIOSYS-1 and PAUP* trees (Figures 1–5).

Within *Mitoura*, five distinct clusters above 0.1 minimum Nei distance are discriminated: *M. loki* (including *M. thornei*), *M. siva* (including *M. mui*ri), *M. nelsoni* (including the “high elevation” *M. nelsoni* population associated with *Juniperus occidentalis*), *M. hesseli*, and *M. gryneus* (including *M. g. sweadneri*). *Mitoura hesseli* is the most distinct member of the *Mitoura* taxa, based on minimum Nei distances, which ranged from 0.297 (*M. loki*) to 0.430 (*M. gryneus* [Arkansas]), while the distances among the other *Mitoura* populations ranged from 0.017 to 0.324. In comparison, the minimum Nei distances between the various *Mitoura* populations and *Incisalia augustinus* ranged from 0.438 to 0.555.

The Arkansas population [perhaps referable to *M. g. castalis* (W. H. Edwards) and hereafter referred to as *M. g. near castalis*] displays an unusual relationship to other *Mitoura* populations. This population branched above 0.1 minimum Nei distance from the other *M. gryneus* populations (Table 4), but clustered with them in all the UPGMA trees created with unbiased Nei, minimum Nei, and unbiased minimum Nei distances plus genetic and unbiased genetic identities (as in Fig. 1). The same cluster pattern was produced by the PAUP* tree constructed through Edwards distances (Fig. 3) and by presence/absence of allelic characters (Fig. 5). The other PAUP* trees placed *M. g. nr. castalis* within the *M. siva* cluster, as sister to all other *siva* populations and quite distant to them (Figs. 2 & 4). The somewhat ambiguous placement of the *M. g. near castalis* population could be due to the low sample size, greater genetic isolation of this population, a combination of both, being a distinct species, or perhaps an intermediate population in a clinal blend zone between eastern *M. gryneus* and western *M. siva* populations.

The minimum Nei distances between *M. mui*ri and *M. siva* populations (0.067 to 0.085) was within the range of genetic distances among the other *M. siva* populations and is less than the minimum Nei distance between *M. mui*ri and the nominate *M. nelsoni* population (0.174). Similarly, the minimum Nei distance between *M. loki* and *M. thornei* (0.050) is within the range of observed minimum Nei distances among the *M. siva* populations (Table 5).

TABLE 4. Genetic distances between *Mitoura gryneus* populations. Locations are the same as those from table 2.

		NJ	TN	AR
1.	FL	0.099	0.063	0.207
2.	NJ		0.032	0.144
3.	TN			0.136

The mean F(ST)s between *loki* and *thornei* was 0.096 and was significant based on contingency chi-square analyses across all 15 loci (P<0.00001). The mean F(ST)s between the two putative San Bernardino Mountains *M. nelsoni* populations was only 0.032 at 11 loci (Table 6) and was not significant based on contingency chi-square analyses (P=0.13707); the other four loci were represented by only a single individual in *M. nelsoni* from Mountain Home. Although the sites of these two *Mitoura nelsoni* populations are separated by 26 kilometers, their ranges may converge where their respective host plant distributions narrowly overlap. The mean F(ST)s of the remaining western southern California *Mitoura* populations ranged from 0.155 to 0.264 (Table 6) and were all significantly different based on contingency chi-square analyses (P<0.00001).

DISCUSSION

Allozyme analyses have been used for over thirty years to illuminate the evolutionary relationships between and within species of Lepidoptera (Brittnacher et al., 1978; Angevine & Brussard 1979; Pashley 1982; Mensi et al. 1988; Brussard et al. 1989; Pratt 1994). Although DNA studies are more frequent in the recent taxonomic literature, allozyme research remains a useful alternative tool for understanding phylogenetic relationships (Nice & Shapiro 2001; Pratt & Wright 2002; Vandewoestijne & Baguette 2002; Pratt et al. 2006; Habel et al. 2008; Habel & Schmitt 2009). Vandewoestijne & Baguette (2002) examined the amount or value of information from Random Amplified Polymorphic DNA (RAPD) compared to allozymes. Not surprisingly they found RAPDs correlated better with geographic differences among Cranberry Fritillary populations than did allozymes. They also found greater genetic diversity in these Fritillary populations with RAPDs than with allozymes. But these comparisons may be somewhat unfair since there were far more polymorphic loci for RAPDs (18) than for allozymes (4).

Recently Pratt & Wright (2002) and Pratt et al. (2006) used allozyme analyses to construct trees that show the evolutionary relationships between North American species of coppers and blues, respectively. Genetic studies using allozymes were used to examine the phylogeography of the marbled white butterfly in

TABLE 5. Matrix of minimum Nei distances of the five *Mitoura siva* and *M. gryneus* (AR) populations. Locations are the same as those from table 2.

	CA2	AZ	NM	UT	AR
CA1	0.050	0.035	0.033	0.090	0.184
CA2		0.028	0.024	0.075	0.127
AZ			0.017	0.047	0.147
NM				0.037	0.136
UT					0.201

TABLE 6. Matrix of minimum Nei distances of the four *Mitoura* taxa found in the San Bernardino Mountains of southern California. Locations are the same as those from table 2.

	<i>M. nelsoni</i> (CA1)	<i>M. loki</i>	<i>M. siva</i> (CA2)
<i>M. nelsoni</i> (CA2)	0.074	0.169	0.159
<i>M. nelsoni</i> (CA1)		0.161	0.164
<i>M. loki</i>			0.159

TABLE 7. Mean F(ST) across 15 loci.

<i>M. thornei</i>	<i>siva</i> CA1	<i>nelsoni</i>	CA2	<i>muiri</i>
<i>M. loki</i>	0.096	0.205	0.220	0.249
<i>M. siva</i> CA1	0.253	XXXX	0.250	0.155
<i>M. CA2</i>	0.264	0.250	XXXX	0.247
<i>M. CA1</i> ¹	0.236	0.178	0.032 [°]	0.194

¹across 11 loci[°]not significant based on chi-square contingency tests

Northern Africa through Europe (Habel et al. 2008). Allozymes were also used to show that genetic differences between populations of two lycaenid species reflect the dispersal differences between populations (Habel & Schmitt 2009).

Analytical considerations. Because allozymes are proteins that differ in size, shape, and charge at a specific pH, they travel at different relative rates through a gel. For consistent results, gels are made with the same percent starch and buffer. Following addition of enzyme substrates (as in recipes of Shaw & Prasad 1970), stained precipitated products result in visible banding corresponding to the differential mobilities of the allozymes. Sometimes more than one enzyme can be identified by the same stain system, since different enzymes may perform the same enzymatic reaction. These allozymes can be differentiated by the banding

patterns in the gel due to 1) subunit differences, 2) distances traveled, and 3) stain intensities. Geiger (1990) discusses much of this methodology in more detail.

Sample size can affect the calculation of genetic distances from allozymes, due to differences in allele frequencies (Nei 1972; Avise 1974; Ayala et al. 1974) and the chance that rare alleles may be included in small samples. It is generally assumed that the larger the sample size the more likely the test sample will accurately represent the frequencies of alleles in the wild population. Another complication can arise when different alleles have indistinguishable mobilities on a given buffer/gel combination. Employing a buffer that can separate as many alleles as possible may reduce this effect. Because we have found that a higher pH selects for greater charge differences in enzyme proteins for

butterflies, we tested a number of buffer systems that could be used at high pH and found Clayton Tretiak pH 8.5 buffer elucidated more alleles with lycaenids than did other buffer systems (Pratt 1994; Pratt & Wright 2002; Pratt et al. 2006).

The use of genetic distance as a measure of phylogenetic distinction and justification for taxonomic decisions is not without controversy. Even the time needed for the speciation process to occur is controversial, with estimates ranging from four to less than a million years (Johnson et al. 1996; Klicka & Zink 1997; Avise & Walker 1998; Avise et al. 1998). Similarly, there has been much controversy over the molecular clock and the amount of time needed to produce a given amount of genetic change in various organisms. Estimates for one Nei unit range from three (Nei 1971) to five (Mensi et al. 1988) and up to 14 millions of years of genetic isolation (Maxson & Maxson 1979; Berlocher & Bush 1982). Certainly, reported differences in Nei distance correlations could be due to different rates of evolution for different organisms under different selection pressures. A Nei distance of 0.1 has been used to distinguish species from subspecies-level differentiation (Ferguson 2002), yet interspecific Nei distance can be well below this number (Berlocher & Bush 1982; Brittnacher et al. 1978; Pratt 1994), while Nei distances among conspecific populations sometimes can be much higher (Mensi et al. 1988). The use of Nei distance in taxonomic/systematic research should be used with caution, especially when applied as a yardstick across more distantly related taxonomic groups.

Because genetic distance does not necessarily equate to genetic incompatibility, the Nei distance alone is not proof of speciation. Nevertheless, genetic distance may be useful in helping resolve phylogenetic relationships and, ultimately, taxonomy among closely-related populations. Where undisputed species-level differentiation of some members of a taxonomic group exists, Nei distances may provide guidance for interpretation of phylogenetic relationships among other populations.

Phylogenetic considerations. In this study, populations within most trees generally clustered within their recognized generic, subgeneric, and/or species groups. Most species within generic clusters, other than *Mitoura*, branched above 0.1 Nei distance from their congeners (Fig. 1). One exception pertains to *Incisalia eryphon*, which either separated near the base of the tree (Figs. 1 & 2) or branched with *I. niphon* separately from other *Incisalia* (Figs. 3, 4, & 5). These differences could be an artifact of low sample size or the western pine elfin (*I. eryphon*) being more genetically divergent. Because the phylogenetic tree in figure 3 has a higher

cophenetic correlation (0.963 vs. 0.917) and a lower percent standard deviation (6.303 vs. 17.656) than the tree in figure 1, it may portray the evolution of the group more accurately. In the Distance Wagner Trees, *Incisalia* was often the most basal branch and, within the genus, the pine elfins (*I. eryphon* and *I. niphon*) may be the most basal (“primitive”) subgroup, correlating with their subgeneric separation (Johnson 1992b).

The relative minimum Nei distances suggest some interesting relationships amongst the Callophryina. For instance, the genetic distance between *C. lemberti* and *C. comstocki* (minimum Nei distance 0.081) is the lowest of all *Callophrys* (s. str.) pairs in this study (Table 4), while *C. dumetorum* is sister to both of them (Figs. 1–4). The former two taxa are considered by some workers to represent subspecies of *C. sheridanii* (W. H. Edwards) (e.g. Howe 1975; Scott 1986; Pelham 2008), while Warren (2005) considers *C. dumetorum* and *C. sheridanii* to be conspecific. Also, the minimum Nei distances for the pairs *M. loki* & *M. thornei* (0.050) and *M. siva* & *M. muii* (0.067–0.085) are quite low compared to other species pairs in this study, suggesting perhaps only infraspecific level distinction.

Loranthomitoura appears to have closer affinities with *Callophrys* (s. str.) (Figs. 1, 2, 4) or *Incisalia* (Fig. 5), or separate from all other genera (Figure 3), than with *Mitoura*, the genus (or subgenus) in which its members were once placed. The systematic position status of *Loranthomitoura* was recently confused by the formal placement of *Callophrys guatemalena* (Clench, 1981) into *Cisincisalia* (Johnson 1992a) and the subsequent recognition by some workers (e.g. Robbins 2004) that *C. guatemalena* is conspecific with *Cisincisalia moeki* Johnson, the type species for *Cisincisalia*. The relationship between *Cisincisalia* and *Loranthomitoura* remains murky, because the description of the former is based largely on plesiomorphic adult alar and genital characters, while that of the latter is based primarily on a suite of autapomorphies of the immature stages including larval host plant usage. Furthermore, the life history and morphology of *C. moeki* immatures remain undescribed; when those factors become known, and/or when the phylogenetic relationship of that taxon to other Callophryina is more directly quantified, its taxonomic status with respect to *C. guatemalena* and the propriety of synonymizing *Cisincisalia* and *Loranthomitoura* will likely be clarified.

Within the *Mitoura* cluster *M. hesseli* exhibited the greatest minimum Nei distances from other populations, supporting at least a minimal two-species genus interpretation consisting of *M. hesseli* and *M.*

gryneus. However, if one used a genetic distance yardstick based on the Nei distances among other callophryine taxa, then one could justify additional species within the *Mitoura* cluster. The genetic distances between sometimes narrowly allopatric and parapatric populations of western taxa (*M. loki*, *nelsoni*, and *siva*), which have been lumped under *M. gryneus* by some workers (e.g. Scott 1986), are often as great as the genetic distances that discriminate each of them from geographically more distant populations of *M. gryneus* (east of the Great Plains).

The presence of a geoclineal blend zone between eastern *M. gryneus* and western *M. siva*, with *M. g. nr. castalis* as an intermediate, is not supported by the genetic distances observed. While the test population of *M. g. nr. castalis* is geographically intermediate between the nearest test populations of *M. g. gryneus* (Tennessee) and *M. s. siva* (New Mexico), it is not part of a smooth geocline between the latter two taxa. The geographic distance between the *M. g. nr. castalis* population and the nearest test population of *M. g. gryneus* (Tennessee) is around 830 kilometers and the minimum Nei distance is 0.136, while the geographic distance between the Tennessee and New Jersey test populations of *M. g. gryneus* is 790 kilometers and the minimum Nei distance between them is only 0.032 (Table 4). Similarly, the geographic distance between the *M. g. nr. castalis* and New Mexico *M. s. siva* test populations is approximately 1320 kilometers and the minimum Nei distance between them is 0.136, while the distance between the same *M. s. siva* and the *M. s. mansfieldi* test populations is 1250 kilometers and the minimum Nei distance between them is only 0.033 (Table 5). Thus, the *M. g. nr. castalis* population presents a disjunction in the east-west pattern of genetic distances (relative to geographic distances) among populations of *M. g. gryneus* and *M. siva*, and appears to be equally genetically distant from both taxa. This suggests a degree of genetic isolation that could justify conferring equal taxonomic status on all three.

Morphologically, *M. g. nr. castalis* presents a mosaic of adult and larval features of eastern *M. gryneus* and western *M. siva*. While *M. g. nr. castalis* closely resembles eastern *M. gryneus* populations in alar characters, first instar chaetotaxy allies it more with western *M. siva*. The first instar larvae of the *M. g. gryneus* and *M. g. sweadneri* test populations have a single well-developed subdorsal (SD) seta, presumably SD1 (Ballmer & Pratt 1992; Ballmer & Wright 2008) on each of segments T3–A7, while *M. g. nr. castalis* has none (G. Ballmer pers obs.). The absence of a well-developed SD1 seta on T3–A7 is shared with populations of *M. g. castalis* from Texas and Durango,

Mexico, as well as all western test populations of *Mitoura* in this study (G. Ballmer & D. Wright pers obs.).

Some uncertainty remains as to the overall relationships among *Mitoura* species because of low sample sizes for some populations and absence of some taxa from the data set. The relatively great range of genetic distances among conspecific populations of recognized species reported here further complicates the picture. This genetic divergence within species is probably due largely to their low vagility and close association with isolated populations of their larval hosts. Because the test sample of *M. g. sweadneri* was the progeny of a single female, the degree of its genetic separation from *M. g. gryneus* populations remains uncertain. The absence of representatives of such taxa as *M. s. chalcosiva* Clench and *Thuja*-associated *Mitoura* could also affect the trees generated. Further allozyme studies of additional populations of *Mitoura* taxa are needed to determine reliability of the frequency differences between them and the effects of including additional taxa. Similarly the inclusion of populations representing additional higher categories within Callophryina [e.g. *Ahlbergia*, *Sandia*, *Xamia*, and *Cyanophrys*] could help clarify the status of such groups, as well as their evolutionary relationships. Studies of additional loci, such as from DNA markers that help distinguish species of the Callophryina may further elucidate relationships.

Some of the *Mitoura* taxa treated here were also subjects of an allozyme study by Nice & Shapiro (2001). In that study unbiased minimum genetic distances from 0.000 to 0.007 were reported for northern California populations of *M. muiri*, *nelsoni*, and *siva*. In contrast, we found unbiased minimum genetic distances for southern California populations of these taxa ranged from 0.009 to 0.164. The differences between the studies could be due to factors such as: (1) our buffer system may have unmasked more alleles, (2) genetic differentiation may be greater among the Southern California test populations, (3) taxonomic decisions based on host plant usage may be fallible, (4) relatively low sample sizes were used in both studies, or (5) some combination of these four factors. A greater diversity of alleles unmasked by the buffer system would increase the measured genetic distances between taxa. Allele diversity could also be affected by different evolutionary histories for the different test populations of the same putative taxa in terms of bottlenecks and/or evolution rates (Soulé 1976). Because both studies employed relatively small sample sizes representing few localities, the reported Nei distances for the relevant taxa may not be definitive. A study using larger sample sizes and

representing additional localities could clarify the genetic variability within and relationships among these taxa.

Larvae. *Callophrys* (s. l.) larvae collectively utilize a wide range of host plants, while host specificity is often a useful correlate of taxonomic boundaries within the group. Thus, the pattern of larval host plant usage among test populations mirrors their allozyme-based cluster patterns (Fig. 2, Table 3). Old World *Callophrys* (s. str.) larvae [e.g. *C. rubi* (L.) and *C. avis* Chapman] collectively utilize at least 17 plant families (Robinson et al. 2010); and, while all North American taxa utilize *Eriogonum* (Polygonaceae), *C. affinis* and *C. perplexa*, respectively, also use *Ceanothus* (Rhamnaceae) and *Lotus* (Fabaceae) (Brown & Opler 1967; Gorelick 1968, 1971; Ferris 1973; Scott 1986; Ballmer & Pratt 1989; Allen et al. 2005). The test populations of *C. affinis* and *C. perplexa* exhibit the lowest minimum Nei and Edwards distances and usually cluster together and apart from those that use only *Eriogonum* (see Figs. 1, 3, & 4).

Incisalia larvae collectively feed on members of at least 11 plant families (Aquifoliaceae, Caprifoliaceae, Cuscutaceae, Crassulaceae, Ebenaceae, Ericaceae, Fabaceae, Liliaceae, Pinaceae, Rhamnaceae, and Rosaceae) (Ballmer & Pratt 1989; Howe 1975; Opler & Wright 1999; Pratt & Pierce 2001; Pyle 1981; Scott 1986; Tilden & Smith 1986). Yet, while a few species have a broad host range (e.g. *I. augustinus* is reported to use at least eight plant families), others are much more host-specific. Thus *I. mossii* uses only Crassulaceae and *I. polios* uses only Ericaceae, while *I. eryphon* and *I. niphon* utilize only Pinaceae (Scott 1986; Robinson et al. 2010). Similarly, taxa assigned to *Loranthomitoura*, *Mitoura*, *Sandia*, and *Xamia* use only Viscaceae, Cupressaceae, Agavaceae, and Crassulaceae, respectively (Bailowitz & Brock 1991; Ballmer & Pratt 1989; Howe 1975; Opler & Wright 1999; Pyle 1981; Scott 1986; Tilden & Smith 1986).

It is noteworthy that larvae of taxa assigned to *Deciduphagus*, *Incisalia*, *Loranthomitoura*, *Sandia*, and *Xamia*, which do not use fabaceaeous hosts in nature, could be successfully reared to maturity on *Lotus scoparius* (Fabaceae) in the lab, suggesting that they may have a retained ancestral ability to feed on legumes (Pratt & Ballmer 1991). However, *Mitoura* larvae, which feed exclusively on Cupressaceae, cannot utilize *Lotus scoparius* (Pratt & Ballmer 1991) and speciation or subspeciation within *Mitoura* corresponds with host shifts strictly within the Cupressaceae (Johnson 1978; Gifford & Opler 1983; Ballmer & Pratt 1989, 1992).

Although host plant specificity in nature is a frequent criterion for recognizing *Mitoura* species (Johnson

1972, 1976, 1978; Ferris 1992) (e.g. *M. hesselli* on *Chamaecyparis* and typical *M. nelsoni* on *Calocedrus*), some taxa are more polyphagous in nature [e.g. *M. siva* on various western *Juniperus* species (Johnson 1978) and *M. loki* on *Juniperus californica* and *Cupressus forbesii* (Ballmer pers. obs.)]. Furthermore, larvae of many *Mitoura* species can be reared on cupressaceous hosts not ordinarily available to them in nature (Comstock & Dammers 1932; Layberry et al. 1998; Guppy & Shepard 2001; Pyle 2002; Forister 2004; Ballmer, Pratt & Wright pers. obs.). In this regard, we report the previously unreported use by *M. loki* (in Orange County, CA) of *Cupressus forbesii*, thought to be used exclusively by *M. thornei* in San Diego County. This observation consisted of numerous phenotypically typical adult *M. loki* in direct association with *C. forbesii* in the upper drainage of Coal Canyon during July 1993 (Ballmer pers. obs.), over a mile distant from the nearest *J. californica* (itself an isolated specimen several miles distant from any other known *J. californica*). The utilization of *C. forbesii* by both taxa further supports their genetic proximity and an interpretation of infraspecific status for *M. thornei*.

Incisalia henrici and *I. irus*, according to all phylogenies (Figs. 1–5) cluster together and appear as sister species. *Incisalia irus* larvae feed only on closely related plants in the Fabaceae (Scott 1986; Albanese et al. 2007), while *I. henrici* larvae utilize many plant species in a number of families, including Fabaceae (Scott 1986; Pratt & Pierce 2001). This suggests that Fabaceae may be the primitive food plant for the ancestor of these two elfins, unless obligate use of Fabaceae is a secondary specialization from a polyphagous ancestor. In this regard it is notable that larvae of a population of *I. henrici* known to use *Ilex opaca* Ait. (Aquifoliaceae) in nature had significantly greater pupal weight and percent survival to adult when reared in the lab on *Cercis canadensis* L. (Fabaceae) than on *I. opaca* and *Prunus serotina* J.F. Ehrh. (Rosaceae) (Pratt & Pierce 2001).

ACKNOWLEDGEMENTS

We thank the following for their invaluable services: Cecilia Pierce helped collect many of the butterflies used in this study, Marc Minno provided the *Mitoura gryneus sweadneri*, Tom Emmel and John Emmel engaged in helpful discussions, Andrew Warren read and provided comments to an earlier version of this manuscript, Tom Wood of the University of Delaware and Clay Sassaman of the University of California at Riverside graciously supplied laboratory space for the electrophoretic studies, and Glenn Gorelick and an anonymous reviewer provided valuable suggestions for improvement of this paper.

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Received for publication 17 January 2009; revised and accepted 11 February 2010

TAXONOMIC UTILITY OF EGG MICROSCULPTURE: MATERNAL EFFECTS AND VARIATION IN EGGS OF *PARNASSIUS SMINTHEUS* DOUBLEDAY (PAPILIONIDAE)

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ABSTRACT. Egg microsculpture has been used at various phylogenetic levels as an informative morphological character set; in particular, the structure of the micropylar rosette has been used to differentiate between closely related taxa at the species-level, but intraspecific variation in this character set remains largely undocumented. Here we show that the number and shape of elements in the micropylar rosette can vary substantially within a population of a single species and even within individual females. A significant proportion (52%) of this variation is attributable to the source female (but uncorrelated with female size), suggesting that some unknown maternal effect influences micropyle structure. Due to this large intrapopulation variation, the utility of this character set and the taxonomic rank to which it is applied should be evaluated carefully.

Additional key words: micropyle, micropylar rosette, taxonomy, morphology, *Parnassius phoebus*, *Parnassius behrii*

Morphological characters are routinely used to make taxonomic and phylogenetic assignments. When using these traits, the premise is that variation among taxa is sufficiently greater than variation within each taxon, thus allowing distinction. For many characters, it is relatively easy to identify sources of variation that need to be accounted for before making comparisons. For instance sexually dimorphic characters would only be compared within sex. However for other morphological characters, such as egg microsculpture, sources of variability may be more inconspicuous.

Although Lepidopteran egg microsculpture has long been documented and described (e.g. Edwards 1872; Peyron 1909; Döring 1955), the use of this morphological character set in Lepidoptera taxonomy has been relatively limited. For example, Häuser et al. (1993) documented egg microsculpture in Parnassiinae, noting that chorion morphology was informative at the genus and sometimes the species-group level. Building on the efforts of Seamans (1933), Salkeld (1975, 1976) used chorionic microsculpture to differentiate between species-groups of the taxonomically difficult cutworm genus *Euxoa*, but noted that species-level differentiation was generally not possible. More recently, variation in micropyle structure has been applied to differentiate among species of the *Papilio machaon* L., 1758 group (Eitschberger 1993) and the *Parnassius phoebus* (Fabricius, 1793) group (Shepard & Manley 1998). Harbich (1996, 1997) and Danner et al. (1998) also used egg microsculpture to elevate several *Hyles* (Sphingidae) taxa to species level, although these

taxa were subsequently re-synonymized (Kitching & Cadiou 2000).

Despite the purported utility of egg microsculpture in sibling species taxonomy, intraspecific variation of micropyle structure remains poorly documented. The purpose of this paper is to examine the variation in structure of the micropylar rosette in a population of *Parnassius smintheus* Doubleday, 1847 (Papilionidae). Of the potential variation in overall egg microsculpture, we focused specifically on the micropyle since this structure was the most obviously variable and it has previously most often been used to make taxonomic inferences in Papilionidae.

MATERIALS AND METHODS

We collected 11 female butterflies from two large contiguous populations (Meadows P & Q, see Matter et al. 2004) along Jumpingpound Ridge, Kananaskis, Alberta, Canada (51°57'N, 114°54'W, ~2100 m). Kananaskis Country butterflies were removed on four dates between 8 and 23 Aug 2002. Upon capture, individual females were placed in glassine envelopes and housed at The University of Calgary's Biogeosciences Institute (~1400 m) under ambient conditions. Under these conditions females will continue to lay eggs (Matter et al. 2006).

We examined the morphology of eggs produced by individual females. Between 11 and 20 eggs were examined for each of the 11 females. For each egg we counted the number of elements around the micropyle (Fig. 1). Additionally we measured the forewing length

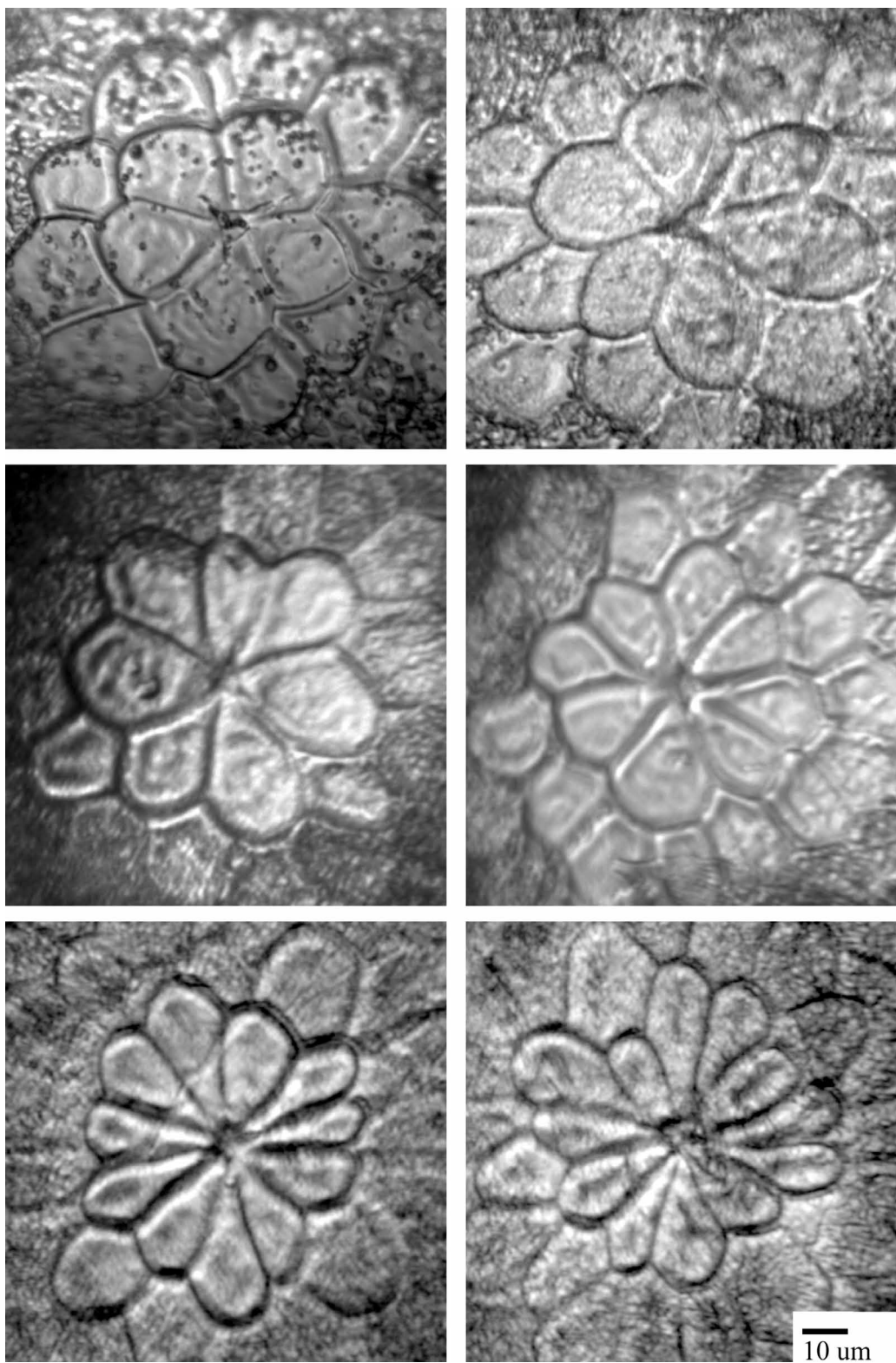


FIG. 1. Variation in micropyle rosette structure of *P. smintheus* eggs, produced by two females. Eggs in the top and middle rows were produced by one female, those in the bottom row by another. The images are centered on the micropyle, with polygon- to pie-shaped elements forming the surrounding rosette. Eggs were photographed at $240\times$ magnification using a Lumenera Infinity 1 digital microscope camera, mounted on a Leica M165C dissecting microscope.

(base to apex) of each female to determine if the number of elements was related to female size. The number of elements around the micropyle was analyzed using a generalized linear model assuming the number of elements had a Poisson distribution. Mean number of elements per egg was regressed against the forewing length of each female to determine if the number of elements was related to female size. To determine if the number of elements around the micropyle varies with egg size, we compared the diameter of eggs to the number of elements around the micropyle. Because the number of elements consists of count data, a Poisson regression was used. Ten additional eggs from six females were used for this analysis. Diameter of the egg was measured at its widest point using the program ImageJ (Abramoff et al. 2004). Digital images for measurements were captured at $60\times$ magnification using a Leica EC3 camera mounted on a Leica M80 dissecting microscope.

RESULTS

Females accounted for a significant amount of variation in the number of elements surrounding the micropyle of *P. smintheus* eggs ($\chi^2 = 18.5$, $df = 10$, $P = 0.04$; Fig. 2). The number of micropylar elements ranged from 5 to 12, (mean 7.6 ± 1.6 (SD)). Element shape varied according to the number of elements, with five- and six-element rosettes exhibiting more polygonal rather than wedge-shaped elements (Fig. 1). Over 52% of the deviance (variation) in the number of elements around the micropyle was explained by the mother. We found no significant relationship between female forewing length and number of elements around the micropyle ($F_{1,8} = 0.14$, $MSE = 0.31$, $P = 0.72$). Similarly, there was no relationship between egg diameter and the number of micropylar elements ($\chi^2 = 0.04$, $df = 1$, $P = 0.84$).

DISCUSSION

The egg surface of *Parnassius* species is highly sculptured compared to other Papilionidae, likely a result of the thick chorion evolved to protect the overwintering egg or pharate larva from predators, parasitoids and adverse environmental conditions (Häuser et al. 1993). The number of elements surrounding the micropyle is due in part to maternal effects; individual females produce eggs with a characteristic mean number, but also show egg to egg variability. We suspect that other characters related to egg morphology show similar trends. Before using such characters in a taxonomic or phylogenetic analysis, variation due to maternal effects should be accounted for. In lieu of this, eggs should be collected from many

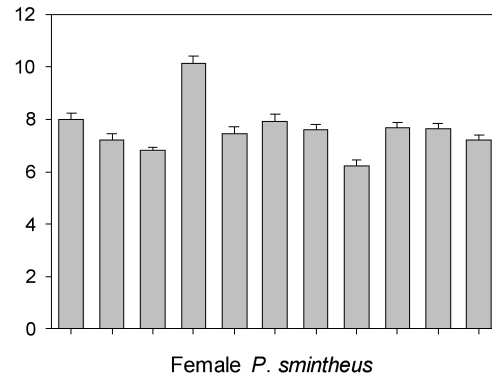


FIG. 2. Variation among female *P. smintheus* in the mean number of micropyle rosette elements. Means are shown \pm SE.

different individuals to overcome this source of variability. Furthermore, infraspecific geographic variation remains undocumented.

The most recent taxonomic revision of the North American *Parnassius phoebus* complex (Shepard & Manley 1998) relied heavily on the morphology of the micropylar rosette as a diagnostic character. Although we are not attempting to discredit the taxonomic assignments of Shepard & Manley (1998), additional work clearly is needed to better understand the taxonomy and biogeography of the North American *P. phoebus* group, since the intrapopulation variation in egg microsculpture of *P. smintheus* exceeds the interspecific variation purportedly diagnostic for the three *P. phoebus* group species (*P. phoebus* (F.) *P. smintheus*, and *P. behrii* Edw.). Subsequent work using multiple independent molecular markers supports the recognition of *P. smintheus* and *P. phoebus* (Omoto et al. 2006, Schoville & Roderick 2009), but the reciprocal monophyly of *P. smintheus* and *P. behrii* is not supported, i.e., the genetic variation within *P. smintheus* as currently defined exceeds the variation between *P. smintheus* and *P. behrii* (Schoville & Roderick 2009), suggesting that the species status of *P. behrii* needs to be re-evaluated. We do argue that care must be taken when examining egg microsculpture because significant variation can be attributed to maternal effects. A mechanism accounting for this variation has yet to be found, but it does not appear to be associated with gross female size or the size of eggs.

ACKNOWLEDGEMENTS

This research was supported by NSF grants DEB-0326957 and DEB-0918929 to SFM and the University of Calgary's Biogeosciences Institute. We thank Thomas Simonsen and an anonymous reviewer for helpful comments.

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Received for publication 04 December 2009; revised and accepted 07 Jan 2011

TEMPERATURE-DEPENDENT DEVELOPMENT IN CAPITAL-BREEDING LEPIDOPTERA

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ABSTRACT. Temperature-dependent development is described by three variables termed thermal characteristics: the developmental zero temperature, below which no development is assumed to occur; the high cutoff temperature, above which development slows; and the developmental index, a measure of physiological time required for a given phase to develop. Physiological time in this study refers to number of degree-days, units that combine temperature and time. The phase of interest here is the entire larval stage. Degree-days track developmental progress more precisely than calendar days and better alert the observer for optimally timing planned interventions. Thermal characteristics are usually derived from simple Type I regressions fitted to the linear portion of plots of rate of development on rearing temperature, where rate is the reciprocal of duration. Existing thermal characteristics for 131 published datasets are revised here using an improved Type II regression proposed by Ikemoto & Takai (2000). These datasets represent species in 11 families and originated between 1927 and 2007 on six continents. Each dataset consists of ≥ 4 associated rates and temperatures. Revised developmental zero temperatures ranged from 3.9 to 16.0. They varied directly with mean annual temperatures at localities of dataset origin, forming a continuum of low to high values between cool and warm climates. Among other relations, the mathematical product of voltinism \times the natural logarithm (\ln) of developmental index, which encompasses multivoltinism, varied directly with developmental zeros. In 91% of datasets, number of degree-days for the larval phase calculated using official mean daily air temperatures agreed within ± 2 calendar days with those using constant laboratory temperatures. Official temperatures were summarized from records at 18 mid-temperate North American weather stations. Thermal characteristics are found to be adapted to climatic regimes, and local weather-station temperatures are usually suitable for degree-day summations.

Additional key words: Degree-day, day-degree, temperature summation, developmental zero, developmental index.

Differing air temperature patterns among growth seasons create differing thermal environments for most insects and other ectotherms. Development may be accelerated if weather is warmer than average, or slowed if cooler. This often makes calendar dates unreliable for forecasting temperature-dependent events such as egg hatching, pupation, or eclosion. A physiological rather than calendrical time scale may offer a more precise and more useful basis for prediction. Degree-days (or day-degrees) combine temperature with calendar days to provide such a time scale. Summing degree-days measures physiological time and tracks temperature-dependent development. It alerts the observer when pest management treatments or other time-sensitive actions are required. It is useful also in other contexts, such as risk analysis, in which thermal characteristics may indicate how successful a pest might prove where it does not yet occur (Gould et al. 2005). The method provides no formal technique for making predictions in advance, but improvisation enables short-range forecasting once a summation has begun.

The present report focuses on the larval stage of development, when capital breeding lepidopterans, including most lepidopteran pests, accumulate the bulk of their reproductive resources (Boggs 1992; Miller 1996; Tammaru & Haukioja 1996). It is typically the longest developmental stage (Honék & Kocourek 1990) and therefore the stage most representative of temperature-dependent development. ‘Development’ refers to progress through set phases of life history, while ‘growth’ refers to increase in biomass.

Practical application of temperature-dependent

development consists of summing degree-days in the field to track and predict developmental mileposts in time or life history. Typical mileposts are egg hatching, adult eclosion, or completion of larval development as in this study. A beginning milepost also might be an arbitrary calendar date. Where it is not possible to introduce a temperature sensor into the larval habitat, official temperatures recorded at a nearby weather station may be substituted. Actually, weather station temperatures can be used in most situations, as in this study.

Three thermal characteristics describe temperature-dependent development: L, the developmental zero temperature, often termed lower developmental threshold, below which no development is assumed to occur; H, the high cutoff temperature above which development slows; and K, the developmental index or physiological time in accumulated degree-days required to complete a particular phase of development. H, usually $>30^\circ$ C, proved least important in this study because such temperatures did not occur for significant periods at mid-temperate North American weather stations used in this study. ‘Lower developmental threshold’ is somewhat misleading, as discussed further on.

Although sometimes referred to in the literature as constants and coefficients, thermal characteristics are not fixed for a species, but often differ from population to population and are subject to local adaptation and evolution, as shown here. Ikemoto & Takai (2000) pointed out that published thermal characteristics for many species, including capital-breeding lepidopterans,

may be suspect because of problems with conventional linear derivation procedure. These problems are detailed further on. Before thermal characteristics were deployed in this study, a major effort was made to revise them for as many capital-breeding lepidopterans as feasible using a derivation proposed by Ikemoto & Takai (2000), here termed the improved linear model procedure.

Surprisingly, degree-day summation from field temperatures, although basic to tracking field development, has been inadequately investigated. Published thermal characteristics for only 22% of the 108 species here were accompanied by field-testing or other evaluation. Moreover, most of the reported testing was based on existing, unrevised thermal characteristics. Field-testing is essential because of the potential for summation errors posed by certain effects to be described.

The three aims of this report are (1) to revise existing thermal characteristics as already noted; (2) to examine how revised thermal characteristics relate to climate, geography, voltinism, and to one another; and (3) to ascertain how well phase durations based on fluctuating field temperatures agree with those based on constant laboratory temperatures. The report also touches on how rate of degree-day accumulation might affect fecundity, a fitness factor influenced by temperature (Miller 2005).

Notable overviews of temperature-dependent development are given by Uvarov (1931), Allee et al. (1949), Andrewartha & Birch (1954), Wilson & Barnett (1983), Wagner et al. (1984), Ratte (1984), Delahaut (2003), Régnière & Logan (2003), and Herms (2007). Taylor (1981) specifically treats physiological time. Baskerville & Emin (1969), Pruess (1983), Higley et al. (1986), and Worner (1992) deal with issues of methodology, and Wang (1960) and Baker (1980) offer methodological critiques. Additional reports are cited later in relevant contexts.

MATERIALS AND METHODS

Degree-day terminology and computation.

Symbols and definitions used are shown in Table 1. All temperatures in this report refer to °C. By definition, one DD accumulates when T for all 24 h of one calendar day is one degree higher than L. During any calendar day, number of DDs accumulated is T - L, assuming T > L. Thus, if T for a calendar day averages 20°, and L is 12°, then 20 minus 12, or 8 DDs, accumulate that day. For n calendar days, number of DDs accumulate as

$$\sum_{i=1}^n (T_i - L)$$

The progression of natural temperatures through daily lows and highs resembles a sine wave, and a sine function (Allen 1976) is built into the summation algorithm used here, as described later. Hourly temperatures can be used, but the 24-h calendar day used here is most common. In the absence of an accurately estimated developmental zero temperature, an arbitrary approximation or 'base temperature' is used.

When DDs have accumulated in field summation to an ending milepost, that number is designated W, the field version of the laboratory developmental index, K (Table 1). Ideally, median-to-median or mean-to-mean individuals in the population should be used when developmental intervals defined by phenological mileposts are attained. Because plant development is also temperature dependent, phenological events such as bud-break or blooming in particular plants can serve as DD indicators if they correspond to mileposts in insect development (Herms 2007).

Thermal characteristics are components of phenological models, which range from the conventional linear type with two parameters to more complex nonlinear, multiparameter types. Linear and nonlinear refer to the straight or curved shape of the line that relates developmental rate to temperature. This study used only linear models—conventional or improved—fitted to datasets with ≥ 4 successive graphed points that formed a reasonably straight line. Most published models for capital-breeding lepidopterans are linear and of the conventional simple Model I regression type. The conventional model, which is the source of 'unrevised' thermal characteristics, is discussed more fully further on.

Phenological models are based on experimental data obtained when D is recorded for an insect at different Ts, usually in the laboratory, but in the field if the subject insect is not amenable to laboratory rearing. Laboratory Ts are usually constant, and were so in nearly all datasets here, but the methodology can accommodate laboratory Ts programmed to fluctuate diurnally. In the event that a field rather than a laboratory study is used to create a model, mean temperatures during the phase of interest in different years or locations, or in manipulated habitats, serve as Ts (Legg et al. 1998b). One dataset here for *Pthorimaea operculella* (Saunders) (Gelechiidae) is of this type (Langford & Cory 1932). The rest used constant laboratory Ts.

In laboratory or field, other environmental factors may influence thermal characteristics, as shown later, and should be monitored or controlled.

TABLE 1. Symbols and definitions used in this study.

DD, degree-day, a measure of physiological time; the mathematical product of units of calendar time x degrees of rearing temperature; further defined in text.
T, temperature in degrees Celsius.
D, duration of larval development—egg hatching to pupation in this study—in median number of calendar days whenever possible as recommended by Legg et al. (2003), otherwise in mean number of calendar days.
R, rate of larval development, 1/D, the fraction of total larval development occurring in one calendar day.
L, 'unrevised' zero developmental temperature; temperature below which, based on the conventional model, no development is assumed to occur; further defined in the text.
L', revised L based on Reduced Major Axis (RMA) Type II regression of the improved linear model; further defined in text.
K, 'unrevised' developmental index, the total number of DDs accumulated during the larval phase usually at constant laboratory temperatures based on the conventional model.
K', revised developmental index, the total number of DDs accumulated usually at constant laboratory temperatures based on the improved linear model.
DA, mean number of DDs accumulating in the field per calendar day; further defined in text.
W, field developmental index, the total number of DDs accumulated based on official weather-station temperatures; mathematical product of DA x D; field equivalent of K'; further defined in text.

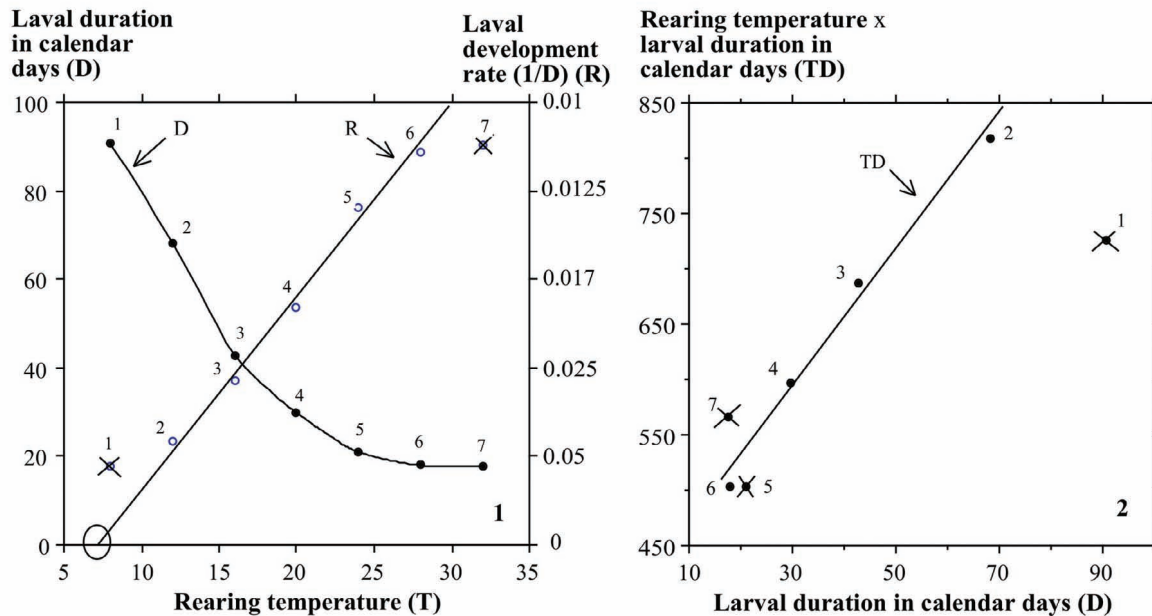
Revision of thermal characteristics. An extensive search yielded 223 published datasets that met the following criteria for admission to the study: ≥ 4 associated D and T values for larval cohorts reared throughout development at the same Ts, same photoperiods, and on the same food. If results were reported by gender, female data were used here; otherwise the combined sexes were used. Humidities were generally uncontrolled. Datasets for which nonlinear models originally had been fitted were admitted if they otherwise met the above criteria. In several datasets multiple observations of D at one T were averaged to obtain a single value to avoid pseudoreplication. The assembled datasets were subjected to additional requirements such as more stringent testing for linearity, as described later. Ultimately, L' and K' were calculated for 131 of the original 223 datasets using the improved linear model of Ikemoto & Takai (2000) (Table 2). Species nomenclature, although updated in a few cases, generally follows that used in dataset sources.

Conventional model. This model begins with the hyperbola, or reverse-J shaped curve, formed when D is plotted on T. It is illustrated here for *Mamestra configurata* Walker (Noctuidae) (Fig. 1: curve D). Next, the reciprocal of D, 1/D, is plotted on T (Fig. 1: line R), an operation that usually transforms at least part of any hyperbola into a straight line (Sokal & Rohlf 1981; Ikemoto & Takai 2000). Finally, simple Model I regression— $y = a + bx$ —is fitted to the straight-line portion of the R–T plot (Fig. 1). The temperature where the reverse-extrapolated regression line intersects the T axis is taken as L (Fig. 1: circle on horizontal axis). L can

be estimated visually, or more exactly from model parameters as $-a/b$. It should be noted for future discussion that L is technically a theoretical value; in practice it cannot be estimated experimentally. The reciprocal 1/b is taken as K. For *Mamestra configurata*, the conventional model gives L as 6.8° and K as 376 (Table 2: No. 36). Unrevised thermal characteristics were recomputed using the conventional model and included in Table 2 for reference. They sometimes diverged from those in original sources because of different rounding, differing interpretations of linearity, or other reasons. Methods of estimating L and K are discussed by Arnold (1959), Pruess (1983), Legg et al. (1998b), and others.

Requisite linearity is based on inspection of the graphed R–T data. Data points are often too scanty for useful statistical evaluation of linearity. Data interpreted as linear in conventional model procedure may not be so interpreted in improved linear model procedure (Figs. 1, 2). As discussed in the next section, the range of effective developmental temperatures usually is defined as those from L to H, and not based on temperatures selected to define it. Curiously, the temperature of fastest development—illustrated here by point No. 6 in Fig. 1—is sometimes wrongly termed 'optimum temperature' (Martin & Huey 2008). Points that depart from linearity are deleted before regression computation.

Problems with the conventional model alluded to earlier are that (1) linear portions of plotted data can be difficult to delimit; (2) variances are not constant along the regression line so that lower and upper y-values are disproportionately weighted; and (3) x is untenably



FIGS. 1–2. **1.** Hyperbolic relation of larval developmental duration, D , to rearing temperature, T , in *Mamestra configurata* Walker (Noctuidae), the line fitted by connecting data points, and its conversion to a conventional phenological model. The model relates larval developmental rate, R , to T , as fitted by Model I regression, $R = 0.000265 T - 0.018$; $r^2 = 0.99$. Data points numbered in order of increasing temperature. Points 1 and 7 (shown crossed out) were omitted from regression because they departed from linearity. Circled intersection of regression line and bottom horizontal axis defines zero developmental temperature, L' . Data of Bailey (1976). **2.** Improved linear phenological model for *M. configurata* relating the mathematical product of rearing temperature, T , \times larval developmental duration, D (TD) to larval duration, D . Line fitted by Model II regression, $TD = 405.1 + 6.19 D$; $r^2 = 0.99$. Data points numbered in order of increasing temperature, which is reversed from the conventional model. Points 1, 5, and 7 (shown crossed out) were omitted from regression because they departed from linearity. Data of Bailey (1976).

treated as error-free, which may depress the slope parameter b . The latter two problems result from usual assumptions of Model I regression (Sokal & Rohlf 1981). Improved linear model procedure addresses these problems, as discussed below.

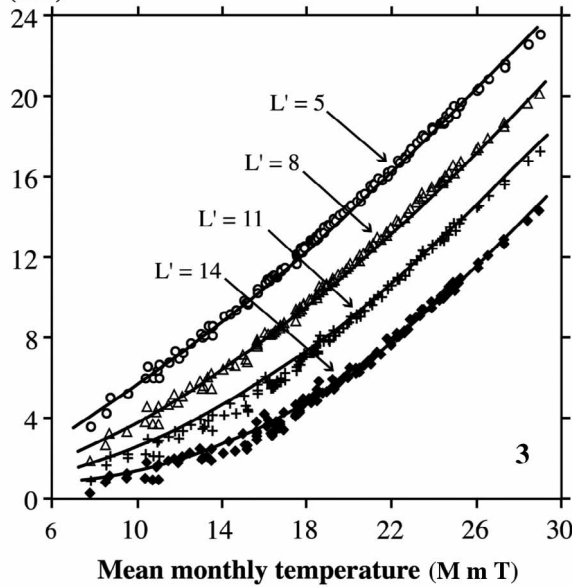
Improved linear model. The form of this model is the same as that of the conventional model, $y = a + bx$, but assumptions are those of Model II reduced major axis (RMA) regression in which both y and x are treated as subject to error instead of y alone (Sokal & Rohlf 1981; Ikemoto & Takai 2000). The same experimental values of D and T are used to create the model, but y becomes the mathematical product $T \times D$ instead of R ($1/D$), and x becomes D instead of T (Fig. 2). The order of plotted data points relative to T is reversed from that of the conventional model (Figs. 1, 2). Linearity is again ascertained subjectively from the graphed $(T \times D)$ – D data, but because of model linearizing, departures stand out and a more stringent test of linearity results. Points excluded due to nonlinearity were mostly in the tails of distributions. The ‘confirmed effective temperature range’ as defined here and displayed in Table 2 does not begin with L' and end with H . It is instead defined by the T s of the highest and lowest values of $T \times D$ on the

straight line, 818.4 and 504.0 (Fig. 2), which yield a range of 12–28°. Although not ideal, this definition seems more realistic because little development occurs in the several degrees between L' and the beginning of the straight line.

Although linear in form, Model II RMA regression was computed by nonlinear methods (Fleury 1991). First, a Model I regression of $T \times D$ on D was fitted, and its parameters a and b then used as start values for iterative computation of final parameter values by means of the nonlinear SYSTAT option (SYSTAT 1992). The final a becomes K' , and the final b becomes L' . For *Mamestra configurata*, L' is 6.19° (rounded to 6.2) and K' is 404 (Table 2: No.36). Not surprisingly, these values differ from their counterparts of 6.8° and 376 obtained with the conventional model. In this report, some variables, notably K' , are sometimes transformed to natural logarithms (\ln) before analysis to improve linearity. Thus far, it seems that only Liu et al. (2002) have used the improved linear model for a capital-breeding lepidopteran.

Summation with official temperatures. Field developmental index, W , was obtained as the mathematical product $DA \times D$, where DA is mean

**Mean No. DDs
accumulating/da
(DA)**



FIGS. 3–4. **3.** Expected number of DDs per calendar day, DA, at mean monthly field temperatures, MmT, for four developmental zero temperatures, L' 5–14, at the 18 weather stations used in this study. Regressions for different L' s follow: $DA L' 5 = 0.315 T + 0.00297 T^2 - 0.000444 T^3 - 0.12$. For $L' 8, 11,$ and $14,$ corresponding coefficients are, respectively, $-0.164, 0.0475, -0.000659T, 1.17; 3.20, -0.646, 0.0623, -0.000795;$ and $-0.771, 0.0559, -0.000568, 3.87$. All r^2 values >0.99 . Further explanation in text. **4.** Map locations of the 18 North American weather stations used in this study. Station names and details are given in Appendix 1.

number of DDs accumulating/day at a given mean monthly field temperature, and D is the median developmental duration at constant T values in the laboratory (Table 1). Thus W is equivalent to K' except that the T s underlying W are weather-station daily minima and maxima. Number of calendar days represented by W and K' were compared, and how closely the number based on W agreed with that based on K' (Table 2) indicates the usefulness of W .

To readily estimate W for the field-based part of the study, Table 3 was prepared to provide DAs necessary for the $DA \times D$ computation. This table gives DAs for mean field T s of 8–31° in 0.5° increments, and for L' of 5.0, 8.0, 11.0, and 14.0°. Only datasets with values of T and L' in the tabulated ranges were used to prepare Table 3, a restriction that excluded 37 datasets with uncommonly large or small values of L' or T (Table 2: Nos. 3, 14–17, 19, 22, 24–29, 43–45, 47–49, 54–56, 79, 80, 82–86, 93, 99, 100, 105–107, 109, 111, and 113). Also excluded were datasets for stored-product capital breeders (Table 2: Nos. 118–124), as W was not estimated for them because they typically develop under artificial storage rather than natural conditions. Table 3 provided expected DAs not only for this study but also for anticipated future studies. Its use often

required linear interpolation to obtain a desired DA because input values of T and L' were often intermediate between tabulated values.

Table 3 was generated in four steps as follows: (1) Accumulated DDs for each of the four selected months (May–August) of each included year were computed from temperature records of the 18 weather stations using a spreadsheet devised by R. D. Moon, Department of Entomology, University of Minnesota. The selected months encompass the period of most larval development. Spreadsheet inputs were daily temperature maxima (daytime) and minima (nighttime) as well as each of the four L' values. The spreadsheet computed DDs between a specified L' and the sine curve of temperatures for that day (Allen 1976). (2) Resulting number of DDs accumulating above each L' for each month were divided by number of days in the month to obtain mean number of DDs accumulating per day per month (DA). (3) Mean DAs for each month of each year for each L' at each weather station were plotted on corresponding mean monthly field T values, to which third-order polynomial regressions were fitted (Fig. 3). (4) Overall mean DAs were estimated with the regression equations in Fig. 3 for each 0.5° T -step and L' , and then assembled as Table 3.

Table 2. Thermal characteristics and related values for datasets used in this study. Dash signifies undetermined or unknown value.

No.	Dataset	Locality	Latitude °N or °S	Mean annual temp.	L	L	K	K	K	W ^a	DA ^a	Voltnism	Confirmed effective T range	Cal. da. diff ^a	Source
Noctuidae															
1	<i>Agrotis ipsilon</i> Hufn.	Japan	39.8 N	10.0	5.6	5.8	388	383	370	20.0	20.0	–	20-30	0.6	Hasegawa & Chiba 1969
2	"	USA IL	40.6 N	10.8	11.0	11.6	341	323	315	10.6	10.6	3.5	18.3-26.7	0.8	Luckmann et al. 1976
3	"	USA OH	40.8 N	12.0	7.3	7.5	495	489	–	–	–	3.0	13-34	–	Archer et al. 1980
4	"	Egypt	25.0 N	20.0	5.6	6.0	645	627	600	16.0	16.0	2.0	15-30	1.7	Nasr & Moawad 1972
5	"	"	30.0 N	19.8	11.3	11.1	267	270	264	9.5	9.5	2.0	17.4-23.4	0.7	Fahmy et al. 1973
6	<i>A. segetum</i> D. & S.	India	53.0 N	16.8	7.4	7.1	613	624	611	12.5	12.5	2.0	10-30	1.1	Singh 1962
7	<i>Pseudaletria unipuncta</i> (Haw.)	Can. ON	45.0 N	5.8	9.5	9.3	292	298	312	9.1	9.1	2.5	13-25	-0.1	Guppy 1969
8	<i>Leucania loreyi</i> Dup. (solit.)	Japan	34.4 N	16.1	9.3	9.4	366	362	285	10.4	10.4	3.0	15-30	7.4	Hirai 1975
9	<i>L. loreyi</i> (Crowd.)	"	34.4 N	16.1	8.0	8.7	432	410	391	13.4	13.4	3.0	15-30	1.4	"
10	<i>L. separata</i> Wlk.	"	43.0 N	8.5	6.7	7.8	397	367	347	14.2	14.2	3.0	15-30	1.4	"
11	<i>Trichoplusia ni</i> (Hbn.)	USA AZ	32.2 N	20.2	8.7	9.0	260	254	240	15.5	15.5	3.0	15-30	0.9	Jackson et al. 1969
12	<i>Stingra henrici</i> (Grt.) (7-instar)	USA IL	40.6 N	11.3	9.9	9.8	481	484	477	12.0	12.0	1.0	18.3-29.4	0.6	Decker & Maddox 1971
13	" (8-instar)	"	40.6 N	11.3	9.5	9.8	545	530	515	12.0	12.0	1.0	18.3-29.4	1.2	"
14	<i>Helicoverpa zea</i> (Bod.) (cotton)	USA AZ, GA	31.8 N	20.2	7.4	8.5	438	416	–	–	–	4.5	22.5-32	–	Butler 1976
15	" (corn)	"	31.8 N	20.2	14.0	14.0	245	245	–	–	–	4.5	22.5-32	–	"
16	" (art. diet)	"	31.8 N	20.2	12.7	12.9	206	203	–	–	–	4.5	17.5-32	–	"
17	"	USA WV	39.7 N	11.4	4.2	5.3	235	219	–	–	–	3.0	10-35	–	Pears 1927
18	<i>H. armigera</i> (Hbn.)	Japan	32.4 N	17.0	11.9	11.7	213	218	217	11.7	11.7	4.0	16.4-30.5	0.1	Jallow & Matsumura 2001
19	"	India	29.2 N	25.1	12.8	12.3	238	248	–	–	–	7.5	20-35	–	Sharma & Chaudhary 1988
20	"	Australia	27.6 S	19.8	11.5	11.1	234	247	212	8.9	8.9	–	13.1-27.1	3.9	Twine 1978

Table 2. Continued.

Noctuidae														
21	<i>Anticarsia gemmatalis</i> Hbn.	Brazil	9.4 S	21.4	14.2	13.4	178	180	175	10.3	3.0	18-30	0.5	Magrini et al. 1996
22	<i>Miythimna connecta</i> (Wlk.)	Australia	37.5 S	14.0	8.1	7.6	390	404	-	-	3.5	15-33	-	Smith 1984
23	<i>Heliothis virescens</i> (F.)	USA AZ	32.1 N	20.2	12.8	12.8	199	199	201	11.8	4.5	17.5-30	-0.1	Butler & Hamilton 1976a
24	"	USA AZ	32.1 N	20.2	13.0	13.7	224	213	-	-	5.0	20-33	-	Fye & McAda 1972
25	<i>H. virescens</i> (10:14 phot.)	USA AZ	33.0 N	22.5	13.5	12.7	178	197	-	-	3.5	15.6-32.2	-	Henneberry et al. 1993
26	" (12:12 phot.)	"	33.0 N	22.5	12.8	13.0	178	175	-	-	3.5	15.6-32.2	-	"
27	" (12:12 phot.)	"	33.0 N	22.5	12.6	13.2	170	161	-	-	3.5	15.6-32.2	-	"
28	"	USA TX	30.8 N	20.3	12.8	12.5	205	211	-	-	-	17.5-34	-	Butler et al. 1979
29	"	USA AZ	33.0 N	22.5	11.7	11.2	212	226	-	-	5.0	15-34	-	"
30	"	USA NC	36.3 N	14.6	11.9	11.5	194	204	211	10.7	-	15-30	-0.6	"
31	" (cotton)	USA AZ	33.0 N	22.5	8.5	8.5	433	433	422	16.0	5.0	20-30	0.7	"
32	<i>H. subflexa</i>	USA MS	33.4 N	17.5	12.9	12.4	214	232	232	9.2	-	16.7-30.3	0.0	"
33	" (gmd.cherry)	"	33.4 N	17.5	9.8	9.6	376	380	375	13.7	-	20.6-30.3	0.4	"
34	<i>Noctua pronuba</i> L.	UK	52.9 N	16.8	6.6	6.6	607	684	661	12.0	1.0	10-25	1.9	Singh 1962
35	<i>Manestra brassicae</i> L.	Romania	47.0 N	7.2	8.8	8.9	344	341	330	13.2	-	15-30	0.8	Stan 1998
36	<i>M. configurata</i> Wlk.	Can. MB	49.6 N	2.6	6.8	6.2	376	404	419	11.5	1.0	12-28	-1.3	Bailey 1976
37	<i>Plathypena scabra</i> (F.)	USA IA	42.0 N	13.0	5.7	5.8	401	397	383	17.5	2.0	15.6-29.5	0.8	Hammond et al. 1979
38	<i>Peritromia saucia</i> (Hbn.)	USA OH	40.8 N	9.8	6.2	7.2	395	355	385	10.8	2.0	12.8-26.7	-2.7	Simonet et al. 1981
39	<i>Autographa gamma</i> (L.)	UK	53.2 N	8.2	7.9	7.9	265	265	264	9.4	2.0	13-25	0.1	Hill & Gatehouse 1992
40	<i>Eudocima salaminia</i> (Cram.)	Australia	27.5 S	19.9	11.9	12.1	280	272	274	8.7	-	15-27	-0.3	Sands et al. 1991
41	<i>Dargida procincta</i> (Grt.)	USA OR	44.6 N	11.6	6.6	6.8	421	414	401	13.8	1.0	15-27	1.0	Kamm 1991
42	<i>Spodoptera cosmioidea</i> (Wlk.)	Brazil	31.8 S	17.5	11.3	11.2	253	256	254	13.3	-	14-30	0.1	Bavaresco et al. 2002

Table 2. Continued. Thermal characteristics and related values for datasets used in this study. Dash signifies undetermined or unknown value.

No.	Dataset	Locality	Latitude °N or °S	Mean annual temp.			K	K'	W ^a	DA ^a	Voltinism	Confirmed effective T range	Cal. da. diff ^a	Source
				L	L'	L''								
Noctuidae														
43	<i>S. exigua</i> (Hbn.)	USA AZ	32.2 N	22.5	12.3	12.2	182	171	-	-	-	20-33	-	Fye & McAda 1972
44	" (art. diet)	USA AL	31.4 N	18.3	13.1	13.1	128	128	-	-	-	18-36	-	Ali & Gaylor 1992
45	" (cotton)	"	31.4 N	18.3	13.5	13.1	159	128	-	-	-	18-36	-	"
46	"	USA AZ	32.2 N	20.2	8.6	9.9	251	220	210	11.5	5.0	15-30	0.9	Butler 1966
47	<i>Spodoptera frugiperda</i> (J. E. Smith) (art. diet)	USA MS	33.4 N	16.4	12.4	12.4	193	193	-	-	4.5	21-33	-	Ali et al. 1990
48	" (cotton)	"	33.4 N	16.4	13.0	13.0	270	270	-	-	4.5	21-33	-	"
49	<i>Spodoptera litura</i> (F.)	India	17.5 N	22.8	10.0	12.3	305	253	-	-	12.0	15-35	-	Ranga Rao et al. 1989
50	"	Japan	35.7 N	15.6	12.0	12.1	258	256	242	11.1	4.0	16-31	1.2	Miyashita 1971
51	<i>Sesamia nonagrioides</i> Lef.	Spain	42.0 N	12.8	12.0	12.1	414	393	415	-	3.0	15-27.5	-1.9	Lopez et al. 2001
52	<i>S. calanistis</i> Hampson (art. diet)	Benin	6.8 N	25.5	12.0	11.3	391	420	434	10.9	6.0	15-30	-1.2	Shanower et al. 1993a
53	<i>Copitarsia decolora</i> (Gn.) (asparagus)	Peru	14.1 S	20.0	5.2	5.1	466	474	467	11.9	-	9.7-24.9	0.6	Gould et al. 2005
54	<i>Orthosia hibisci</i> Gn.	Can. BC	49.6 N	6.0	4.5	4.4	263	257	-	-	1.0	7.5-20	-	Judd et al. 1994
55	<i>Papaipema nebris</i> (Gn.)	USA IL	40.5 N	10.4	4.0	4.0	1391	1395	-	-	1.0	12.8-23.9	-	Levine 1983
56	<i>Panolis flammea</i> D. & S.	Germany	49.0 N	7.8	4.0	3.9	434	436	-	-	1.0	10-22	-	Zwölfer 1931
Tortricidae														
57	<i>Adoxophyes honmai</i> Yasuda	Japan	35.8 N	16.0	8.9	8.6	257	262	245	13.9	4.5	15-28	1.2	Nabeta et al. 2005
58	<i>Epiphyas postvittana</i> (Wlk.) (Rumex)	Australia	37.7 S	19.8	7.1	7.8	334	310	294	10.2	3.0	11.5-25	1.6	Danthanarayana 1975
59	" (<i>Plantago</i>)	"	37.7 S	19.8	8.0	8.2	310	304	295	9.9	3.0	11.5-25	0.9	"
60	" (<i>Malus</i>)	"	37.7 S	19.8	7.1	7.8	351	327	304	10.2	3.0	11.5-25	1.7	"
61	" (1st)	"	35.5 S	22.0	9.9	9.6	404	417	482	11.4	3.0	15.7-25	-5.7	Dumbleton 1939
62	" (2nd)	"	35.5 S	22.0	7.8	7.2	510	541	549	12.0	3.0	11.7-25	-0.6	"
63	"	"	37.7 S	16.6	7.7	7.7	319	325	320	9.1	3.0	10.3-25.2	0.5	Danthanarayana et al. 1995

Table 2. Continued.

Tortricidae														
64	<i>Epichoristodes acerbella</i> (Wlk.)	S. Africa	25.7 S	18.7	7.0	7.0	413	416	423	12.4	2.5	9.5-25	-0.5	Bolton 1979
65	"	Italy	43.9 N	15.0	10.2	10.2	341	341	324	1.4	4.0	16-28	1.5	Quaglia 1983 (1985)
66	<i>Homona magnanima</i> Diak.	Japan	37.0 N	15.6	9.4	9.8	276	274	258	9.9	4.0	15-28	1.6	Mao & Kumimi 1990
67	<i>Ancylix comptana</i> (Froel.)	USA IA	41.7 N	9.9	9.8	10.0	255	251	237	11.7	3.0	14-30	1.2	Gabriel & Obrycki 1990
68	<i>Crocidosena plebejana</i> Z.	Australia	27.0 S	22.0	11.2	11.1	208	212	192	9.7	8.0	14-31	2.0	Hamilton & Zalucki 1991
69	<i>Argyrotaenia splaleropa</i> (Meyr.)	Brazil	31.8 S	17.5	10.8	11.6	245	236	196	10.2	4.0	14-30	4.0	Manfredi-C. et al. 2001
70	<i>A. velutinana</i> (Wlk.) (1st)	USA NC	35.2 N	15.2	6.3	6.4	471	469	459	10.8	4.0	10-25	0.9	Hawthorne et al. 1988
71	" (2nd)	"	35.2 N	15.2	7.0	6.7	410	425	427	10.6	4.0	10-25	-0.2	"
72	<i>Parabestia viteana</i> (Clem.)	USA PA	42.0 N	9.5	6.8	6.6	240	242	236	15.0	2.5	15-30	0.4	Tobin et al. 2001
73	<i>Platynota idaeusalis</i> (Wlk.)	USA NC	35.3 N	12.9	10.5	9.7	324	365	373	8.3	4.0	13-27	-0.9	Rock 1985
74	"	USA VA	38.5 N	13.3	7.1	7.9	437	403	423	2.7	2.0	12.8-26.7	-1.6	David et al. 1989
75	<i>P. flavedana</i> Clem.	"	38.5 N	13.3	8.7	8.9	370	360	391	11.8	2.0	12.8-26.7	-2.6	"
76	<i>Zeiraphera canadensis</i> Mut. & Free.	Can. NB	47.6 N	7.3	6.4	6.6	265	260	262	12.5	1.0	12.3-27.7	-0.2	Régnière & Turgeon 1989
77	<i>Choristoneura pinus</i> Free.	Can. ON	-	-	9.9	9.7	366	380	398	9.0	1.0	12.5-25	-2.0	Lysik & Nealis 1988
78	<i>C. fumiferana</i> (Clem.)	"	48.4 N	9.0	7.0	6.4	501	534	547	10.9	1.0	10-25	-1.2	Régnière 1987
79	<i>Rhyacionia frustrana</i> (Comst.)	USA AR	34.3 N	16.8	10.8	9.8	309	355	-	-	3.0	14-34	-	Haugen & Stephen 1984
80	<i>Cydia pomonella</i> (L.)	USA CA	38.7 N	16.0	11.6	12.0	239	224	-	-	3.0	15.6-32.2	-	Pitcairn et al. 1991
81	<i>Merophyas divulsana</i> (Wlk.)	Australia	27.4 S	19.9	8.1	7.9	278	283	285	11.1	7.0	11.3-27.5	-0.2	Allsopp et al. 1983
Gelechiidae														
82	<i>Pectinophora gossypiella</i> (Saund.) (sooty)	USA AZ	33.5 N	22.5	15.4	15.5	167	165	-	-	4.5	22.5-30	-	Bartlett et al. 1980
83	" (wild-F)	"	33.5 N	22.5	12.7	12.4	217	222	-	-	4.5	17-32	-	"
84	" (sooty-F)	"	33.5 N	22.5	12.3	11.9	220	227	-	-	4.5	17-32	-	"

Table 2. Continued. Thermal characteristics and related values for datasets used in this study. Dash signifies undetermined or unknown value.

No.	Dataset	Locality	Latitude °N or °S	Mean annual temp.	L	L'	K	K'	W ^a	DA ^a	Voltinism	Confirmed effective T range	Cal. da. diff ^b	Source
Gelechiidae														
85	<i>Phorinaea operculella</i> (Z.)	Japan	33.6 N	16.3	11.1	10.9	162	164	-	-	-	15-33	-	Koizumi 1955
86	"	USA MD	38.0 N	14.2	12.0	12.0	184	182	-	-	5.0	17.5-31.9	-	Langford & Cory 1932
87	"	Peru	12.1 S	11.0	13.6	9.2	174	252	254	14.8	-	16.1-30	0.1	Sponleder et al. 2004
88	<i>Pectinophora gossypiella</i> (Saund.) (WCRL)	USA AZ	32.2 N	20.2	13.2	13.4	240	234	236	8.9	4.5	17.5-30	-0.2	Butler & Hamilton 1976b
89	" (wild)	"	32.2 N	20.2	13.6	13.5	235	236	224	10.0	4.5	17.5-30	1.2	"
90	" (orange)	"	32.2 N	20.2	13.5	13.4	202	202	193	10.0	4.5	17.5-30	0.9	"
91	" (APHIS)	"	32.2 N	20.2	11.4	11.5	253	251	-	-	4.5	20-32	-	"
92	" (WCRL-7S)	"	33.5 N	22.5	13.3	14.0	232	214	201	9.5	4.5	16.7-30	1.4	Bartlett et al. 1980
93	" (4-instar)	"	33.5 N	22.5	12.3	12.3	226	227	-	-	4.5	20-32.5	-	Huchison et al. 1986
94	" (5-instar)	"	33.5 N	22.5	12.2	12.3	274	272	262	11.1	4.5	20-27.5	0.9	"
95	<i>Scrobipalpus absoluta</i> (Meyr.)	Uruguay	35.0 S	13.0	6.3	6.6	265	262	246	13.0	-	12-30	1.2	Bentancourt et al. 1996
96	<i>Keiferia lycopersicella</i> (Wlsm.)	USA CA	33.7 N	18.3	10.8	11.4	211	191	207	7.2	7.5	14-26	-2.3	Lin & Trumble 1985
Crambidae														
97	<i>Diatraea saccharalis</i> (F.) (1st)	USA MS	33.3 N	17.2	11.5	11.9	348	338	323	14.7	4.5	22-30	1.0	King et al. 1975
98	" (2nd)	"	33.3 N	17.2	9.6	10.0	390	381	362	17.5	4.5	22-31	1.1	"
99	"	Cuba	22.0 N	25.0	15.7	14.9	324	361	-	-	6.6	19-28	-	Jasic 1967
100	<i>Diatraea grandiosella</i> Dyar	USA MS	38.0 N	14.4	8.9	9.9	575	539	-	-	2.0	18.3-32.2	-	Whitworth & Poston 1979
101	<i>Udea ferrugalis</i> (Hbn.) (1st)	Korea	37.6 N	11.8	11.3	11.5	162	159	154	9.8	-	15-27	0.5	Lee et al. 2002
102	" (2nd)	"	37.6 N	11.8	12.2	12.0	140	144	150	8.0	-	15-25	-0.7	"
103	<i>Maruca vitrata</i> (F.)	China	31.6 N	-	10.9	11.1	184	180	173	11.1	2.0	15-30	0.6	Chi et al. 2006
104	<i>Ostrinia nubilalis</i> (Hbn.)	USA ND	46.9 N	10.8	12.3	13.6	334	293	284	11.2	1.0	17-30	0.8	Calvin et al. 1991
105	"	USA MO	36.4 N	15.5	13.0	13.7	310	293	-	-	3.0	17-32	-	"
106	"	USA DE	38.7 N	12.7	13.2	14.3	326	295	-	-	1.0	17-32	-	"
107	<i>Ostrinia nubilalis</i> (Hbn.) ^b	USA IL	40.1 N	10.8	11.2	11.3	263	262	-	-	2.0	15.7-32.2	-	Matteson & Decker 1965
108	<i>Cnaphalocrocis medinalis</i> Guenée	Japan	33.6 N	16.3	12.2	12.0	204	208	203	9.1	5.0	15-27.5	0.6	Wada & Kobayashi 1980
109	<i>Chilo auricilius</i> Dugd.	India	26.8 N	24.0	15.1	16.0	501	458	-	-	5.0	20-30	-	Mitra & Verma 1981
110	<i>C. sacchariphagus</i> (Bojer)	Réunion	21.0 S	23.7	12.7	12.8	582	577	575	9.5	4.5	17-30	0.2	Goebel 2006
111	<i>Crambus trisectus</i> (Wlk.)	USA IL	40.1 N	10.8	10.5	11.0	539	522	-	-	2.0	21.1-32.2	-	Banerjee 1969

Table 2. Continued.

Crambidae														
112	<i>Diaphania nitidalis</i> (Stoll)	USA NC	27.2 N	25.0	10.5	10.8	214	208	197	11.3	2.5	15.5-29.4	1.0	Elsley 1980
113	<i>D. indica</i> (Saund.)	India	11.4 N	27.0	13.1	14.0	240	229	-	-	-	25-40	-	Peter & David 1992
Plutellidae														
114	<i>Plutella xylostella</i> (L.)	China	30.2 N	16.2	8.4	8.0	134	139	143	11.6	-	12-30	-0.3	Liu et al. 2002
115	"	Japan	35.0 N	16.7	9.6	10.0	146	141	142	12.1	-	17.5-27.5	0.0	Yamada & Kawasaki 1983
116	" (cauliflower)	Iran	35.8 N	17.1	6.2	6.7	152	146	154	12.9	-	10-28	-0.6	Golizadeh et al. 2007
117	" (cabbage)	"	35.8 N	17.1	8.2	6.9	138	159	170	12.7	-	10-28	-0.9	
Pyralidae ^b														
118	<i>Ephesitia kaelniella</i> Zell. (70% RH)	UK	-	-	6.8	7.0	1136	1115	-	-	-	12-25	-	Jacob & Cox 1977
119	<i>E. figulifella</i> Gregson	Cyprus	-	-	12.2	11.7	533	560	-	-	-	17.5-30	-	Cox 1974
120	<i>Cadra cautella</i> Wlk. (60% RH)	India	-	-	13.0	11.7	437	512	-	-	-	15-30	-	Tuli et al. 1966
121	" (75% RH)	"	-	-	11.1	11.2	500	500	-	-	-	15-35	-	"
122	" (90% RH)	"	-	-	10.3	11.6	435	390	-	-	-	15-35	-	"
123	" (70% RH)	Argentina & Nigeria	-	-	12.8	12.8	358	356	-	-	-	17.5-30	-	Burges & Haskins 1964
124	<i>Plodia interpunctella</i> (Hbn.) (50% RH)	Nigeria	-	-	12.1	12.4	298	290	-	-	-	17.5-30	-	Prevelt 1971
Arctidae														
125	<i>Hyphantria cunea</i> Drury (P2L)	Japan	35.7 N	16.0	9.8	9.1	474	503	480	13.5	2.0	17-26.5	1.7	Itô & Miyashita 1968
126	" (P1L)	"	35.7 N	16.0	9.4	10.0	505	473	473	11.0	2.0	14-26	1.3	"
127	" (1CL)	"	35.7 N	16.0	9.1	8.9	515	521	514	15.9	2.0	19-30	0.5	"
Galactiidae														
128	<i>Homadula anisocentra</i> Meyr.	USA IA	42.0 N	9.9	11.8	10.9	208	233	245	10.4	2.0	14-30	-1.2	Bastian & Hart 1991
Cossidae														
129	<i>Zeuzera coffeae</i> Nietner	Taiwan	24.5 N	23.6	12.1	12.0	1401	1411	1345	13.6	2.0	20-30	4.8	Chang 1987
Carposinidae														
130	<i>Carposina sasakii</i> Mats.	Korea	37.3 N	12.3	9.4	9.1	272	280	279	13.0	1.5	16-28	0.0	Kim et al. 2001
Lasiocampidae														
131	<i>Streblota panda</i> Hbn.	Spain	37.3 N	19.2	12.5	11.9	521	563	607	10.0	4.0	16-28	-4.4	Calvo & Molina 2005

^a Values for T and L' given only within ranges included in Table 3.

^b All Pyralidae here infest stored products and develop in artificial environments; only their thermal characteristics, localities of origin, and revised effective Ts are given.

TABLE 3. Mean DD accumulations per day (DA) at weather-station mean monthly temperatures from 8-31° C (T) and L' from 5-14° C based on equations in Fig. 3.

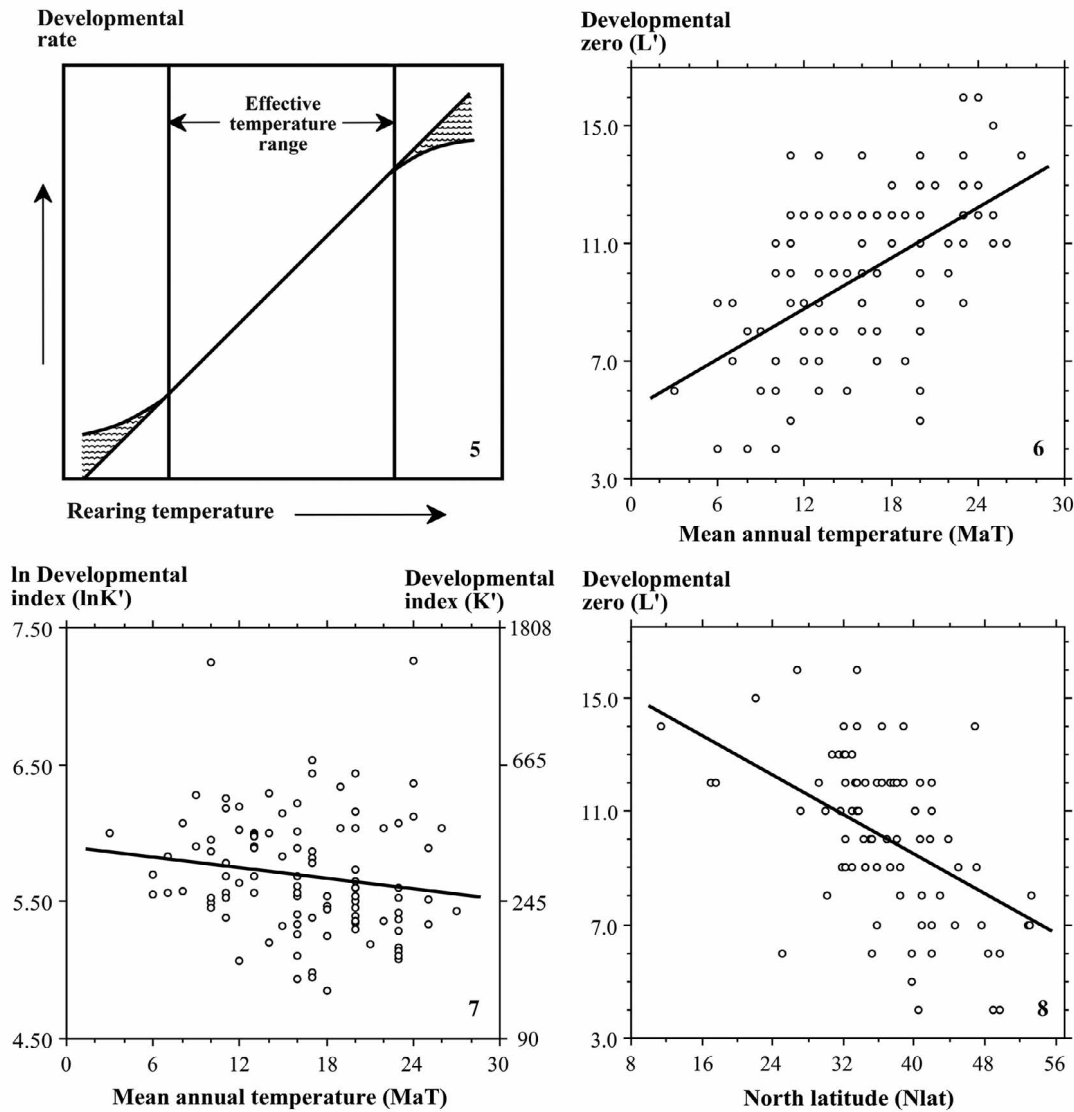
T	L'=5	L'=8	L'=11	L'=14	T	L'=5	L'=8	L'=11	L'=14
8	4.08	2.56	1.61	0.99	19.5	14.03	11.15	8.39	5.87
8.5	4.44	2.80	1.72	1.01	20	14.51	11.62	8.83	6.26
9	4.80	3.06	1.85	1.04	20.5	15.00	12.10	9.28	6.65
9.5	5.18	3.34	2.00	1.10	21	15.48	12.58	9.74	7.06
10	5.56	3.62	2.17	1.18	21.5	15.97	13.06	10.20	7.48
10.5	5.95	3.92	2.36	1.28	22	16.46	13.54	10.66	7.90
11	6.35	4.24	2.57	1.39	22.5	16.95	14.03	11.14	8.34
11.5	6.76	4.57	2.80	1.53	23	17.43	14.51	11.61	8.78
12	7.18	4.91	3.04	1.68	23.5	17.92	15.00	12.09	9.24
12.5	7.60	5.26	3.30	1.85	24	18.41	15.49	12.58	9.70
13	8.02	5.62	3.58	2.04	24.5	18.89	15.98	13.06	10.17
13.5	8.46	5.99	3.87	2.25	25	19.38	16.47	13.55	10.64
14	8.90	6.38	4.18	2.47	25.5	19.86	16.95	14.04	11.12
14.5	9.34	6.77	4.50	2.71	26	20.34	17.44	14.53	11.61
15	9.79	7.18	4.84	2.96	26.5	20.82	17.92	15.02	12.11
15.5	10.25	7.59	5.19	3.23	27	21.29	18.41	15.51	12.61
16	10.71	8.01	5.55	3.51	27.5	21.76	18.88	16.00	13.11
16.5	11.17	8.44	5.92	3.81	28	22.23	19.36	16.49	13.62
17	11.64	8.88	6.31	4.12	28.5	22.70	19.83	16.97	14.13
17.5	12.11	9.32	6.71	4.44	29	23.16	20.30	17.45	14.65
18	12.59	9.77	7.11	4.78	29.5	23.61	20.76	17.93	15.17
18.5	13.06	10.22	7.53	5.13	30	24.06	21.22	18.41	15.69
19	13.54	10.69	7.95	5.50	30.5	24.51	21.67	18.88	16.22
					31	24.95	22.11	19.34	16.75

As the calendar day is usually the ultimate currency in DD applications, how closely W approximated K' was compared in terms of calendar days (Table 2), which were computed as (K'-W)/DA.

Mamestra configurator can again serve to illustrate how W was estimated and how Table 3 assisted. In laboratory rearing of this species, the four surviving values of T and D yielded an L' of 6.19° (rounded to 6.2) (Fig. 2, Table 2: No. 36) and T and D medians of 18.0° and 36.4, respectively. Turning to Table 3, T of 18.0° and L' of 6.19° indicates a DA intermediate between 12.59 for L' = 5 and 9.77 for L' = 8, which after interpolation is 11.5. The mathematical product of 11.5 × 36.4 (DA × D) yields W of 418.6 (rounded to 419) (Table 2: No. 36). Then K' of 404 less W of 419 equals -15 DDs, which,

when divided by 11.5 DDs per calendar day (DA), gives a difference of -1.3 calendar days between W and K' (Table 2: No. 36).

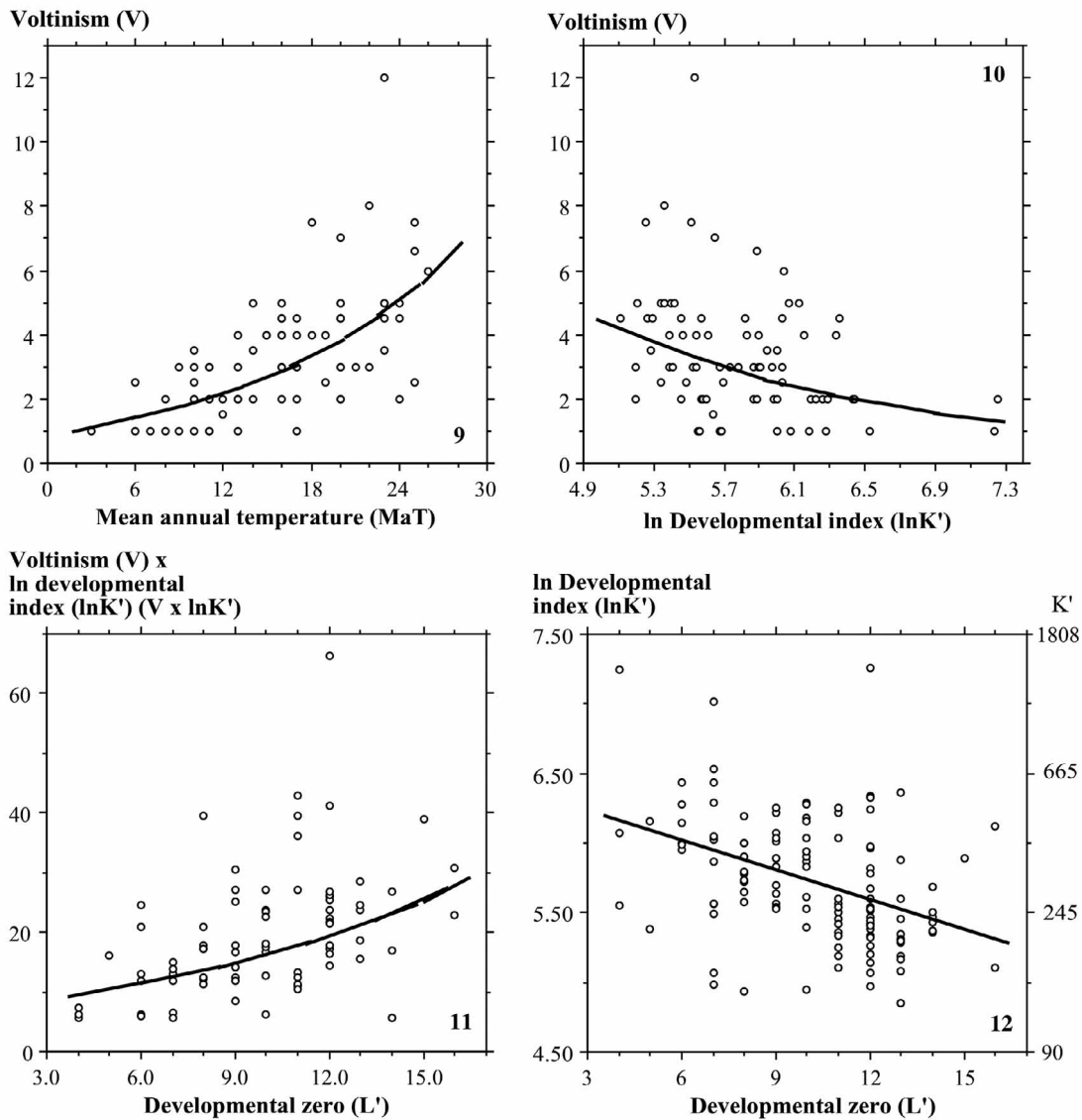
Field temperatures used in this study originated from 1920–2000, and the 18 official weather stations providing them were believed representative of temperatures where more than 25 North American datasets originated (Fig. 4, Appendix 1). U. S. temperature records, except those for Minnesota, were obtained at <http://cdo.ncdc.noaa.gov/CDO/cdo>; temperatures for the one Minnesota station were obtained at <http://climate.umn.edu/MnlocMS/MNlocNEW.asp>. Official Canadian temperature records were obtained at http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html.



FIGS. 5–8. **5.** Diagram illustrating the Kaufmann or rate summation effect. The effect occurs when field temperatures used in summation fall beyond the limits of the straight line (lower and upper shaded areas). Further explanation in text. **6.** Empirical relation of developmental zero, L' , to mean annual temperature, MaT, at or near localities of dataset origin; n reduced to 101 by unavailability of some values of MaT, and by dataset removals to avoid pseudoreplication. $L' = 5.37 + 0.287 \text{ MaT}$, $r^2 = 0.29$, $P < 0.001$. **7.** Empirical relation of ln developmental index, $\ln K'$, to mean annual temperature, MaT, at or near localities of dataset origin for 101 n ; n reduced as in Fig. 6. $\ln K' = -0.013 \text{ MaT} + 5.9$, $r^2 = 0.01$, $P = 0.12$. Arithmetic equivalents of $\ln K'$ are on right-hand vertical axis. **8.** Empirical relation of developmental zero, L' , to latitude at or near origins of northern hemisphere datasets, Nlat; n reduced to 85 by unavailability of Nlat for some datasets, and by dataset removals to avoid pseudoreplication. $L' = 16.5 - 0.173 \text{ Nlat}$, $r^2 = 0.26$, $P < 0.001$.

Mean annual temperatures, latitudes, and voltinism were compiled from dataset source publications, maps, and on-line resources. These variables as well as others appear in or are derivable from Table 2. Temperatures originally reported in °F were converted to °C before use. Statistics were generated with SYSTAT software (SYSTAT 1992), and SE refers to standard error.

Kaufmann and related effects. A fundamental problem with all linear phenological models is that the full range of any R–T relation is actually curvilinear at its lower and upper ends (Ruel & Ayres 1999; Worner 1992; Liu et al. 1995). At the lower end, the curve is accelerating or concave-upward, and at the upper end, decelerating or concave-downward (Fig. 5). Nonlinear



FIGS. 9–12. **9.** Empirical relation of voltinism, V , to mean annual temperature, MaT , at or near localities of dataset origin; n reduced to 77 because of unavailability of V and MaT for some datasets. $V = 0.950 \times 10^{0.0304 MaT}$, $r^2 = 0.43$, $P < 0.001$. **10.** Empirical relation of voltinism, V , to \ln developmental index, $\ln K'$, for 81 n ; n reduced as in Fig. 9. $V = 60.51 \times 10^{-0.231 \ln K'}$, $r^2 = 0.14$, $P < 0.01$. **11.** Empirical relation of mathematical product of voltinism, V , times \ln developmental index, $\ln K'$ [$V \times \ln K'$] to developmental zero (L') for 81 n ; n reduced as in Fig. 9. $V \times \ln K' = 6.054 \times 10^{0.0430 L'}$, $r^2 = 0.23$, $P < 0.001$. **12.** Empirical relation of \ln developmental index, $\ln K'$, to developmental zero, L' , for 131 n . $\ln K' = 6.43 - 0.0697 L'$, $r^2 = 0.18$, $P < 0.001$. Arithmetic equivalents of $\ln K'$ are on right-hand vertical axis.

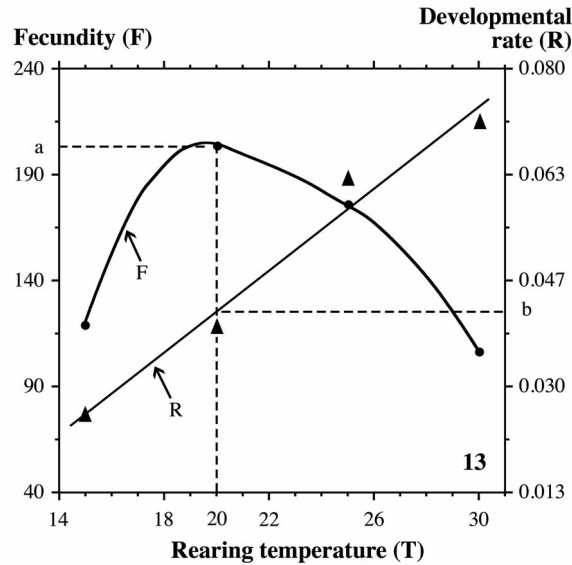


FIG. 13. Fecundity, F , as related to rearing temperature, T , and rate of larval development, R , in *Adoxophyes honmai* Yasuda (Tortricidae). Curve F fitted by connecting data points. Line R fitted by the improved linear model; $R = 0.00435 T - 0.0462$. Data of Nabeta et al. (2005).

models can be fitted to the full range of data, but linear model simplicity is thereby lost, and more parameters must be estimated—their number often approaching the number of observations in small datasets. Temperatures that fluctuate beyond the effective temperature range (Fig. 5: shaded areas) create summing errors. These errors constitute the Kaufmann or rate-summation effect (Kaufmann 1932). The straight line underestimates developmental rate in the lower shaded area, and overestimates it in the upper shaded area. Total summation error will depend on how often unusually low and high temperatures occur. The possibility of such occurrences underscores the importance of generating adequate numbers of T–D points for modeling to ensure that removal of points that later prove to depart from linearity does not reduce model accuracy.

The Kaufmann effect is encompassed by Jensen's Inequality (Ruel & Ayres 1999), a broader phenomenon involving more than the tails of the rate response distribution. The inequality refers to the difference between actual and predicted DDs when the rate response to temperature is nonlinear. In such cases, field temperature variance acts independently of average field temperature. As discussed further on, these potential effects appear to have had minimal impact in this study.

RESULTS AND DISCUSSION

Revision of thermal characteristics. Among the 223 datasets originally meeting study criteria, 16 (7%) were convincingly curvilinear and therefore dropped. Most of the dropped datasets had been fitted with nonlinear models. One or more of these were published by Bari & Lange (1980), Butler et al. (1975), Butturini & De Berardinis (1992), Chen & Su (1982), Daoud et al. (1999), Gould et al. (2005), Johansen (1997), Jones (2005), Legaspi & Legaspi (2007), Madge (1962), Miner (1947), Snyder (1954), and Tiso et al. (1991–92).

More significantly, 207 (93%) of the 223 datasets had ≥ 4 linear points. However, application of the improved linear model during thermal characteristic revision resulted in reductions to < 4 linear points in more than 70 of the 207 datasets, causing them also to be dropped (Appendix 2). Such wholesale exclusion of datasets again underscores the importance of generating sufficient numbers of T–R points for modeling—at least six, preferably eight or more (Legg et al. 2002)—in case points must be removed because of nonlinearity.

The 131 surviving datasets (Table 2) originated from 1927–2007 on six continents, and represent species in 11 families. Across all families, L' ranged $3.9 \pm \text{SE } 0.58$ – $16.0 \pm \text{SE } 0.62$. The statistical distribution of revised thermal characteristics shifted only slightly: L

and L' medians were 10.8 and 10.9, respectively, L' exceeding L in 62 (47%) of datasets, and medians of K and K', each 280, K' exceeding K in 55 (42%) (Table 2). Lack of greater overall differences is of little consequence, however, as the focus of thermal characteristic revision was the individual dataset and its purpose the improvement of dataset quality and reliability.

L' tended to reflect local climates, values changing by nearly 1° for each 3° change in mean annual temperature at the site of data collection, forming a continuum of low to high values from cool to warm conditions (Fig. 6). Low L' values enable earlier development in short, cool growth seasons. K' appeared uninfluenced by mean annual temperature (Fig. 7), but it assumed more importance when combined with other variables, as shown further on.

Noctuidae was the best-represented family among surviving datasets. Although many noctuids are capable of feeding as adults, and a few may approach butterfly income breeding in amount of adult feeding, all were considered capital breeders here because not enough is known at present to classify them more accurately. Mean values of L' and K' for Noctuidae (56 n) were 9.9 and 336 (ln = 5.817), and for the next three best represented families were Tortricidae (25 n), 8.6 and 341 (ln = 5.832), Crambidae (17 n) 12.4 and 320 (ln = 5.768), and Gelechiidae (15 n) 12.0 and 220 (ln = 5.394).

Trudgill & Perry (1994) suggested that high L values serve to delay development until lethal spring frosts have mostly passed. Theoretical and empirical studies of unrevised thermal characteristics have further suggested that tropical ectotherms would have higher L and lower K values than temperate ones (Honék 1996b; Trudgill & Perry 1994; Trudgill 1995). The revised thermal characteristics strongly support these suggestions.

Not surprisingly, L' declined with increasing north latitude (Fig. 8) as it did with decreasing mean annual temperature, the former also noted for unrevised values of L by Honék (1999). The trend in L' relative to increasing south latitude was positive but not statistically significant. The south latitude sample was small (14 n), but a different trend is perhaps to be expected because of the larger ratio of ocean to landmass in the southern hemisphere. Response of one species to differing geography is well illustrated by *Agrotis ipsilon* (Hufnagel) (Noctuidae) with L's ranging 5.8–11.6 among three continents (Table 2: Nos. 1–5), although for reasons thus far unclear, relations between L' and climatic variables in this example do not mirror statistical outcomes across families.

Voltinism across all families ranged from 1–12 (Table 2). It declined like L' with declining mean annual temperature (Fig. 9), but increased with decreasing K' (Fig. 10). Joint relations among voltinism, V, ln K', and L' are readily understandable at high values of L' where multiple generations develop in one growth season (Fig. 11). It is assumed in Fig. 11 that L' for a species at a given locality does not change appreciably between generations during the same growth season.

As emphasized by Honék (1996a) for unrevised thermal characteristics L and K, revised thermal characteristics L' and K' were themselves inversely correlated among species (Fig. 12). For a 1° change in L' near its median, K' changed by ~31 DDs.

Body size is not addressed in the present study, but it is noteworthy in passing that Honék (1999) showed that body mass and unrevised K are directly correlated. Honék concluded that, other things being equal, larger bodied species require longer developmental durations than smaller ones and thus have larger Ks.

Examples of environmental factors other than temperature influencing thermal characteristics include humidity where at 90–60% R. H. K' at the same Ts ranged 390–512 (Table 2: Nos. 120–122); photoperiod where K' among photophases of 10–14 h of daylight ranged 161–197 (Table 2: Nos. 25–27); and food quality where L' and K' were, respectively, 8.5° and 416 on cotton, and 14.0° and 245 on corn (Table 2: Nos. 14 and 15).

In summary, thermal-characteristic revision does not appear to have overturned any broad conclusions based earlier on unrevised values, but instead strengthens such conclusions with higher quality surviving datasets. For instance, the inverse correlation of L' and mean annual temperature (Fig. 6) confirms that thermal characteristics of populations are adaptive and subject to evolution.

Summation with official temperatures. Agreement of calendar-day differences between W and K' here for a period as long as the entire larval phase exceeded expectations. Some 91%—all but 9—agreed within ±2 calendar days (Table 2). Agreement was doubtless enhanced by the exclusion of datasets that were weak or had extreme values. Most divergences of >2 calendar days were from coastal or near coastal oceanic and lacustrine environments in Japan, Australia, Taiwan, (Table 2: Nos. 8, 20, 38, 61, 69, 75, 96, 129), the U. S. and Canada (Table 2: Nos. 38, 75, 96), Brazil, and Spain (Table 2: Nos. 69, 131). Association of divergences with these environments may be coincidental, however, as other datasets from similar environments did not diverge excessively (Table 2: Nos. 2, 9–10, 18, 50, 57, 62, 66, 108, 115, 125–127).

Methods of estimating W gave values that agreed reasonably well with K' even in warm-climate species. Most W estimates involved temperatures in the confirmed effective range of ~ 15 – 28° ; only 32 datasets (26%) had upper values $>31^\circ$ (stored product lepidopterans excluded) (Table 2). During the May–August period of official temperatures used to estimate DA and W , field temperatures averaged $\sim 20^\circ$. In 64 datasets that included a T of 20° , resulting D values ranged 11.9–173.6, with a median of 29.2 days, or ~ 1 month. Mean monthly temperatures $\geq 29^\circ$ were never observed in the North American weather-station records used (Fig. 3). For instance, the 30-yr July ‘normal’ (long-term average) temperature at Sioux Falls, SD, was $23.5 \pm SD 1.7^\circ$, with 99 percent confidence limits of 19.1 – 27.9° . This makes a warmer average July temperature unlikely to occur there oftener than once a century. Of course, very warm temperatures may occur for less than monthly durations, and even regularly in the Torrid Zone ($<24^\circ$ latitude on either side of the equator). Six surviving datasets originated in the Torrid Zone, and in all four for which W was calculated (Table 2: Nos. 21, 52, 53, 87), agreement of W with K' proved to be well within ± 2 calendar days. As emphasized by Ruel & Ayres (1999), however, hourly or other less than half-day field temperatures would provide more accurate results. The degree of agreement between W and K' suggests that Jensen’s Inequality had little adverse impact on DA s in Table 3 and thus on W .

Lack of attention to egg and pupal development probably detracts little from the uses of this study, as previous surveys found that unrevised thermal characteristics were similar for egg, larval, and pupal stages of Lepidoptera (Honék 1996a, b).

Interestingly, Podolsky (1984) has written at length about a forecasting method that bypasses thermal characteristics. This method first assembles phase durations at different habitat temperatures. As the temperatures in this first step are uncontrolled, many more observations may be required than with a thermal-characteristic approach. Next, mean temperatures at the location of interest are assembled by successive 10-day or other intervals. Finally, the two assemblies are combined into a nomogram allowing phase durations to be predicted starting on any of a range of dates. Perhaps because of the large amount of data required and uncertainty whether results are transferable between localities, Podolsky’s method has not been widely adopted.

Finally, agreement of W with K' seems sufficient to warrant exploration of weather-station temperatures for new developmental applications. One topic for study is the effect of temperature on growth-related population

fitness factors such as fecundity. Capital-breeding Lepidoptera reared at different temperatures differ in maternal body size and fecundity (Honék 1993; Miller 2005) with, surprisingly, cooler rather than warmer temperatures having the greater positive impact (Atkinson 1994; Miller 2005) (Fig. 13). Martin & Huey (2008) explained why cool, seemingly suboptimal temperatures are actually optimal. What may have been overlooked in past work on capital-breeding Lepidoptera is that rate of development also may affect fecundity. For instance, fecundity in *Adoxophyes honmai* Yasuda (Tortricidae) peaked at a rather slow developmental rate of ~ 0.042 (Fig. 13: b) and midrange T of 20° (Fig. 13: a). Because developmental rate, R , is the reciprocal $1/D$, peak fecundity occurred at a larval duration of 24 calendar days, which is protracted compared with the shortest D of 14 days at a T of 30° . This suggests that DD summation, in so far as it indicates developmental rate, might predict fecundity. In future work, fecundity and other fitness factors relative to temperature-dependent developmental rates and associated population indices such as capacity for increase should be explored.

ACKNOWLEDGEMENTS

I thank the following for generous assistance: M. P. Ayres, whose review of an earlier manuscript on temperature-dependent development inspired this study; R. D. Moon for making available his degree-day calculator, and for statistical guidance; R. D. Moon, M. A. Roberts, Peijian Shi, and an anonymous reader for very constructive reviews of the manuscript; J. L. Moe and C. W. Muggli-Miller for computer support and figure transcriptions; C. W. Muggli-Miller for Japanese translations; and M. A. Borg for bibliographic help.

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Received for publication 10 January 2011; revised and accepted: 2 August 2011.

Appendix 1. Weather stations and data used in this study

Station	Months and years
Canada	
Fredericton, NB	May-Aug. 1940, 1950
Montreal River, ON	May-Aug. 1933, 1965
Timmins, ON	May-Aug. 1930, 1953
Guelph Arboretum, ON	May-Aug. 1985, 1990
Sioux Lookout, ON	May-Aug. 1920, 1932
Brandon CDA, MB	May-Aug. 1927, 2000
Regina CDA, SK	May-Aug. 1970
United States	
Harrisburg, PA	May-Aug. 1935, 1940
Cincinnati, OH	May-Aug. 1990, 2000
Lansing, MI	May-Aug. 1960, 1965
Le Roy, MN	May-Aug. 1950, 1960
Lincoln AFB, NE	May-Aug. 1950, 1955
Sioux Falls, SD	July-Aug. 1995
Oklahoma City, OK	June-Aug. 1950, 1960, 1970
Minot, ND	July 1936
Rapid City, SD	July-Aug. 1955, July 1980
Cheyenne, WY	May-Aug. 1990, 2000
Boise, ID	May-Aug. 1955

Appendix 2. Sources containing one or more datasets dropped from the study because fewer than four linear points survived thermal characteristic revision with improved linear model procedure.

Adati et al. (2004), Åsman (2001), Atkinson (1980), Barker & Enz (1993), Beckwith (1982), Berkett et al. (1976), Bues & Poitout (1980), Calvin et al. (1991), Chen & Su (1982), Cox (1974), Danthararyana (1975), Danthararyana et al. (1995), De Bernardinis et al. (1991-92), Deshmukh et al. (1982), Doerr et al. (2002), El-Shaarawy et al. (1975), Foerster (1996), Fye & McAda (1972), Gal (1978), Galán & Rodríguez (1992), Ghulam-Ullah (1955), Goebel (2006), Gomma (1973), Hardy (1938), Hasegawa & Chiba (1969), Herfs (1963), Hirai (1975), Howell & Neven (2000), Huang & Peng (2002), Jacob & Cox (1977), Janisch (1933), Jones (2005), Kamata & Igarashi (1995), Kinjo & Arakaki (2002), Koizumi (1955), Kwon et al. (2005), Li et al. (1990), Maksimovic (1963), Mawby et al. (1976), Milonas & Savopoulou-Soultani (2000), Miyashita (1971), Oliveira et al. (2004), Parrella & Kok (1977), Prevett (1968), Rahman & Khalequzzaman (2004), Richmond & Bachelier (1989), Roberts & Mahr (1986), Roltsch et al. (1990), Shanower et al. (1993b), Shields (1983), Sidibé & Laugé (1977), Sinchaisri & Sögawa (1969), Singh (1962), Takeda & Chippendale (1982), Taveras et al. (2004), Weinberg & Lange (1980), and Yamada & Koshihara (1976).

OBSERVATIONS ON THE SEASONAL BIOLOGY AND APPARENT MIGRATION OF *ARGYNNIS*
(*SPEYERIA*) *CORONIS* (NYMPHALIDAE) IN CENTRAL WASHINGTON

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Abstract. Opportunistic observations on the seasonal biology of *Argynnis coronis* in central Washington obtained over 40 years, suggest that the ecology of this species is characterized by well defined seasonal population movements between low and high elevations. Unfed first instar larvae diapause and overwinter in lithosol shrub-steppe areas immediately east of the Cascade Mountains feeding and developing on sagebrush violets (*Viola trinervata*) during March-May. Males eclose 7-14 days before females in mid-late May. After mating, females delay egg development and migrate 50–100 km westward reaching high elevations in the Cascades by late June-early July. Most males die in the shrub-steppe but a few migrate with the females. Female-dominated populations over-summer at 2,000-2,500m and are active feeding on flowers but remain non-reproductive. Ovaries develop in early August and females begin an eastward downslope movement returning to shrub-steppe areas by early September. Oviposition on soil, stones, rocks and ground level woody plants where violets grow in spring, occurs during September and early October.

Additional key words: overwintering, migration, reproductive diapause, oversummering, oviposition, shrub-steppe, Cascades

Argynnis (*Speyeria*) *coronis* (Behr) ranges throughout much of the western US from Washington and South Dakota to Colorado, Arizona and southern California (Scott 1986). In Washington and Oregon it occurs primarily along the Cascade Mountains and eastern foothills but also east of the Cascades in Oregon (Dornfield 1980; Hinchliff 1994, 1996; Pyle 2002; Warren 2005). A single generation flies from May to October in favored habitats, which include canyons, hillsides, shrub-steppe, forest margins and mountain meadows from 300–2500m (Dunford 2009). The ecology of this species is of great interest with altitudinal migration, adult reproductive summer diapause and hibernal larval diapause, apparent components of the annual life history in Washington and Oregon (Pyle 2002; James & Nunnallee 2011). Opportunistic observations on the biology of *A. coronis* obtained over 40 years in central Washington are summarized and presented here.

RESULTS

Overwintering and spring larval development.

In common with all *Argynnis* spp., *A. coronis* overwinters as an inactive unfed first instar larva hidden in detritus, soil or under rocks on the ground (Fig.1). (Pyle 2002; James & Nunnallee 2011). In central Washington (Figs. 2 and 3), overwintering appears to occur in the eastern foothills of the Cascades, primarily in the shrub-steppe zone where the primary larval host plant, *Viola trinervata* (T.J Howell) T.J Howell ex Gray

(Sagebrush Violet), occurs (Fig. 4). It is possible that some individuals overwinter at slightly higher elevations associated with other violet species (e.g. *V. nuttalli* Pursh.). Overwintering larvae diapause and do not readily respond to normally favorable stimuli (e.g. warmth and host plants), for ~ 3 months after hatching (James 2008). In the laboratory, larvae exposed to cold conditions (5 °C) and darkness for 80 days broke diapause and commenced development reaching adulthood after 54 days at 25 °C (James 2008). Larvae of *Argynnis* spp. are notoriously difficult to find, especially early instars, thus it is unclear when larvae of *A. coronis* begin feeding. Third instar larvae were discovered under rocks in the vicinity of *V. trinervata* (Schnebly Coulee, north ridge, Kittitas County (46.95 ° N, 120.09 °



FIG. 1. Overwintering unfed first instar larva of *Argynnis coronis*.

W, 625m) on 26 April 1971 and 16 April 1988. They were also found in Ryegrass Coulee, Kittitas County (46.93° N, 120.06° W, 457m) on 30 April 1973 and at the south fork of Ahtanum Creek, Yakima County (46.51° N, 120.91° W, 762m) on 10 April 1969). A developmental duration of ~8 weeks at 25 °C (James 2008), suggests that adults eclosing in May likely commence larval development in late February or early March. Ambient maximum temperatures during March–April in eastern Washington shrub-steppe average 13–18 °C (Yakima) indicating that heliothermic warming in the exposed habitat must play a major role in the rapid development of *A. coronis* larvae.

Adults: Ecdysis, courtship and mating. Adults eclose from early May to late June, depending on temperatures experienced during March–May. Males appear 7–14 days before females, similar to that reported for Californian populations of *A. coronis* (Sims 1984). In a seasonal survey at Schnebly Coulee, Kittitas

County in 2004, males were first observed on 6 June nectaring heavily on *Salvia dorrii* (Kellogg) Abrams (Purple Sage). Females were first observed on 19 June. On 3 July, no adults were seen at this location. During 2005–2010, first adults were seen annually between 17 May and 26 May in the Yakima-Naches district of central Washington. In 2007, adults began eclosing around 26 May and on 3 June ~50 males were seen on the slopes of Umtanum Canyon, Kittitas County, 27 km N of Yakima (46.85° N, 120.48° W, 508 m) nectaring and searching for females. In 2010 adults began eclosing around 20 May continuing for ~4 weeks. On 3 June ~25 newly eclosed adults were seen near Cowiche Mt, 19 km west of Yakima, Yakima County (46.65° N, 120.75° W, 620 m) during 1 hour. Most were males engaged in rapid, low, searching flight (hazy sunshine, 17–21 °C), seeking females. A number of individuals (3–4) were resting on small shrubs with unhardened wings. Newly eclosed individuals resting on the ground or on small

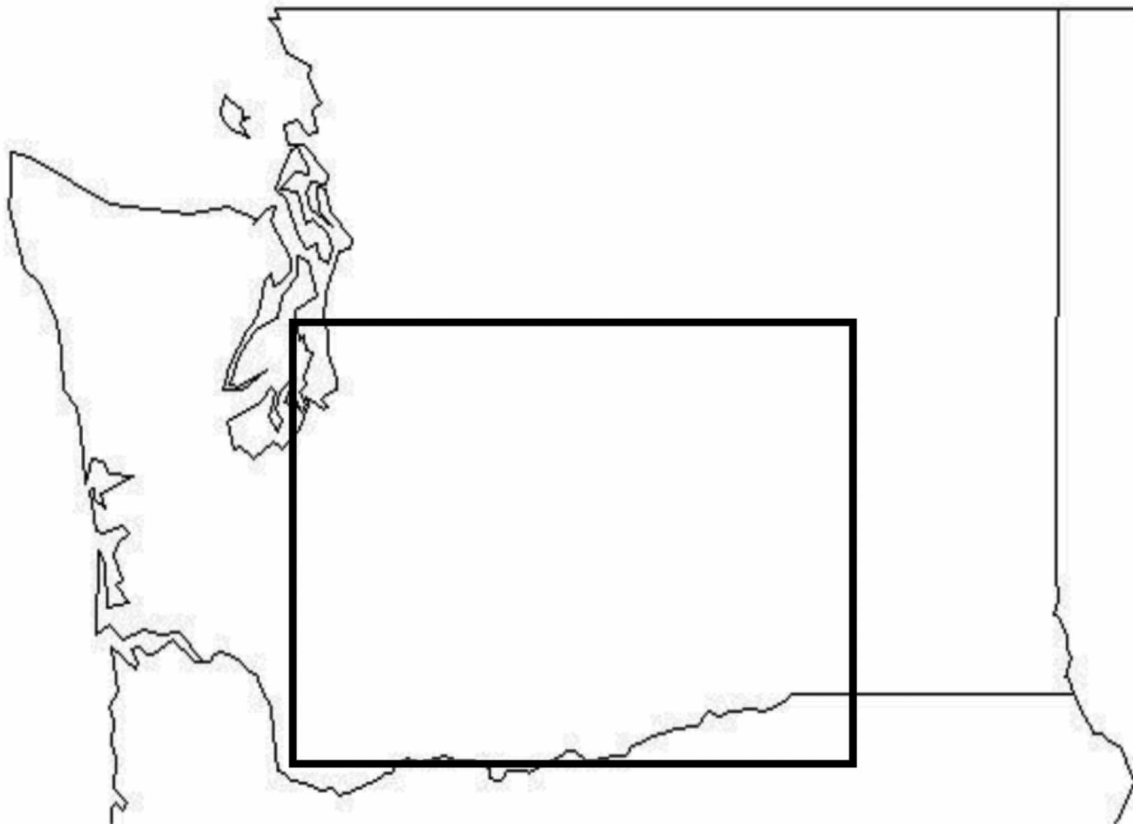


FIG. 2. Approximate area of Washington State in which observations on *Argynnis coronis* were made 1969–2010.

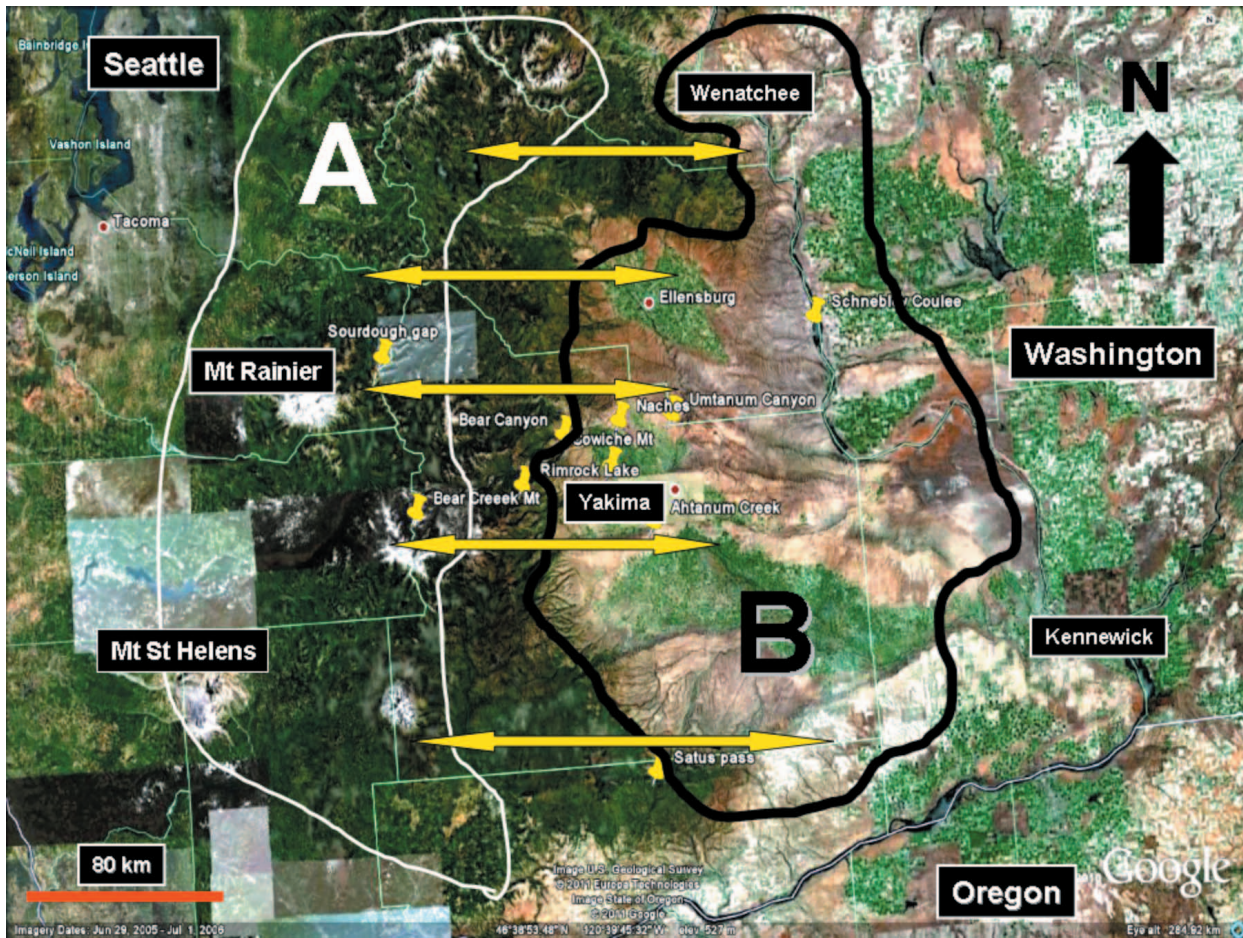


FIG. 3. Approximate summer (A- Cascade Mountains) and fall-spring (B-shrub-steppe) ranges of *Argynnis coronis* in central Washington. Putative late spring (west) and early fall (east) migration indicated by arrows.



FIG. 4. Larval host of *Argynnis coronis*, Sagebrush Violet (*Viola trinervata*), in spring.



FIG. 5. Copulating pair of *Argynnis coronis* in June.



FIG. 6. Male *Argynnis coronis* imbibing from damp mud in June.

shrubs showed a 'startle response' when disturbed, rapidly opening and shutting wings with no flight. Two copulating pairs were seen with females carrying males (Fig. 5). Nectaring occurred primarily on two buckwheat species, *Eriogonum heracleoides* Nutt. (Parsley Buckwheat) and *Eriogonum compositum* Dougl. ex Benth (Northern Buckwheat). Males were also seen during June imbibing from damp mud in groups of up to a dozen (Fig. 6). Eclosion was still occurring at this site on 17 June with fresh females clinging to low shrubs and conspicuous mate-searching by males. Four hours of observation on 1 July resulted in ~40 males seen searching for females which appeared to be absent. Most individuals showed significant wing wear on this date. By mid June all *Viola* host plants have withered and desiccated, remaining dormant until the following March.

Adults: Westerly upslope migration by females.

Once mated, females appear to undertake an upslope, westerly migration, which ultimately takes them to the highest peaks of the Cascade Mountains (Fig. 3). On 4 June 2009, an estimated 150 *A. coronis* females were seen flying rapidly westward in a number of meadows and along roads and trails near Rimrock Lake, 53 km west of Yakima, Yakima County (46.62° N, 121.18° W, 1134 m) during 4 hours (10:00–14:00h, sunshine, 25–27 °C). During 13:00–14:00h, females passed through a meadow at a rate of 2–3 per minute. Flight was purposeful and mostly uninterrupted by nectaring. Limited nectaring was observed mid morning on dandelion and *Ceanothus*. Captured females (35) had plump abdomens and all continued westward flight after release. No males were seen. On 17 June 2010, 32 females were seen flying westward at Cowiche Mt in partial sunshine and temperatures of 18–21 °C during two hours.



FIG. 7. Desiccated Sagebrush Violet (*Viola trinervata*) at Cowiche Mountain in October.

Adults: Non-reproductive females overwintering at high elevations. *Argynnis coronis* adults are generally absent in shrub-steppe localities from about the end of June until September. No *A. coronis* adults were observed from 8 July–30 August 2005–2010 on multiple field excursions at Cowiche Mt, Bear Canyon, Umtanum Canyon and many other shrub-steppe locations near Yakima, Ellensburg and Wenatchee. In 2010 the last individuals (worn males) were seen at Cowiche Mt on 7 July. The majority of males appear to remain in sage-steppe until death, although a small number apparently follow females upslope. Males and females were present at Satus Pass, Klickitat County (45.92° N, 120.65° W, 987 m) on 13 June 2007, likely en route to higher elevations, but this location is only ~5 km from the nearest shrub-steppe habitat (500–600 m). Female-dominated populations of *A. coronis* occur at high elevations (2000–2500 m) in the Cascade Mountains during July–August, for example at Bear



FIG. 8. Female *Argynnis coronis* ovipositing on wood of *Artemisia rigida* on the ground at Cowiche Mountain on 12 October 2010.

Creek Mountain, Yakima County, 68 km west of Yakima, (46.53° N, 121.34° W, 2200 m) and Sourdough Gap, Pierce County, 85 km west north west of Yakima (46.90° W, 121.50° W, 2000 m). Males are rarely seen at these sites (e.g. five seen at Bear Creek Mt during 6 years of observation). High elevation summer populations are flight-active and individuals spend much time feeding from the flowers of alpine plants like phlox, buckwheats, asters and bistort. At Bear Creek Mt, females in July are found in lower meadows (~2000 m) but by early August they only occur on the highest ridges (~2200 m) usually the only sites where phlox and asters are still blooming. There is no evidence that females aestivate at these high elevations. Although no dissections have been made, it is clear that females at these high elevations are non-reproductive and likely contain undeveloped ovaries at least until August. Sims (1984) showed that females of Californian *A. coronis* are in reproductive diapause for a 3–5 week period in summer. Four females collected from Bear Creek Mt on 30 July 2005 oviposited after 7 days (James 2008). However, large numbers of eggs were not produced until the second half of August.

Adults: Easterly downslope migration. Easterly movement of females downslope begins in mid-late August. In the seasonal survey at Schnebly Coulee, Kittitas County in 2004, females were first observed on 15 August, nectaring on *Chrysothamnus* sp. In most years *A. coronis* is hard to find at Bear Creek Mt in the second half of August. On 31 August 2009 ~20 females showing uninterrupted strong easterly flight were seen on the access road (FR 1204) to Bear Creek Mt at ~1500–1800 m. On 31 August 2005 large numbers (25–30) of females reappeared in Bear Canyon, Yakima County, 32 km west north west of Yakima (46.70° N, 120.89° W, 644 m) after an absence of 2.5 months. On 11 September 1999, numerous females were observed nectaring on garden blooms in the city of Ellensburg, Kittitas County (47.01° N, 120.5° W, 486 m), on the floor of the Kittitas Valley. During 2007–2010 *A. coronis* females appeared in Yakima City during the last days of August or first days of September, becoming common visitors to gardens visiting *Buddleia* and asters during September.

Adults: Autumn reproductive populations in shrub-steppe. On 10 September 2007, ~50 females were seen on blooms of *Chrysothamnus nauseosus* (Gray Rabbitbrush) in Umtanum Canyon during 2 hours. On 29 September 2006 at the same location only 12 were seen plus one male. On 13 September 2010 an estimated 100 *A. coronis* females were seen nectaring on *C. nauseosus* in a canyon near Cowiche Mt (sunshine, 26 °C, 13:00–14:00h). A further 20–30 were observed on adjacent hillsides and plateaus searching

for oviposition sites. Similarly, on 27 September ~75 were seen nectaring and ~25 ovipositing (sunshine, 25 °C, 13:30–15:00h). One very worn male was caught. On 29 September ~50 were seen nectaring and ovipositing. On 12 and 13 October, 6 and 9 females were seen respectively, ovipositing or searching for oviposition sites. On 18 and 21 October no females were seen ovipositing, but on each date 2 were seen nectaring on rabbitbrush. Late October females had thin bodies and faded, worn wings.

On 27 and 29 September and 12 October the oviposition behavior of a total of 25 ovipositing females was studied at Cowiche Mt. Females flew close (~0.3 m) to the ground alighting only in areas lacking in grass and comprised mostly of rocks, stones and bare earth. These areas supported densest concentrations of desiccated sagebrush violets (Fig. 7). Areas with abundant cheat grass (*Bromus tectorum* L.) were inspected by females but rejected for oviposition. Fire-affected areas (a wildfire on 18 July 2010 produced a mosaic of burned and unburned patches) appeared to be ignored by female *A. coronis*, which flew over them at heights > 2 m. Eggs were laid singly on rocks, soil and plant material including ground level trunks and stems of Stiff Sagebrush (*Artemisia rigida* (Nutt.) A. Gray) (Fig. 8). After an egg was laid the female either crawled or flew briefly 5–25 cm before ovipositing again. In most instances 2–3 eggs were laid in close proximity before the female flew to another location 5–30 m away. One female laid 8 eggs, each separated by 5–8 cm before flying to a new patch. Bouts of oviposition were occasionally interrupted by visits to flowers for nectar. Oviposition continues into early October in most years but is usually finished by mid month.

DISCUSSION

Collectively, the observations reported here indicate that the life history of *A. coronis* in central Washington is characterized by seasonal, well defined population movements between low and high elevations (Fig. 3). Early workers recognized the vagile nature of *A. coronis* populations (e.g. Hammond 1974, 1981; Sims 1984) but did not report the seasonal migration strategy we describe here.

Oviposition, overwintering and development of immature stages occurs in shrub-steppe areas east of the Cascades with adults (and eggs) escaping the summer drought and heat of this low elevation zone by migrating 50–100 km to spend the summer months in alpine environments (Fig. 3). Most of our evidence is circumstantial and confirmation of the strategy must await more detailed and experimental studies. For example, marking of significant numbers of newly-

eclosed spring adults is needed to confirm migration to alpine zones and back again.

While summer diapause and altitudinal migration appears to be a feature of *A. coronis* ecology in central Washington, studies are needed to determine whether this strategy also occurs in other parts of the species' range. In Oregon, populations in eastern basin lands and ranges of the south-east also appear to 'move about' although populations on the eastern slopes of the Cascades and in the Ochoco-Blue-Wallowa Mountains appear to be sedentary. The observations of Shapiro & Manolis (2007) suggest seasonal movements occur in the Sacramento valley of northern California where *A. coronis* 'disappears' during summer, females reappearing and laying eggs in September. Sims (1984) showed in laboratory studies that female *A. coronis* in California spend 3–5 weeks during summer in reproductive diapause but did not provide information on behavior during this period. Confirmation of reproductive status is needed for Washington female *A. coronis* during June–August as well as information on mechanisms and/or environmental cues responsible for induction and termination of dormancy. Is induction cued by increasing daylengths or is it genetically programmed? Sims (1984) showed that short daylengths (12 hrs) hastened diapause termination in Californian *A. coronis* females. Our observations in Washington indicate females commence oviposition when daylengths decline from 15 to 14.5 hrs (early August).

Female reproductive diapause was also described for Kansas populations of *Speyeria* (*Argynnis*) *idalia* (Kopper et al. 2001), which breeds at low elevations in native grasslands. In common with the shrub-steppe/*A. coronis* system, violet host plants in Kansas grasslands occur only in spring and female *A. idalia* have an extended non-reproductive life span during summer before laying eggs during autumn. Unlike *A. coronis*, *A. idalia* does not migrate out of the hot, dry grasslands during summer. Females, instead show reduced activity and avoidance of sunlight (Kopper et al. 2001), suggesting aestivation. Aestivation serves the same function as migration to a cooler zone by limiting exposure to high temperatures.

Females of a number of *Argynnis* species have been reported as mating only once (Shields 1967; Burns 1968; Kopper et al. 2001). Three freshly emerged *A. coronis* females examined by Sims (1984) each had a single spermatophore, so it is likely that single matings are characteristic of this species too. However, although rare, males were seen in alpine summer and autumn shrub-steppe populations so it is possible that some females may mate more than once. The importance and phenology of males in the annual life history of *A.*

coronis needs further study.

The observations on oviposition indicate that female *A. coronis* are able to distinguish between areas that have violets and those that do not. Female avoidance of cheat grass covered areas corresponds to the relative scarcity of *V. trinervata* plants in grassy areas (James unpubl. obs.). The absence of oviposition in burned areas suggests that the cues guiding site selection by females (volatiles from senesced violets?) are absent in these areas. Our observations of *A. coronis* ovipositing on ground level parts of sagebrush are similar to those of Durden (1965) who found females of *Speyeria* (*Argynnis*) *callippe* Boisduval ovipositing on woody parts of sagebrush in Wyoming. Durden (1965) considered *Artemisia* as a possible host plant for *A. callippe* but it is more likely that sagebrush simply provided a convenient oviposition site near to violets as it does for female *A. coronis* in central Washington. Observations on oviposition by *A. coronis* have rarely been made by us or others and the extent of breeding grounds in central Washington is unclear. *Viola trinervata* occurs strictly in lithosol shrub-steppe habitats (sagebush flats, rocky hillsides) in a narrow band east of the Cascades. In similar habitats in Oregon *V. trinervata* is replaced by *V. beckwithii* Torr. And Gray (Great Basin Violet), a recorded host for *A. coronis* in Oregon (Warren 2005). The limited areas of lithosol shrub-steppe habitats present in Washington and Oregon may be critical to the survival of *A. coronis* and conservation of this species should feature in shrub-steppe management and conservation (Wooten 2002).

A prolonged female life span and associated reproductive dormancy appears to be characteristic of a number of species in the greater fritillary genus *Argynnis* in North America, primarily those occupying habitats with hot, dry summers. In contrast, species resident in higher elevation, cooler habitats like *A. mormonia* Boisduval and *A. egleis* Behr show no delay in female oviposition (Boggs 1987; James & Nunnallee 2011). *Argynnis coronis* is the only *Argynnis* species to date for which altitudinal migrations have been described, although *A. callippe* has been observed migrating downslope in late August and September in central Washington and may have a similar ecology. The ecological rationale for *A. coronis* delaying oviposition in shrub-steppe is likely tied to better survival of eggs and first instar larvae from September onwards than during July–August. Air temperatures in shrub-steppe areas of central Washington during summer routinely exceed 35–38 °C with relative humidities often < 10% and little or no cloud cover resulting in day time ground temperatures in excess of 50 °C. Kopper et al. (2001) suggested a similar rationale for the adoption of

aestivation and reproductive dormancy by females of *A. idalia* in Kansas.

Our observations suggest *A. coronis* populations in central Washington are highly adapted to exploiting an ephemerally favorable environment in spring then vacating it during the harsh conditions of summer. Escaping an unfavorable environment along with suppression of reproductive development is characteristic of the oogenesis-flight syndrome first postulated by Johnson (1969). In most butterfly examples of this syndrome, escape is from a cold environment (e.g. *Danaus plexippus* L.). The shrub-steppe habitat and the endemic violet species, *V. trinervata* are clearly critical to the ecology of *A. coronis* in central Washington. *Viola trinervata* is locally common but it is absent in many seemingly suitable shrub-steppe sites, particularly those with abundant cheat grass. Additionally, shrub-steppe is one of North America's most imperiled and neglected ecosystems (Noss & Peters 1995) with areas in Washington rapidly declining with the development of irrigated agriculture and urbanization (Wooten 2002). Efforts are required to expand and consolidate our understanding of the ecology of *A. coronis* and to highlight the vulnerability of this species to continued degradation of the shrub-steppe ecosystem.

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Received for publication 2 December 2010; revised and accepted 29 August 2011.

Journal of the Lepidopterists' Society
65(4), 2011, 256-258

OUTBREAK OF *EACLES IMPERIALIS* (SATURNIIDAE) ON COCOA AND FRUIT TREES IN MILAGRO – ECUADOR

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ABSTRACT. The account represents the first record of *E. imperialis* (Drury) as cocoa pest. The moth was founded in Milagro - Ecuador producing prominent defoliation on cocoa and other fruit trees.

Additional key words: defoliation, host range

A marked eruption of larvae of the saturniid *Eacles imperialis* (Drury) occurred in late March 2010 in the Milagro Canton, Guayas Province, Ecuador. In Ecuador there are no official records of this moth on cocoa (*Theobroma cacao* L., clon CCN-51) or national or fine flavor cocoa (*T. cacao* L.), nor in other commercial trees, severe enough to cause major defoliation. According to local farmers, larval numbers erupted for the first time, causing up to 95% defoliation of commercial cocoa cultivars.

Relatively few groups of insects from Ecuador have been well studied (e.g. Papilionoidea (Lepidoptera) and Carabidae (Coleoptera)), and thus knowledge of other families lags far behind (Barrera 2008). Dangles et al. (2009) mentioned that the paucity of entomological knowledge was due to the great diversity of Ecuadorian species living in a wide range of habitats and the difficulty of identifying the majority of tropical insect species, and in spite of the national effort to gather information on the taxonomy and ecology of insects, progress has been slow (Jiggins et. al. 2006; Moret 2005). Due to this lack of knowledge of Ecuadorian insects and their biology, control techniques are often inappropriate.

It is likely that the saturniid that is the focus of this report, *E. imperialis*, was resident prior to the planting of commercial crops. Changes in factors such as relative humidity, temperature and photoperiod, as well as the excessive use of chemicals contributed to “seasonal” pest emergence in high population levels; according to some authors, those factors affect the population of Lepidopteran natural enemies (Tanada & Fuxa 1987; Carrero & Planes 2008). Below we describe an outbreak caused by *E. imperialis* larvae in Ecuadorian cocoa and fruit tree plantations.

METHODS

Percentage of defoliation was estimated using the Townsend-Heuberger formula. Evaluation of defoliated trees was carried out using a scale from 0 to 5 (where 0 = 0% and 5 = 90–100% defoliation). Larvae were identified by Kirby Wolfe (personal communication), and compared to descriptions of Ferguson (1971) and Tuskes et al. (1996). In order to understand the current status of this pest in Ecuador as well as historical records of macrolepidopteran outbreaks, the official Ecuadorian registers of pests on economically important crops and available publications and newsletters were reviewed.

RESULTS

The outbreak of *E. imperialis* larvae on cocoa and other fruit trees caused prominent defoliation (Figure 1A), reaching an average of 95% over 313.82 acres. High densities of larvae were found in plantations, with a minimum of more than ten individuals per tree. The use of expensive doses of chemicals to eliminate the pest provoked economic losses due to the increment of production costs and environmental damages not yet evaluated.

Macrolepidoptera larvae were collected in the “La Garganta” community (Milagro, Guayas Province), located at 2°06'52.66" south latitude and 79°34'12.30" west longitude (Figure 1B). Existing records do not document the presence of this insect in the province of Guayas.

According to Tuskes et al. (1996), the most frequent host plants of *E. imperialis* are pine (*Pinus*), oak (*Quercus*), box elder (*Acer negundo*), and other maples, sweet gum (*Liquidambar styraciflua*), and sassafras (*Sassafras albidum*). In addition, Ferguson (1971) cited that larvae of *E. imperialis* in North America have been reported to feed on many tree species: *Tabebuia*, *Juglans*, *Cupressus*, *Cedrus*, *Gleditsia*, *Rhus*, and *Fagus*. Other common hosts cited in Wagner (2005) include *Tilia*, *Acer*, *Betula*, and *Ulmus*. The recent outbreak of *E. imperialis* in Ecuador occurred in commercial cultivars of cacao (*Theobroma cacao*, clon CCN-51) and on national cocoa (“arriba” fine or flavor cocoa). In addition, we recorded the caterpillars feeding on several fruit trees species, including mango (*Mangifera indica* L.), plum (*Spondias purpurea* L.), zapote [*Quararibea*

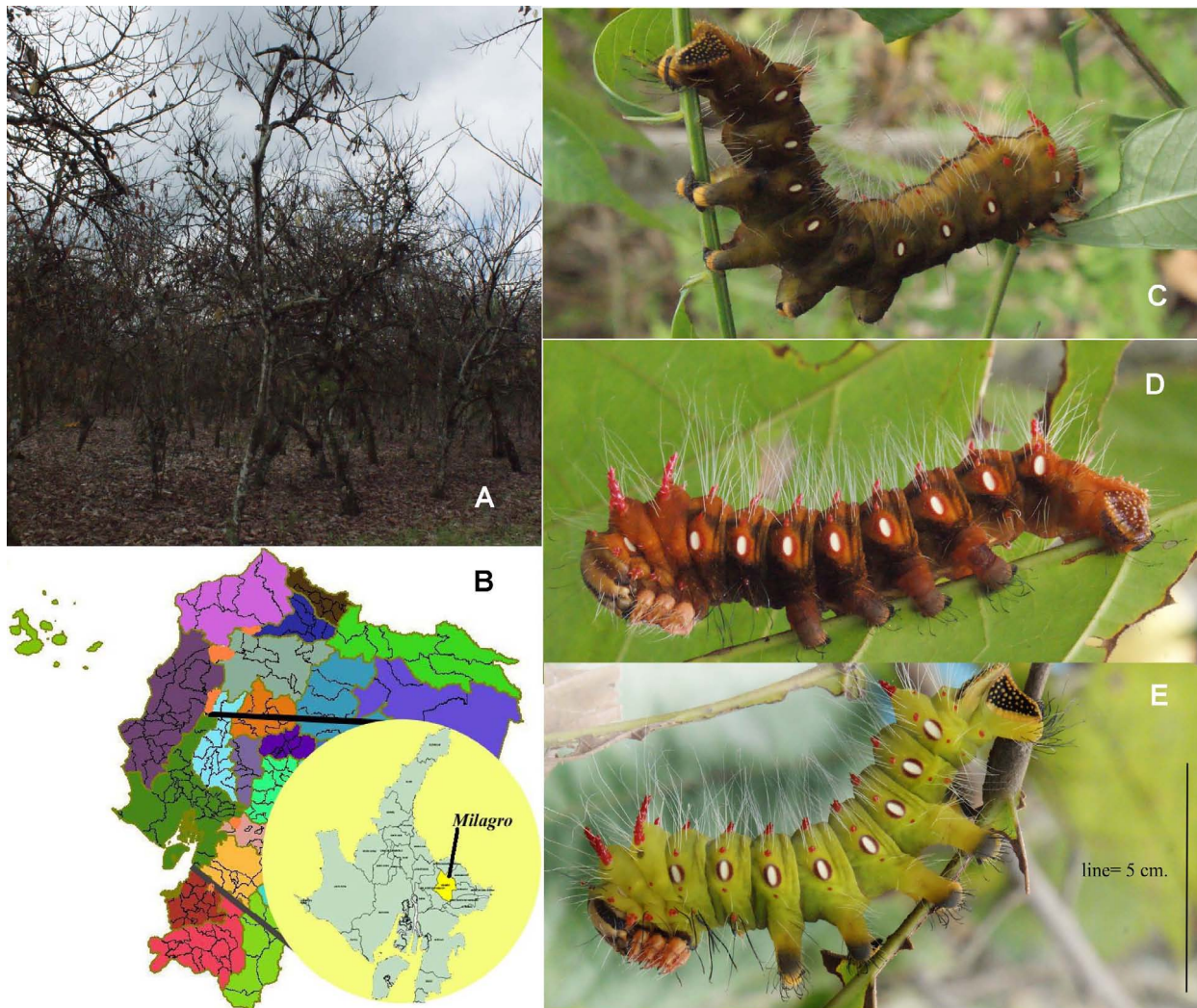


FIG. 1. (A) Defoliation caused by *E. imperialis*. (B) Location of outbreak. Region affected by defoliator presence indicated in yellow. Polymorphism of *E. imperialis* larvae: (C) brown, (D) reddish brown, (E) light green, 2010 CIBE - ESPOL©

cordata (Humboldt & Bonpland) Vischer], guava (*Psidium guajava* L.), “guaba” (*Inga edulis* Mart) and banana (*Musa* sp.). Severe defoliation, of at least some individual plants, was noted on all of these hosts.

DISCUSSION

This report may show how the effect of global warming and other factors including agronomic practices could contribute to new pest emergence. On the other hand, the information about the status of the pest in Ecuador is characterized by the lack of official records of this pest species on cocoa cultivation and other fruit trees. INIAP, Ecuador's agricultural research institution, mentioned in its “Guide for cocoa cultivation in the Amazon” (2001) that pests such as *Apatelodes costariseni*, *Dimorpha quaesita* and *Eacles masoni* cause defoliation damage on cocoa crops in the Ecuadorian Amazon region. Barrera (2008) listed *E. masoni* and *E. imperialis* as coffee pests in Brazil. The Andean inventory of pests and plant diseases (1996) includes *E. masoni* as a cocoa defoliator with a limited distribution in certain regions, that is sometimes responsible for moderate levels of impact and which sometimes requires obligatory control (=application of pesticides).

Racheli & Racheli (2006) indicated the presence in Ecuador of two subspecies of *E. imperialis*: *E. i. cacicus* (Drury, 1773) in the provinces of Sucumbíos, Orellana, Napo, Tungurahua and Morona-Santiago; and *E. i. anchycayensis* (Lemaire, 1971) in the provinces of Esmeraldas, Manabí and Pichincha. In addition, two subspecies of *E. masoni* have been recorded in the Coastal provinces, mountains, and Eastern Ecuador: *E. m. tyrannus* (Draudt, 1930), in Pichincha, Manabí and Cañar, and *E. m. fulvaster* (Rothschild, 1907), distributed in Sucumbíos, Orellana, Napo and Morona-Santiago. None of these have been previously recorded in Guayas.

ACKNOWLEDGEMENTS

We thank Dr. David Wagner from University of Connecticut for providing bibliographic material and for his suggestions on a first draft, Drs L. Kirby Wolfe from Natural History Museum of

Los Angeles and John Heppner from University of Florida for collaborating in the confirmation of the species, Drs Lyle Buss and Jorge Peña from University of Florida for being interested in this account. Thanks also to the farmers from “La Garganta” (Milagro, Guayas) for allowing specimen collections, and Engs. Pablo Chong, Lisbeth Espinoza and Eduardo Chávez from CIBE for their cooperation in field sampling and survey.

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Received for publication 23 August 2010; revised and accepted 15 August 2011.

FEMALE ABDOMEN POSITION SIGNALS RECEPTIVITY DURING COURTSHIP IN THE JAPANESE SULFUR BUTTERFLY, *COLIAS ERATE* (PIERIDAE)

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Abstract. Females of the Japanese sulfur butterfly, *Colias erate*, eclose in the grassland vegetation in early morning and copulate immediately after their wings fully expand. Once mated, females lose their interest in mating with males that approach them. Receptive females exposed their abdomens toward courting males, whereas unreceptive females hide their abdomen. Field observation of males' courtship behavior to females was carried out by focusing on the abdomen posture of females. We glued the wings of females together, forced their abdomen to be either exposed from their wings or hidden in their wings, and presented them to males. When males found the females, most males hovered around the female. Males alighted near the females when their abdomens were exposed. However, when their abdomens were hidden, males stopped courting and left the females. Females with exposed abdomens, irrespective of mating experience, copulated with the male courting. Thus, the female posture of hiding her abdomen was a signal of unreceptivity, as well as the rejection of the courting males.

Additional key words: Exposed abdomen, mate refusal, mating experience, receptivity

Although sexual communication in butterflies involves the use of multiple signals at different phases of mate recognition and choice, constituting a complex scenario (Klein & Araujo 2010), initial attraction usually takes place by visual channels (Scott 1972). The information contained in the male visual signals alters or controls the female's behavior, and vice versa. Alfalfa fields in the USA and Canada are a favorable habitat for sulfur butterflies, *Colias* spp., providing abundant food for the larvae and adults (Hovanitz 1944), showing mating behavior within the field in which they emerged (Stern & Smith 1960). In Japan, there are few single alfalfa fields, while a lot of ski slopes are abundantly covered with clovers such as *Trifolium repens* and *T. pratense* in the cool temperature zone (Watanabe & Nakanishi 1996). In this region, the Japanese sulfur butterfly, *C. erate*, is abundant and can be seen flying and courting in the middle of the day during summer.

Flight is essential to butterflies in looking for mates, food and new habitats. Silberglied & Taylor (1978) clarified that males of *C. eurytheme* and *C. philodice* search for females visually, using UV-reflectance, and that some pheromone is vital to the females. In *C. erate*, Watanabe et al. (1997) showed that males can easily encounter females when they fly around the habitats, probably using visual cues. Irie & Watanabe (2009) also reported that newly-emerged females are, in general, easily found by mate-searching males on the wing in the early morning. Such virgin females simply accept

courting males and then copulate. On the other hand, mated females become unreceptive to male courtship advances (Hasegawa & Watanabe 2008). Consequently, almost every flying female in the daytime seems to be unavailable for searching males. Mated females showed a pierid-specific mate refusal posture at the perching site (wings-spread, abdomen nearly vertically upturned) or do a so-called ascending flight followed by the courting males (Hasegawa & Watanabe 2008). However, the dissection of females captured in the fields indicates that females mate a couple of times during their life span (Nakanishi et al. 1996), indicating that mated females do not always refuse males. Therefore, males have a chance to mate with experienced females as well as with virgin females.

In the daytime, males actively patrol on the wing, searching for females in grasslands (Watanabe & Imoto 2003). When they find a female perching or flying, they visit the female to start courtship behavior. Males hover around the perching female, or near the flying female inhibiting her flight course. When the female accepts the courtship of the male, she alights on the vegetation, remains motionless on the perch site, and extends the abdomen toward the male from her hindwings for copulation, as reported in other *Colias* butterflies (Rutowski 1980). The male follows her, alights next to the female, and bends his abdomen to the tip of the female's abdomen for copulation. However, when the female shows mate refusal behavior due to a recent copulation,

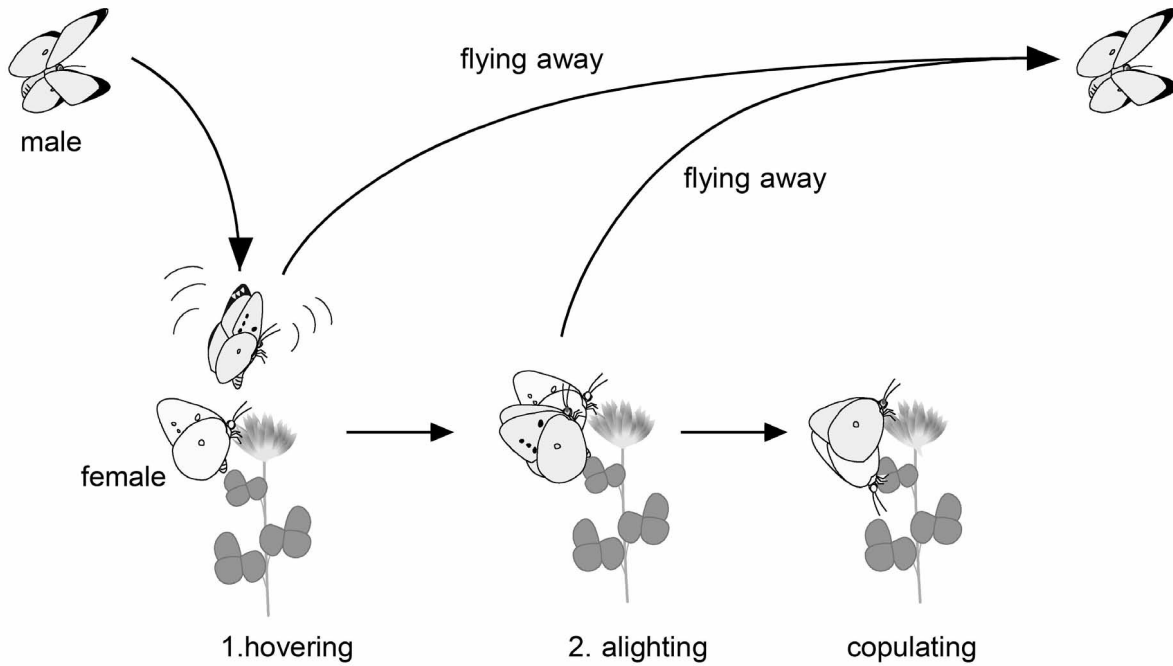


FIG. 1. A schematic representation of the courtship behavior of the *Colias erate* male to the presented female (c.f., Hasegawa & Watanabe 2008). **1.** A flying male visits the presented female and starts to hover in front of the female. **2.** The male alights on the edge of female's wings, walks down, and bends the abdomen towards the female, then the male and the female copulate. In each stage, when the males abandon copulation, they fly away from the female.

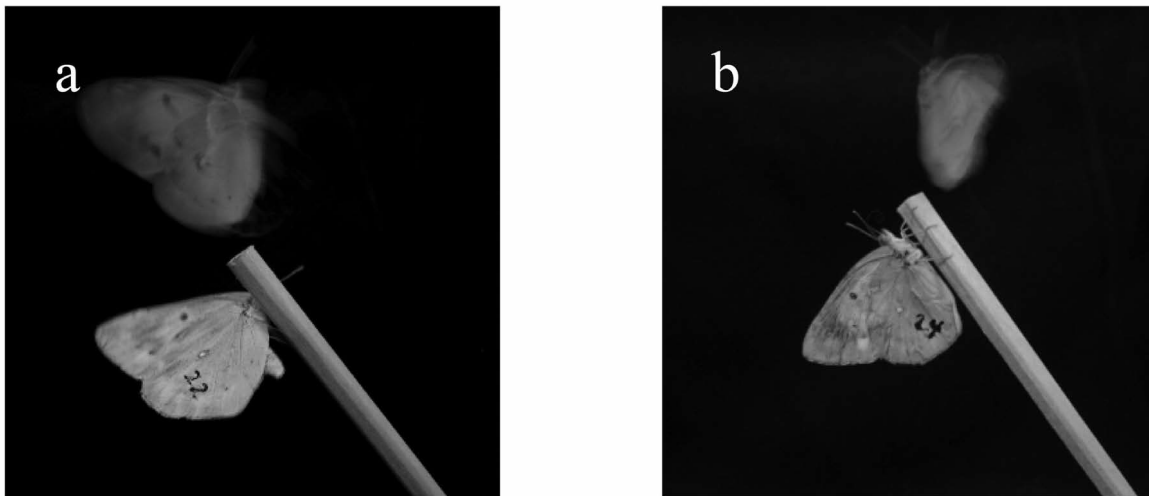


FIG. 2. The female response to the fluttering male. **a.** the virgin female showed the exposed abdomen to the male. **b.** the unreceptive female did not expose the abdomen.

males stop their courtship behavior and fly away from her (Hasegawa & Watanabe 2008). In order to identify the counter behavioral signal of the mated females against the male courtship behavior, inhibition of such behaviors was useful in the field experiment (Irie & Watanabe 2009). In the present study, in order to understand the adaptive significance of female refusal, we quantitatively investigated the courtship behavior of males to females whose abdominal movements were manipulated.

MATERIALS AND METHODS

Female response to male courtship. The abdominal responses of females to courtship behavior of males were investigated using virgin and non-virgin females in August 2008. Virgin females were the offspring of wild *C. erate* females captured in July 2008 in Tsukuba, Ibaraki Prefecture, Japan (36°7'N, 140°30'E, alt. 30 m). Immediately after emergence, they were kept in a refrigerator (10°C) until the experiment. Non-virgin females, young ones judging by the degree of wing wear (Watanabe & Nakanishi 1996) to exclude any age effect on the male behavior, were captured in the fields, Tsukuba, Ibaraki Prefecture in June 2008. In addition, we used young laboratory-reared females that had mated on the day prior to the experiment. They were also kept in a refrigerator (10°C) until the experiment. After the experiment, each female was dissected to confirm the number of spermatophores in the bursa copulatrix, because the number of spermatophores indicates the number of copulations in the females (Nakanishi et al. 1996).

Young males were the offspring of wild females captured in Tsukuba, in June and July 2008. They were tethered with a fine black cotton thread tied to the neck. A thread of 20 cm did not seem to inhibit courtship behavior of the male because they fluttered freely within the reach of the thread. Forewings of each female were glued together in order to inhibit mate refusal posture and flying away. Then, the females were put on the tip of a 10-cm wooden stick to perch and were presented to tethered males fluttering in the laboratory. The distance between male and female was about 5 cm. Each trial lasted at least 30 seconds, which is longer than the duration of hovering in wild males (Irie & Watanabe 2009). Abdominal responses of the females were observed.

Male courtship behavior in relation to abdominal posture of females. Courtship behavior of males to perching females with different abdominal postures was observed for the summer generations in the grasslands of ski slopes in Sugadaira, Nagano Prefecture (36°31'N, 138°20'E, alt. 1320 m) in late July 2008. The grasslands were flat, and were covered with dense *T. repens* and *T.*

pratense, which are the host and nectar plants for the larvae and adults of *C. erate*, respectively. In the middle of the day, most males flew above the grass searching for a mate while females were resting, nectaring, and being courted.

On the day before the experiment, resting or nectaring young females were captured with a net in the grassland. Virgin females reared in the laboratory were also used for the experiment. Both forewings and hindwings of each female were glued together to become a closed wing posture, and their abdomen was forced to be either exposed, out from their wings, or hidden in their wings. The females were not able to move their abdomen. Thus, four groups of females were prepared (virgin/non-virgin, and abdomen hidden/ exposed). From 1200 to 1500 on sunny days, females were placed on a *T. pratense* flower in the grassland. When males visited the perching female their courtship behavior was observed.

The courtship behavior of males was divided into hovering and alighting (Hasegawa & Watanabe 2008). In the present study, as shown in Figure 1, when males noticed and visited the female perching, they began to hover around her. Next, the male alighted on the edge of the female's wings, walked down and bent his abdomen toward the female in order to copulate. The duration of hovering and alighting was measured using a stopwatch (accuracy 0.01 s) and a voice recorder. Each trial was carried out until a total of 10 males alighted; by that time, the scales of the female were detached by the males. Females that experienced fewer than 10 instances of male alightings were withdrawn 90 minutes after the start of the experiment. This was because of an increase in their body temperature due to direct sunlight and non-feeding. After the experiment, each female was dissected to confirm the number of spermatophores in the bursa copulatrix.

RESULTS

Female response to male courtship. Each female whose forewings were fixed remained motionless on the tip of a stick soon after being placed there. At this time, their abdomen was hidden in their hindwings. Immediately after the male appeared with their wings fluttering, the virgin female pushed her abdomen out of the hindwings toward the male (Fig. 2a). In contrast, when the male stopped fluttering, the female put her abdomen back into her closed wings. When the male resumed the fluttering, the female again exposed her abdomen toward the male from the hindwings. Table 1 shows that all virgin females exposed their abdomen in response to male fluttering. On the other hand, mated females that were reared in the laboratory did not expose the abdomen when a fluttering male was presented (Fig.

TABLE 1. The number of females that extended or hid their abdomen when presented with fluttering males.

Mating experience of the females	Extended Abdomen	Hid Abdomen	Total
Virgin	11	0	11
Mated (laboratory reared)	0	4	4
Mated (captured in the field)	0	7	7

2b). They remained perched in place without showing any behavior to the fluttering males. Mated females captured in the fields also perched and did not display their abdomen.

Male courtship behavior in relation to abdominal posture of females. When females were placed on flowers in the grassland, searching males visited them. Out of 13 visits to virgin females whose abdomen was hidden in hind wings, 10 males stopped hovering and left the female, while 3 males attempted copulation. When the female's abdomen was exposed, more of the visiting males attempted copulation (16 out of 22 males). Before leaving, the males hovered for 2.8 ± 0.4 s (\pm SE, $n=10$) for virgin females with their abdomen hidden, and for 1.7 ± 0.3 s (\pm SE, $n=7$) for virgin females with their abdomen exposed.

When mated females were presented the same results were obtained. Out of 18 visiting males, 14 stopped hovering after 1.9 ± 0.2 s (\pm SE, $n=10$) when the female's abdomen was hidden. Out of 35 male visits to females with exposed abdomens, 19 males attempted copulation. The duration of hovering was 7.8 ± 2.7 s (\pm SE, $n=11$). Hovering duration was different between the groups, though the statistical test indicated only marginal significance (log-transformation to normalize the data distribution, abdomen hidden: $Z=-1.784$, $p=0.07$; abdomen exposed: $Z=-1.859$, $p=0.06$). When the female's abdomen was hidden in the wings, about one fourth of the males that visited the females alighted on them after hovering, irrespective of the female's mating experience. Alternately, when males hovered around a female whose abdomen was exposed, more than half of them alighted on the females. Thus, the proportion of males that alighted was not affected by the female mating experience, but by the female abdomen posture (Log-Linear Model, female mating history: $Z=-0.911$, $p=0.15$; female posture: $Z=1.428$, $p<0.01$; interaction: $Z=-0.911$, $p=0.36$).

It was physically impossible for males to copulate with females whose abdomen was hidden. Males that alighted on a virgin female with the abdomen hidden continued to attempt copulation for 19.0 ± 11.4 s (\pm SE, $n=3$), which

was not significantly different from the time spent by males that alighted on a female with the abdomen exposed (8.3 ± 2.4 s, \pm SE, $n=9$) ($U=10$, n.s.). After that time, they gave up attempting to copulate and flew away from the female. Attempts to copulate with non-virgin females with the abdomen hidden lasted for 21.1 ± 10.8 s (\pm SE, $n=3$), which was not significantly different from the time spent by males that alighted on virgin females with the abdomen hidden (19.0 ± 11.4 s, \pm SE, $n=3$) ($U=3$, n.s.). The males flew away after copulation ended.

Some females with the abdomen exposed mated with the males. Out of 8 virgin and 12 non-virgin females with their abdomen exposed, 5 and 5 females copulated, respectively. The proportion of remated females in the non-virgin females was not significantly different from that in the virgin females ($\chi^2=0.29$, n.s.).

DISCUSSION

In the Lepidoptera, there are many reports on the sequence of nuptial communication between males and females before copulation, including the female's response to the male's courtship behavior (e.g. Scott 1972; Rutowski 1984; Wiklund 2003). The female posture of exposing her abdomen towards the male during the courtship behavior has often been reported as a signal of female receptivity in butterflies such as *Precis coenia* (Scott 1975), *Leptidea sinapis* (Wiklund 1977), *Eurema lisa* (Rutowski 1978), *C. eurytheme* and *C. philodice* (Grula et al. 1980). In the present study, receptive virgin females of *C. erate* also exposed their abdomen for males approaching them.

In *C. erate*, females show ascending flight or mate refusal posture when they do not want to copulate (Nakanishi et al. 1996). Interference due to males courtship attempts drives ovipositing females off of the flight areas (e.g. Shapiro 1970). In addition, because copulation of *C. erate* lasts for about an hour, females suffer from restricted oviposition time (Watanabe et al. 1997). Thus, females gain benefits by avoiding harassment by males and excessive matings. Mate refusal posture or ascending flight in females often leads to interruption of the male courtship behavior (Hasegawa & Watanabe 2008), functioning as signals of unreceptivity. However, even when the female shows the mate refusal posture, some males continue courtship behavior, achieving copulation. Female abdominal position must play a role in altering or controlling the male's behavior.

In the present study, ascending flight and mate refusal posture in females were artificially restricted by fixing their wings, such that males could alight near the female and attempt to copulate. The proportion of the non-virgin females that copulated is clearly higher than the proportion of females in the field, compared with the

mating frequency of young females reported by Nakanishi et al. (1996). Thus, the abdomen extension of females is vital behavior for copulation. Alternatively, females can refuse copulation by hiding their abdomen, suggesting that females can choose males. While wing fluttering at the perching site functions as refusal of males courting in *Lycaena phlaeas* (Watanabe & Nishimura 2001), the posture of hiding the abdomen is the male avoidance behavior in *C. erate*. It is physically impossible for the male to put his genitalia onto the tip of female's genitalia in the hindwings for copulation. Suzuki (1981) reported that unreceptive *Pieris rapae* females can avoid copulation by lifting their abdomen into their wings.

Watanabe et al. (1997) suggested that females could identify the age of males copulating, and that females may choose males based on the sex pheromones and scales on the wings of males. Males can gauge the receptivity of a female by abdominal posture, and they will not attempt to copulate while females hide their abdomen. Although polyandrous *Colias* females accept remating depending on the intensity of the male's courtship (Rutowski 1985), prolonged courtship behavior costs males by reduced searching time. Thus, interrupting courtship behavior to an unreceptive female is adaptive for males. Courtship proceeded along the behavioral sequence in which male and female recognize each other as a suitable mate. Successful copulation occurs only after the typical courtship behavior is achieved (Silberglied & Taylor 1978). More specifically, male and female behaviors in *C. erate* mutually release the next behavior. In order to evaluate female receptivity during courtship behavior, *C. erate* males exploit the positive female behavior. The present study clarified that the abdominal extension towards the male is an indication of female receptivity, and that it may be a releaser of male alighting. Birch et al. (1989) demonstrated that when the females move off against the male courtship behavior in *Mamestra brassicae*, the males fly away from the female without any attempt for copulation. Therefore, these studies suggest that butterfly females readily repel male's attempts so that antagonistic coevolution between males and females does not escalate in butterflies.

ACKNOWLEDGEMENTS

Thanks are due to Brett Setmoore, Arizona State University, who greatly improved the original text with his comments.

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Received for publication 16 April 2010; revised and accepted 15 August 2010.

LOCAL POLYPHAGY IN *THEOPE LYCAENINA* BATES, 1868 (RIODINIDAE: NYMPHIDIINI)

Additional key words: *Azteca*, immature stages, host-plants, myrmecophily, *Theopeina*

The genus *Theope* Doubleday, 1847 comprises 77 species occurring from central Mexico to Northern Argentina (Hall 1999, 2008; Jauffret & Jauffret 2009). Life histories have been described for only about of 12% of the species in the genus (Hall 1999, 2002; Kaminski 2006). All known larvae are myrmecophilous, and nearly always associated with ants of the genus *Azteca* Forel (Dolichoderinae) (DeVries et al. 1994; DeVries 1997; Hall 1999; but see Kaminski 2006 for *Theope thestias* Hewitson, 1860 being facultatively tended by several Formicinae and Myrmicinae). Reported larval host plants for the genus *Theope* include thirteen plant families (DeVries et al. 1994; Hall 1999; Kaminski 2006; Beccaloni et al. 2008; Janzen & Hallwachs 2010).

The present paper reports additional food plant records for a population of *Theope lycaenina* Bates, 1868 (Fig. 1A) and larval feeding behavior in a coastal locality in Southeastern Brazil. Observations were carried out in the region of the Praia da Fazenda, in the Nucleo Picinguaba of the Serra do Mar State Park, in Ubatuba municipality, Sao Paulo State (23°21'S, 44°50'W), from 2004 to 2007 (always in January), and July 2010. Most observed larvae were brought to the laboratory and reared to adults for species identification. Ant vouchers are deposited in the following Brazilian museums: Museu de Zoologia da USP, Sao Paulo, and Museu de Zoologia da Unicamp, Campinas. Adult vouchers of the butterfly are deposited in the Museu de Zoologia da Unicamp, Campinas.

In January 2004, two larvae were observed feeding on an unidentified Sapindaceae vine, tended by workers of *Azteca chartifex* Forel, 1896 (Fig. 1B), which lived in a large carton nest on a tall nearby tree. In 2005 to 2007, eight additional larvae were observed, always using the same host plant species, and likewise tended by *A. chartifex* ants. In July 2010, an intensive 10 days search was done on three additional plants (distant more than 10 m from each other) that each harbored a colony of *A. chartifex*, which were easily found by the presence of carton nests and by the large number of ant workers patrolling the whole plants. Each of these plants also harbored larvae of *T. lycaenina*, including *Andira fraxinifolia* Benth. (Fabaceae) (on which three third instars were found), *Morus nigra* L. (Moraceae) (one second instar) and *Malvaviscus arboreus* Cav.

(Malvaceae) (two first instar and one third instar). The last two plant families are also new plant family records for *Theope*, and these new records increase to four the number of families used by this species in the study area, and to eight the number of plant families used by *T. lycaenina* over its range (Beccaloni et al. 2008; L. A. Kaminski pers. comm.). In all cases the larvae, including the small first instars, always were observed being tended by at least two *Azteca* workers (Fig. 1B). No larvae were ever found on neighboring host-plants of the same species that had no ants present. In *A. fraxinifolia*, two third instars were observed on the surface of dead twigs consuming the superficial tissues.

Results strongly suggest that larvae of *T. lycaenina* are highly dependent on *Azteca* ants to develop and grow, confirming the pattern observed for most known species in the genus *Theope*. In addition, by using hosts belonging to four different plant families in a single place, *T. lycaenina* can be considered locally polyphagous. Since the presence of larvae is apparently highly correlated with the presence of *Azteca* ants, it is

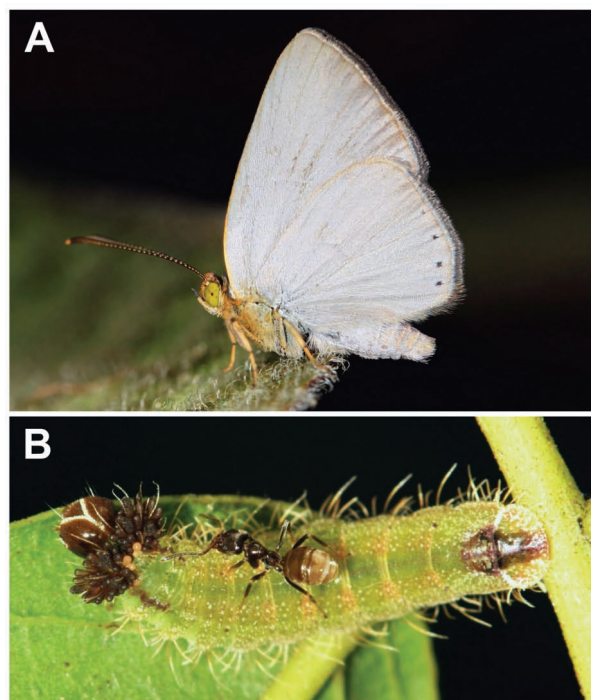


FIG. 1. **A**, Adult male *Theope lycaenina*. **B**, Last instar larva of *Theope lycaenina* tended by one worker of *Azteca chartifex* (photos by Lucas Kaminski).

possible that polyphagy in *T. lycaenina* is mediated by the presence of particular ant taxa, similar to what has been proposed for other myrmecophilous riodinids (DeVries et al. 1994; DeVries 1997; Hall & Harvey 2001; Kaminski 2008). Also, the behavior of feeding on dead plant tissues could be an additional factor that promotes polyphagy in this species, because they would be less selective by specific secondary substances of their host plants. These observations reinforce the idea that ant presence can be a major factor influencing host plant choice in species with obligate symbiotic associations (e.g., DeVries et al. 2004; Kaminski 2008).

ACKNOWLEDGEMENTS

Lucas A. Kaminski, Curtis Callaghan, Luisa L. Mota, Carla Penz, Keith Willmott and Niklas Wahlberg made useful comments on the manuscript. Rogério R. Silva identified the *Azteca* species and Curtis Callaghan identified the *Theope* species. This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 04/05269-9), CNPq (300282/2008-7), and the National Science Foundation (DEB- 0527441).

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Received for publication 2 October 2010; revised and accepted: 1 June 2011

A SIMPLE TECHNIQUE TO SAMPLE POLLEN FROM MOTHS AND ITS APPLICATIONS
TO ECOLOGICAL STUDIES**Additional key words:** palynology, natural history, ecology

Pollen is a physical link between many plant-animal interactions and can be a useful tool for biologists in ecological studies. Pollen found on field collected moths can be sampled and identified. Location of pollen, number of pollen grains, and pollen identification can be recorded for individual specimens. These data can be applied to explore a number of life history phenomena such as nectar plant use and pollination ecology. Further, with the option of using museum specimens, researchers can explore ecological questions from a larger geographic and temporal scale otherwise not easily obtained in a limited field season.

Moths and their interactions with their nectar plants are diverse yet notoriously difficult for researchers to investigate. Apart from larger hovering moths (e.g. Sphingidae), moths that visit flowers at night are often small and cryptic. They are known to seek nectar from small white or pale colored flowers often situated at the tips of branches (Oliviera et. al. 2004; Makholela & Manning 2006; Okamoto et. al. 2008; Tasen et. al. 2009). Still, moths nectaring on plants is a phenomenon rarely observed and documenting plant-moth interactions in the field is a formidable task. To complement such studies, investigating pollen found on moth specimens can be applied to further document nectar plants and quantify pollination efficiency.

Researchers have dabbled into exploring these methods to investigate life history questions relating to Lepidoptera pollination ecology (Darwin 1885; Wiklund et. al. 1979; Courtney et. al. 1982; Jennerston 1984; Tasen et. al. 2009), migration (Mikkola 1971; Lingren et. al. 1993), and even overwintering (Berkhouson & Shapiro 1994). Many of these studies used similar techniques to identify pollen with Scanning Electron Microscopy (SEM) and often removed the head of the specimen in order to extract the pollen. The techniques described here offer a less expensive and invasive alternative to using SEM to sample and identify pollen from field collected fresh or museum specimens.

Materials. Stereo microscope (7–15x magnification), hot plate, probe with fine point (see below), glycerin gel stained with pigment (see below), glass microscope slide with cover slips, small weights (~40 grams), slide warmer, reflective microscope with 10–60x objectives,

camera attachment and imaging software (AxioVision Rel. 4.5), pollen keys and regional identification manuals, and/or plant samples from the field.

Probe. Wooden dowel 14 cm long and 0.2 cm in diameter, with an embedded tip of a # 2 insect pin.

Glycerin gel with stain. Add 20 g of gelatin (Crescent Chemical Company, Cat. # 23310.02) to 70 ml boiling distilled water, once thoroughly mixed, add 60 ml glycerin (Fisher Scientific, Cat. # 633-4) and 1.2 g phenol (Fisher Scientific, Cat. # G33-4), then after crystals dissolve, add 22 drops of Safranin-O stain (Fisher Scientific, Cat. # S670-25).

Collecting and Preserving Specimens. Field collected specimens should be kept in separate glass vials to prevent pollen contamination. Store moths in vials in a freezer until use in pollen analysis. This will preserve the specimen and help reduce loss of scales or pollen.

Scanning for pollen. To scan a moth for pollen, remove from the vial and pin the specimen through the thorax with an appropriately sized insect pin. Using a small piece of foam to stabilize the pinned specimen, scan the specimen for pollen under a stereomicroscope, paying special attention to the mouthparts (Fig. 1-I). If the proboscis is not visible, use a minuten insect pin to gently bend up from the base of the proboscis until fully exposed. If the specimen is not hydrated, you may need to rehydrate the specimen in a relaxing chamber long enough to rehydrate the proboscis.

Extracting pollen from specimen. Heat the glycerin gel on a hot plate at 52°C in a water bath until it has reached liquid form. Place microscope slide(s) on a slide warmer. Locate pollen on the moth using a stereomicroscope. Pour a small portion of the glycerin gel onto the microscope slide. Gently dip the probe tip into the glycerin gel. For pollen clumps, a small drop of glycerin gel works well. For individual pollen grains, a short streamer of glycerin gel works well, especially for grains embedded between the scales. Once the pollen is adhered to the probe, transfer the pollen from the specimen to the microscope slide into the drop of glycerin gel. Cover the drop with a cover slip (Fig. 1-II), and prepare a label for the microscope slide. Allow the slide to rest on the slide warmer long enough to enable the gel to stain the pollen (at least 24 hours). Seal the edge of the coverslip with clear fingernail polish.

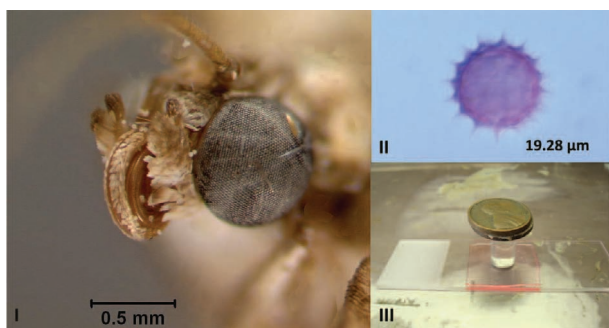


FIG. 1. **I**) Pollen grains found on proboscis and palpi of *Samea ecclesialis* (Pyraloidea). **II**) Photograph of pollen grain, *Eupatorium* sp. (Asteraceae), taken with AxioVision imaging software. **III**) Microscope slide with pollen sample in glycerin gel stain, cover slip, and weight on warming plate.

Identifying pollen: Using a reflective microscope at 40X (objective) and 1.25X (optivar), locate the pollen on the microscope slide and photograph using an attached digital camera and imaging software (Fig. 1-III). Using this image, identify the pollen with either a pollen key (Kapp et al. 2000), pollen identification manual (Jelks 2001), and/or matching pollen with pollen sample extracted from a properly identified plant (i.e. use of pollen collections can be arranged by collection managers at existing palynology institutions).

If the use of a palynology collection is not feasible, it is also possible to create a pollen library by which to match pollen. Pollen can be sampled from flowers observed in bloom within the same time and vicinity that the moths were collected. This is most beneficial when working in a habitat where the majority of plants are not yet represented in a palynology collection. Creating a pollen library with the plants in the area of study is not only beneficial for identifying pollen found on the moths, but can also be preserved in a local palynology library as a resource for future researchers.

ACKNOWLEDGEMENTS

The authors would like to thank David Jarzen for helpful comments on pollen identification and the use of the pollen collection. Jacqueline Y. Miller, Deborah L. Matthews, and Christian Salcedo provided constructive comments on the manuscript.

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Received for publication 14 December 2010; revised and accepted 31 May 2011

A SIMPLE NUMERICAL INDEX FOR ASSESSING THE SPRING MIGRATION OF MONARCH BUTTERFLIES USING DATA FROM JOURNEY NORTH, A CITIZEN-SCIENCE PROGRAM

Additional key words: Monarch butterfly, *Danaus plexippus*, spring migration, Journey North, citizen science

Monarch butterflies, *Danaus plexippus*, in eastern North America are unique among butterflies, not only because they undergo a famous long-distance migration from breeding sites in Canada and the northern United States to the mountains of Central Mexico, but also because they are cherished by the general public to a large degree. As such, their population status is monitored closely each year by scientists, the media, and concerned citizens. Most people are aware that monarchs face many threats; in recent years scientists have documented deforestation of their wintering sites (Brower et al. 2002), chemical exposure in their breeding sites (Oberhauser et al. 2006), and reduced numbers of females throughout the population (Davis & Rendon-Salinas 2010). Thus, even though monarch populations tend to fluctuate over time (Swengel 1995; Walton et al. 2005), members of the public become especially concerned when the number of overwintering monarchs reaches an unusually low point, as occurred in 2002 following a catastrophic winter storm (Brower et al. 2004), or recently when their numbers reached an all-time low (since tracking began in the 1970s) in the winter of 2010 (Rendón-Salinas et al. 2010).

When low winter numbers occur, there tends to be a flurry of articles in the popular press, multiple interviews with scientists, and importantly, a close watch of the spring migration northward to look for signs of population recovery. One way this is done is by viewing online maps created by the Journey North program (www.learner.org/jnorth). This is a citizen-science program whereby school children, naturalists and interested citizens report the first sighting of an adult monarch butterfly in the spring, and these reports are displayed on a map online so that the spring recolonization of the monarch's breeding range can be tracked in real time (Howard & Davis 2004).

When viewing maps of the spring migration on the Journey North website and trying to assess the general health of the migration for any given year, it is tempting to compare the number of reports (i.e. sightings) of monarchs from year to year, and indeed, this is what many people (scientists and non-scientists) do. However, there is a problem with this approach that most overlook, in that the number of participants of this

program has been increasing steadily each year (Figure 1). Thus, the number of sightings may not necessarily reflect the true status or health of any given spring migration, since the number of observers has not remained constant over time. Here, we describe a simple numerical index that is not largely influenced by the number of observers per year, and takes into account both the speed of the migration and its geographical breadth, which are both important components of a successful recolonization. This index can be easily graphed, which should allow for visual comparisons of the health of the spring migration from year to year.

Data Set. We used the spring adult sightings database from the Journey North program, selecting all data from 1997 through 2010. The details of this data set are described elsewhere (Howard & Davis 2004; Davis & Howard 2005). Briefly, school children and interested citizens report the date and location to Journey North when they observe their first adult monarch butterfly each spring throughout N. America. The sightings are verified and compiled by Journey North staff, and added to an online map of North America. The majority of sightings in this program come from the monarch population east of the Rocky Mountains, and for simplicity, we confined the current study to those data from this population (i.e. excluding all sightings west of -100°W). Moreover, we also excluded all sightings from the state of Florida, since those reports are likely of monarchs dispersing from the resident populations there (Howard et al. 2010).

Spring migration index. Since the primary goal of the spring migration for monarchs (and for many other migratory organisms) is to recolonize their breeding range, and to do it as rapidly and efficiently as possible, the two components of the migration that would most reflect its progress would be the timing and the geographic spread. We derived values for both of these components for monarch migration in the following way. First, we selected all Journey North sightings that were reported at 30°N latitude ($\pm 0.5^\circ$) and calculated the average Julian date for these sightings each year (an index of timing). This latitude represents the approximate midpoint of the recolonization journey by the returning adults, which generally progress as far as

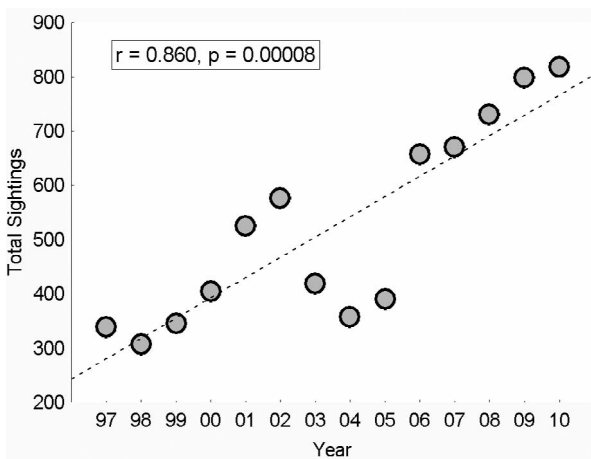


FIG. 1. Number of sightings of adult monarchs in the spring reported to Journey North from 1997–2010.

35°N (Cockrell et al. 1993); the offspring of this generation then continue the northward spread (Howard & Davis 2004). Then, we selected all sightings reported up to the end of April, and counted the number of US states occupied by this time each year (an index of geographic breadth). These two scores are negatively related, so that in years when the migration reached 30°N sooner, there were more states occupied by the end of April, and vice versa (Figure 2). Finally, we divided the number of states occupied (the values on the y axis) by the index of timing (the values on the x axis) to derive a single (unitless) numerical score that describes each year's migration. In this score, higher numbers reflect years when the initial migration wave proceeded rapidly and covered more states, and low scores the opposite.

The spring migration index calculated above can be easily graphed for visual comparisons between years (Figure 3). In doing so, one can see that the score for the spring of 2010 was low, as might be expected since the overwintering population was extremely low prior to this migration (Rendón-Salinas et al. 2010). In other years, such as in 2000, the migration arrived at 30°N latitude early and was spread over a large area by the end of April, leading to a high index (0.39). We point out though that this index, as we have calculated it, represents the progress of only the initial phase of the spring recolonization, or that of the returning adult generation. While this generation is arguably the most important for setting the stage for the breeding season to come, one could also argue that the true success of the migration might be better indexed at a later stage, when the migration/recolonization is winding down. However, at these later stages (i.e. in June and July), it

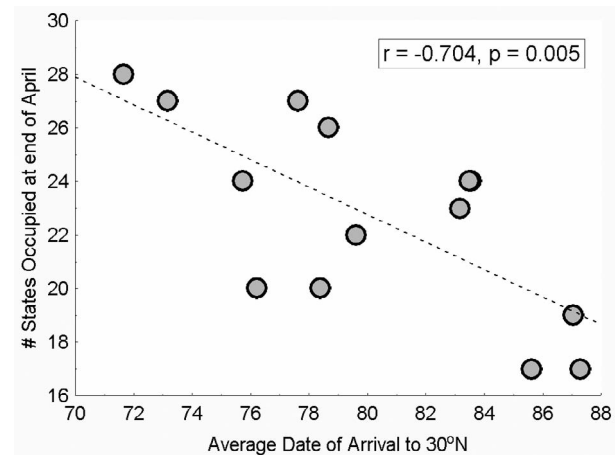


FIG. 2. Relationship between monarch arrival to 30 degrees north latitude and number of states occupied by monarchs at the end of April, for all years (14).

becomes increasingly difficult to tease apart actual migration from the commencement of breeding at any given site, and to know for certain which generation is being observed from the citizen-science reports. That is why we chose to focus on the reports from March and April, which are unquestionably of migratory adults returning from Mexico.

We reiterate that this spring migration index does not necessarily reflect the size of the returning generation, merely the spatial spread of the migration wave. One could argue that with larger numbers of monarchs in any given year, the spatial breadth occupied may in fact be greater, although this would likely be confounded by the varying numbers of observers in the data set (Fig. 1). Thus, it may be nearly impossible to index the 'size' of the returning generation with these data. We argue that this numerical migration score represents a useful

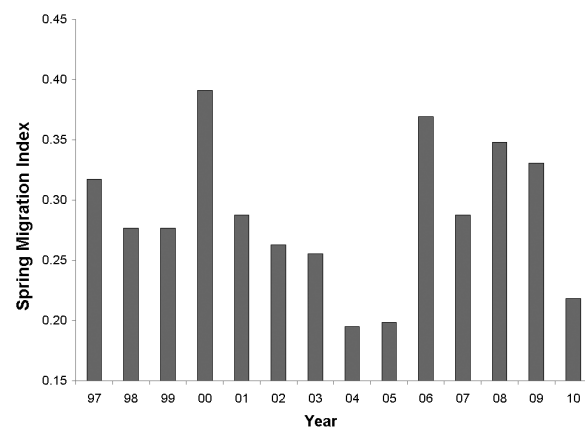


FIG. 3. Annual spring migration indices from 1997–2010. See text for description of index calculation.

alternative approach for monitoring the progress of the spring migration.

From a research standpoint, we hope that this simple scoring system will be useful to those interested in evaluating the progress of the monarch spring migration in terms of timing and breadth. It should allow for a rapid assessment of migration progress following winters with low population size, or perhaps for evaluating the effects of weather on spring migration progress. This index also will be helpful for tracking long-term trends in migration success. Given what many believe to be the precarious status of monarch populations in North America, any improvement in our ability to document and interpret how variations in the spring migration may reflect or impact population numbers should be welcome.

ACKNOWLEDGEMENTS

We thank the thousands of Journey North participants who submit their monarch sightings each year, and who made this study possible. We also thank Sonia Altizer, and the steering committee of MonarchNet for helpful discussions about monarch biology, and for providing the stimulus for writing this paper.

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Received for publication 2 October 2010; revised and accepted 4 March 2011

DROPPING FROM HOST PLANTS IN RESPONSE TO PREDATORS
BY A POLYPHAGOUS CATERPILLAR**Additional key words:** defensive behavior, Lepidoptera, anti-predator defense, *Orgyia leucostigma*, predation risk

Induced behavioral responses to predation risk have been viewed as a consequence of a decision-making process juxtaposing the benefits of escaping predation and the costs of this anti-predator defensive response (Lima & Dill 1990). A large number of studies have shown that caterpillars can reduce mortality from predation by a diverse array of induced behaviors, including hitting (Awan 1985), biting (Soares et al. 2009), regurgitating (Gentry & Dyer 2002), performing defensive displays (Evans 1986) such as aposematic sounds (Brown et al. 2007), thrashing (Iwao & Wellington 1970), ceasing movement (Castellanos et al. 2010), hiding (Rota & Wagner 2008), crawling away (Stamp & Bowers 1988), changing feeding schedules (Stamp & Bowers 1993), dropping on silk threads (Castellanos & Barbosa 2006), and dropping off the plant (Castellanos et al. 2011). However, studies addressing the potential costs of these induced anti-predator behaviors are relatively rare (but see Stamp & Bowers 1988, 1991).

Among the potential behavioral responses of prey, dropping provides an effective adaptation in that it eliminates, or reduces the risk of predation, although it can expose caterpillars to starvation and predation on the ground (Cain et al. 1985; Weseloh 1988; Mathews et al. 2004). Any assessment of ecological costs and benefits of dropping would require a complete understanding of larval post-dropping behavior. One important component of the determination of costs and benefits is the behavior involved in returning to a host plant. After dropping, individuals may land on host foliage underneath the leaf from which they drop or they may fall to the ground where they can either move randomly until a host plant is found or efficiently locate hosts, reducing the likelihood of on-ground mortality (Bierzychudek et al. 2009). Thus, in this study we conducted a series of experiments in order to determine whether *Orgyia leucostigma* (J. E. Smith) (Lymantriidae) caterpillars that drop in response to predatory wasps are able to grasp a leaf or branch underneath the leaf from which they drop, and whether larvae that fall to the ground exhibit orientation behavior that is likely to return them to a tree host.

Orgyia leucostigma caterpillars are polyphagous and external solitary feeders primarily on the foliage of

deciduous trees (Raffa & Powell 2004). Larvae used in the experiments originated from a laboratory colony established using larvae collected from *Acer negundo* L. (Aceraceae) (box elder) trees in Patuxent Research Refuge (PRR), Laurel, Maryland, USA. Larvae eclosing from egg masses were reared individually in 437-ml plastic containers, and fed box elder foliage. *Polistes fuscatus* (Fabricius) (Vespidae) is a major predator of caterpillars, which they capture and process to feed their larvae (Gould & Jeanne 1984), and has been observed attacking and consuming larvae of *O. leucostigma* in the PRR (J. Kemper, Dept. of Entomology, University of Maryland; pers. com.). Wasps used in this experiment belonged to a laboratory colony started with individuals collected from the same PRR site.

Predation experiments with wasps. Individual third instar *O. leucostigma* caterpillars were placed on a central apical leaf of potted 2.5 m box elder trees and exposed to a single *P. fuscatus* wasp, which flew from its nest to the experimental trees. Before a wasp was allowed to forage on the experimental tree, the caterpillar was allowed to acclimate for one hour; during this period, caterpillars often selected a different leaf from the one it was placed on. For each encounter, the behavioral responses of surviving caterpillars were recorded; if caterpillars dropped, we also recorded whether they landed on a leaf or branch underneath the leaf from which they dropped, or on the ground. A total of 30 trials were conducted in 2.8 m x 1.5 m x 1.5 m plexiglas cages inside a greenhouse using six box elder trees and different predator and prey individuals.

Orientation behavior of caterpillars. Since several species of caterpillars are known to orient to vertical objects such as tree trunks (Lance & Barbosa 1982; Harris et al. 1995; Rieske & Townsend 2005), we conducted an experiment to study the orientation behavior of third instar *O. leucostigma* to vertical silhouettes of various widths (representing "tree trunks" of various sizes). From this experiment we determined the narrowest tree silhouette that the caterpillars were able to discern. We also determined if caterpillars that drop and fall on the ground in the field exhibit orientation behavior that is likely to return them to a host tree.

TABLE 1. Orientation of *Orgyia leucostigma* caterpillars to black bands of various widths. Band = caterpillars climbing the band. Side with band = caterpillars climbing the half side of the arena with the band. Opposite side = caterpillars climbing the half side of the arena on the opposite side of the band (band-less side). The numbers between parentheses represent percentages.

	Width of band (cm)			
	15	7.5	3.5	1.75
Band	17 (60.7)	15 (68.2)	12 (46.1)	9 (27.3)
Side with band	8 (28.6)	6 (27.3)	14 (53.8)	15 (45.4)
Opposite side	3 (10.7)	1 (4.5)	0 (0)	9 (27.3)

Cylindrical arenas were constructed with white cardboard sheets measuring 70 cm in height and forming a ring 90 cm in diameter. A single silhouette consisting of a black paper rectangular band of 15, 7.5, 3.5, or 1.75 cm wide and 70 cm high (representing the silhouettes of trees) was attached to the interior wall of the arenas. The black bands were rotated to eliminate any position effects. Different individual third instars were stimulated to drop from a box elder leaf by bending their hairs to simulate a predator attack (Castellanos et al. 2011), and the path of the larva was observed until it reached the edge of the arena and climbed up its side. The orientation of a caterpillar was recorded as either climbing the band of black paper, the half side of the arena with bands (but not the band itself), or the opposite half (i.e., the band-less) side. If a caterpillar did not reach the edge of the arena within a period of one hour, it was not included in the analysis. Sample size for the 15, 7.5, 3.5, and 1.75 cm wide bands was 28, 22, 26, and 33, respectively. The orientation behavior to the different rectangular silhouettes was analyzed with Chi-square tests for independence (SAS Institute 1990).

Field experiment. At the PRR field site, trees within two 10 m x 10 m quadrats were identified to species. Within the quadrats, six *A. negundo* trees with trunk diameters between 25.5 and 58.6 cm at a height of 70 cm from the ground were chosen as probable hosts from which a caterpillar could fall on the ground. Twelve caterpillars were stimulated to drop from a randomly selected box elder leaf from the canopy of the selected trees. Caterpillar movement after dropping was visually observed and the time it took larvae to reach the trunk of a tree was measured.

All caterpillars that escaped predation from wasps did so by dropping; twelve (71%) landed on the ground and five (29%), on a branch underneath the leaf from which they dropped. All dropping larvae that had subtending vegetation below were able to avoid landing on the ground. Thus, dropping is a primary response to predatory wasps, but dropping did not always result in caterpillars landing on the ground. Some caterpillars

actively grasped the foliage under the leaf from which they dropped (I. Castellanos personal observation). Landing on plant foliage after escaping predation risk by dropping may reduce the risk of mortality in the leaf litter.

The presence of black bands in experimental arenas had a significant effect on caterpillar orientation ($P < 0.05$), except when the band was 1.75 cm wide ($P = 0.058$) (chi-square test: $\chi^2 = 10.22, 11.46, 17.32,$ and 3.59 for the 15, 7.5, 3.5, and 1.75 cm wide bands, respectively) (Table 1). Thus, the 3.5 cm band was the smallest silhouette that had a significant effect on caterpillar orientation. A band width of 3.5 cm seen from a distance of 45 cm (the radius of the arena) corresponds to a visual acuity of 4.45° . Five percent of the caterpillars did not reach the edge of the arena within one hour and were not included in the analyses.

All twelve caterpillars that landed on the ground under the canopy of box elder trees in the field oriented to and reached the trunk of the same tree from which they were stimulated to drop. It took caterpillars $70.08 \text{ min} \pm 14.31$ (mean \pm SEM), ranging from 9.1 to 145.5 min to reach the tree.

Our results show that *O. leucostigma* caterpillars exhibit orientation behavior that is likely to return them to a host, which should reduce the likelihood of on-ground mortality. However, larvae could spend more than two hours in the leaf litter before reaching a tree, which could represent a cost in terms of exposure to ground predators. Without data on the rate of encounter with predators in the litter it is not possible to affirm that the travel time required to reach a host tree subjects the larva to significant predation, therefore it becomes difficult to determine costs. However, the data provided in this study do indicate that dislodged larvae of *O. leucostigma* that land on the ground are likely to find a host tree. Since all tree individuals mapped in the quadrants, except one (*Poplar* sp.), were host-plants for *O. leucostigma* (I. Castellanos, unpublished), there is a high probability that any tree reached after crawling in the leaf litter will be an adequate food source for a polyphagous species such as *O. leucostigma*. Specialist

herbivorous insect species that drop will be in a disadvantage in stands where there is a mixture of plant species. However, specialist species may possess other mechanisms that may allow reducing the costs associated with dropping in response to predators. For example, larvae of *Macaria aemulataria* (Walker) (Geometridae), which are specialists on *Acer* species in the PRR, dislodge from plant surfaces in response to predators, but do not reach the ground because they suspend themselves from a silk thread (Castellanos & Barbosa, 2006). An exciting area for further studies would be to compare the propensity to drop for other free-feeder caterpillars that vary in host range.

ACKNOWLEDGEMENTS

We acknowledge the able assistance of Holliday Obrecht of the Patuxent Wildlife Research Center for his invaluable support of this research. I. Castellanos thanks the Programa de Mejoramiento del Profesorado (PROMEP), Secretaría de Educación Pública, México, and FOMIX Hidalgo 95828 segunda fase, for their generous support. We acknowledge the helpful suggestions provided by D. G. Bottrel, M. C. Christman, R. F. Denno, B. Scholtens, D. D. Yager, I. Zuria and two anonymous referees to improve the manuscript.

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Received for publication 2 December 2010; revised and accepted 5 September 2011

PROFILE

Journal of the Lepidopterists' Society
65(4), 2011, 273–276

CHARLES F. HARBISON (1904-1989)

PIED PIPER OF SOUTHERN CALIFORNIA JUNIOR NATURALISTS

For more than 30 years, Charles Harbison—‘Harbie’ to his friends, which included everyone who knew him—was the primary spokesman for natural history inquiries in San Diego County, California. Possibly no person was more instrumental in the early development of young biologists on the Pacific Coast, and several of his protégés became lifelong lepidopterists. Although he was not primarily a lepidopterist, he was a member of our Society for 24 years beginning in the 1950s and attended many Pacific Slope Section meetings. He organized and hosted two of these at the San Diego Natural History Museum (SDNHM), in 1955 and 1965. The former was just the second meeting following organization of the Pacific Slope group. It was well attended and featured an immensely successful and memorable field trip to the desert following late summer rains, and it was instrumental in establishing the popularity of the meetings. In 1982, Harbison was the Comstock Award honoree at the Pacific Slope Meeting.

Charles Harbison was born in National City, just south of San Diego, in January 1904, into a pioneer family in San Diego history. His grandfather, John S. Harbison, was one of the earliest successful beekeepers in California, in the Sacramento Valley in the 1850s. In 1869 he moved to San Diego and by 1874 moved to the area that is now called Harbison Canyon, in the foothills east of San Diego, where he developed a large bee range. He eventually operated six apiaries with 2,000–3,000 hives, and according to Wikipedia, within seven years he was the largest producer of honey in the world, shipping carloads to Chicago and other eastern cities. Charles’ father settled in National City in the 1870s, and in 1908 moved the family to the Imperial Valley, where he owned a turkey ranch near Seeley. Harbie recalled that his interest in insects began in his first year at El Centro High School, and he made his first collections there and at the Johnson Ranch near the town of Boulevard in the mountains of eastern San Diego County, where the family spent parts of the summers. His father also operated tourist trips, and young Charles helped run trips into northern Baja California, where he had his first experiences in the region. By 1919, the family returned to the San Diego area, and he graduated from Sweetwater High School

in Chula Vista in 1923. Harbie then took courses at San Diego Normal School (now California State University, San Diego), before moving to Los Angeles, where he spent five years working for the Division of Nature Study of the Los Angeles City Schools. There he continued his formal education, taking night courses at the University of California, Southern Branch (now UCLA).

Harbison entered U. C. Berkeley, in spring 1932 as a junior and studied entomology under Profs. E. O. Essig, S. B. Freeborn, and W. B. Herms and invertebrate zoology under Joseph Grinnell. Economic times were so poor that the summer field course, which was required of entomology major undergraduates from the 1920s until 1970, consisted of an encampment near Vacaville in the Sacramento Valley, where the students studied effects of Buckeye blossoms on honeybees, using pollen grain comparisons. Even so, after retirement Harbie said he often wondered if he ever had a happier time than his first year at Berkeley. He graduated with a B. S. in entomology in May 1933;



he had hoped to continue in the Masters program, but in April word came from the Federal Government that there would be no student grants that year. This, coupled with the recent death of his father, forced him to leave school and return to help support the family in Chula Vista, where he lived for most of the rest of his life. He packed his books and other belongings and *walked* back to Los Angeles due to lack of funds. He left behind a girlfriend, with the intent they would reunite when his financial situation improved, but it did not for many years, and he never married.

Harbie found work with Federal job programs (SERA and WPA), at first as a pick and shovel laborer, and beginning in 1934, in the Entomology section at the SDNHM, which could not afford the \$90 weekly salary that the WPA provided. During the 1930's he maintained the entomology collections with the help of Ian Moore, a college student and later noted coleopterist. More than anything, Harbie desired permanency at the museum, not imagining that it would be 17 years before he would obtain a full time position.

During that era he participated in many field trips. His first opportunity to explore Baja extensively came in 1935, when he and C. M. Brown were financed by a sphingid collector in the eastern U. S. to make an expedition through much of the northern peninsula, and this led to a lifelong fascination with Baja. Other expeditions during the pre-WW II years included several as an assistant to the museum mammalogist, L. M. Huey, to Organ Pipe National Monument and other sites in southwestern Arizona, and to Baja California.

Between 1939 and 1949, Harbison also was employed part time by the San Diego City Schools to teach nature study programs through the museum, and in that role he achieved his most influential success, for his enduring talent lay in encouraging younger students. In 1943, the U. S. Navy took over the Natural History Museum for use as a hospital, but Harbie was not deterred from his junior naturalist programs. He moved his materials, along with part of the entomology collections, to Brooklyn Grammar School, in south San Diego, and maintained a "Children's Museum," where he continued to offer classes. He taught summer courses and classes on Saturdays during the school year. These were highlighted by local field trips, each emphasizing a different subject of natural history. Enhanced by the seacoast to mountains to desert diversity of San Diego County, we stalked marine fossils, birds, spring wild flowers, insects, or intertidal animals (and low tide always seemed to occur at 5:00 A.M.). Students spent half the time off looking for their own special interests, tolerated and converted into

added lessons by Harbie's patience and versatility as a naturalist. Later, in high school and as undergraduates at SDSC, we often visited the museum to identify specimens and to be encouraged in our particular pursuits by Harbie. Whereas city residents could ride the streetcar, which stopped across the street from the museum, John Heppner recalls bicycling in the early 1960s with a beetle collector friend 15 miles from his home in Santee to visit Harbie.

When the museum was reopened in 1949, the junior program was turned over to young instructors who had been high school junior specialists of Harbison's program. Unable to survive on his woefully underpaid, part-time curatorship, Harbie worked night shifts at Solar Aircraft and resumed taking college courses to obtain a teaching credential from SDSC. He taught grammar school in rural Jamul, but never having been able to administer discipline of any kind, resigned in exasperation after one year. Finally, in 1952, he was able to return to the SDNHM as a fully salaried staff member, with three days per week spent in the Entomology Department and two weekend days assisting in the Library and conducting nature walks for the public. Most of the time he worked seven days a week, so he was always available to visitors in the Entomology Department, and scarcely a day went by that he did not spend hours responding to phone inquiries or showing recent collections to visitors, especially kids, answering their questions about everything from moths that came to the porch lights to tarantulas and fossils. Hence, his influence on young naturalists continued until his retirement in 1969.

Trips of the early post WWII era were made in an adventurous atmosphere, mostly in dilapidated 1930's cars, and we all remember digging and pushing them from the sand, for which Harbie never seemed to gain proper respect. Or we waited apprehensively while he walked or rode a bus back to civilization for parts or assistance, or until he trudged back to camp at dusk, burdened with net, trowels, plant presses festooned with palm fronds and bags of succulents, looking like a burro in baggy pants. Vivid in my memory is an incident when I hiked part way back down the precipitous trail into Tajo Canyon because it was getting dark, to find Harbie struggling up the slope with a heavy backpack and plant presses under both arms; he refused to hand over any of his load, but instead instructed me to dig up specimens of an onion growing along the trail. A few years later in similar circumstances he fell and broke a tooth because he was carrying his flashlight in his mouth.

The list of Harbison's protégés who went on to make contributions to biology, as amateurs and professionals,

is remarkable and includes professors, writers, and curators at several California universities, the State University of New York at Stony Brook, the University of North Carolina, as well as the Executive Director of the Entomological Society of America and educators and naturalists in the San Diego area. Persons he assisted to a greater or lesser extent and went on to publish on and/or make significant collections of Lepidoptera include John W. Brown (Ph.D. Entomology, U.C. Berkeley); David K. Faulkner (M.S. Entomology, CSU Long Beach); J. O. Hunt (B.A., SDSC); R.A. Mackie (M.S. Entomology, U. Idaho); Gordon Marsh (B.S. Entomology, U. C. Berkeley); the late M. J. McKenney (M.D., Stanford U.); the late D. M. Peterson (M.S. Entomology, U. C. Riverside); J. A. Powell (Ph.D. Entomology, U.C. Berkeley); Paul A. Rude (M.S. Entomology, U.C. Berkeley), Oakley Shields (Ph.D. Entomology, U.C. Davis), Paul Spade, and Ray Stanford (M.D. UCLA). Many other Harbison students later held professional positions as biologists or taught in high schools.

Harbison's own research interests were in the insects, especially Odonata, and plants of Baja California. He made many trips during 1949 to 1969, in an effort to visit the type localities of dragonflies and plants, especially succulents and cacti, with which Harbie had expert familiarity. He traveled the length of the peninsula in 1952 with Museum botanist Ethel Higgins (who was in her 80's) and again in 1967 with Darley Howe and Gordon Marsh. He was particularly fascinated by Cañon del Cantil (Cantillas and its north fork, Tajo Cañon) in the Sierra de Juarez, which he visited many times, leading expeditions of naturalist hikers. On the last of these, in 1973, he had to be virtually carried out of the canyon after he fell and injured his head. The nucleus of insects collected by Harbie in Baja stimulated generations of entomologists to focus efforts on that fauna.

Harbison made 27 extended research trips between 1952 and 1968, including five visits to Guadalupe Island, Mexico, and the 1958 Scripps Institution of Oceanography expedition to Barro Colorado Island, Panama, and Clipperton Island, Mexico. The latter is a harsh and desolate atoll 900 miles south of Baja California, which had 30 species of weedy plants (Sachet 1962). Harbie found 13 orders of insects as well as spiders and other arthropods and was quoted as calling it "an apex of my career." He was a member of the 1962 Belvedere Expedition to the Gulf of California, which visited 32 islands, where he collected more than 10,000 arthropod specimens (Lindsay 1962).

Harbison's published productivity was limited but began with an enchanting description of the vegetation

in one of the steep, north slope ravines of Santa Cruz Island in 1927, and included at least two articles on type localities and rediscovery of plant species and descriptions of two new Agave-feeding megathymid skippers from Baja California. The versatility of his interests is reflected in patronyms proposed in his honor. They include at least two plants, *Verbena Harbisonii* Moldenke, 1940, and *Astragalus Harbisonii* Barneby, 1953; a mouse, *Peromyscus guardia harbisoni* Banks, 1967; a bee, *Centris harbisoni* Snelling, 1974; a butterfly, *Euphyes vestris harbisoni* Brown and McGuire, 1983; a yucca skipper, *Megathymus yuccae harbisoni* J. & T. Emmel, 1998; and a scorpion, *Vaejovis* (now *Serradigitus*) *harbisoni* Williams, 1970, named for his collecting in Baja California and "having encouraged many biology students to study the arthropod fauna of this region."

Upon his retirement in 1969, Harbison remained active at the SDNHM for another six months—and continued to teach occasional classes to young students as a volunteer until 1972—but then felt it best to separate himself in fear that the Museum would not feel a need to recruit a replacement in the Entomology Department. This was a well-founded apprehension; in fact, a full time curator was not hired for many years. However, it was a shame that Harbie's contributions to the SDNHM, especially the public relations value he provided, and his own interest in working with the collection were terminated with many potentially productive years remaining.

As the city's best-known resident naturalist, Harbie frequently appeared in the news media, often to help dispel fear of insects and spiders. On one occasion, under the incredulous, slack-jawed gaze of a TV host, Harbie prodded a shaggy tarantula into biting his finger, and then held it up with the beast still attached, to show parents in chaparral suburbia that the pain was endurable and the venom feeble. Harbison maintained a steady, determined expression and seemed undaunted by the cameras. Whether any viewers were convinced is unknown. Of all things that Charles Harbison did or did not accomplish, he can never be forgotten for the time he spent with people, especially young naturalists, answering their questions. Certainly many of us will never forget our introduction to a microscope, professional books and monographs, and adventures trying to match our catches with identified specimens in a museum collection. It is to be hoped that Harbie was repaid in satisfaction in seeing so many of his students contract an incurable interest in natural history from his infectious enthusiasm, which we carried into professional lives.

ACKNOWLEDGMENTS

This tribute was facilitated by David Faulkner, who researched data from records at the SDNHM and conducted interviews with Harbie in 1981-82 (Faulkner 1987) and by a letter of personal recollections written by Harbison the year he retired (Chemsak & Batiste 1969). Further memories and anecdotes were contributed by R. Mitchel Beauchamp, John Brown, W. Darryl Hansen, John Heppner, Paul Rude, Ray Stanford, and Stan Williams. Several passages were enhanced by Wikipedia.

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Received for publication 23 Jan 2011; revised and accepted 1 June 2011.

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PRINTED BY THE ALLEN PRESS, INC., LAWRENCE, KANSAS 66044 U.S.A.

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