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GEOGRAPHIC VARIATION IN ADULT AND LARVAL *LOPHOCAMPA MACULATA* HARRIS 1841

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ABSTRACT. The spotted tussock moth, *Lophocampa maculata* Harris 1841, inhabits a wide band of North America on both sides of the United States/Canadian border from coast to coast and extends southward along the Pacific coast and in the major mountain ranges of the United States. Within this large range, the species exists as several geographic variants characterized by a number of phenotypic differences, most notably last instar larval coloration. Other distinctions include voltinism, with the California coastal variety being uniquely bivoltine, and larval food preference. While considered a generalist feeder on broadleaf trees, some geographic variants show strong preferences for a particular genus of food plant. Over most of its range, *L. maculata* exists in one of two larval color patterns. Last instars of the Eastern and California Coastal forms are a combination of black and yellow, while the Western Interior form is a combination of orange and black. However, in the Pacific Northwest there is a stable population exhibiting wide variation in larval coloration, with features of the color patterns of both the other two varieties. An additional, and perhaps unique, feature of larval coloration is the rare occurrence of individuals with partial depigmentation for one or two instars, followed by reversion to normal coloration in the last instar and the adult. These individuals are found in all of the geographic populations, although there are small distinctions in coloration that appear to be population-specific. Larval coloration results from three pigments. Exogenous xanthophylls, obtained from the diet produce the yellow color, whereas the endogenous black pigment is eumelanin. The orange pigment is most likely pheomelanin, produced endogenously.

Additional key words: bivoltinism, geographic range, pigmentation, melanin, xanthophylls

The New World genus *Lophocampa* is composed of about 70 species, most of which are tropical (Powell & Opler, 2009). There are 11 species north of the Mexican border, of which the Spotted Tussock Moth, *Lophocampa maculata* Harris 1841, (Erebidae, Arctiinae (Lafontaine 2010)) is the most widespread. It is found across North America on both sides of the United States/Canadian border and south in the United States in the Appalachian, Rocky and Pacific (Sierra Nevada, Cascade and the Coastal Range) mountains. It is also found along the immediate Pacific coast as far south as Los Angeles, CA. The species was first described from Massachusetts in 1841 (Harris 1841). In the late 19th century it was placed in the genus *Halysidota* and various forms were described differing in geographic location and larval appearance (Dyar 1892 a,b). These were regarded by some to represent separate species. Currently, the moth is placed in the genus *Lophocampa* and all the geographic variants are considered the same species.

Standard field guide descriptions of this species belie the considerable variation in phenotypic characteristics of the different populations across North America (Wagner 2005; Beadle 2012). This paper will describe the adult and larval characteristics of the various geographic races of *Lophocampa maculata*. For the purposes of this paper all varieties of this organism are considered to be the same species and the different forms and geographic segregates will be referred to as populations to avoid premature decisions concerning taxonomic levels. The species is considered to be univoltine and a generalist feeder on hardwood trees (Robinson 2002). However, as will be described, the various geographic populations differ significantly in many phenotypic and life history features. The genetic basis for these variations and the evolutionary history of the species giving rise to the present geographic variants are currently under active study.

Materials and Methods

Range Data

Adult data were obtained from collections at Oregon State University, Los Angeles County Natural History Museum, Burke Museum at the U. of Washington, Spencer Museum, Royal British Columbia Museum, and private collections. Voucher specimens may be found at Oregon State University, Corvallis, OR.

In addition, the online databases BAMONA (butterfliesandmoths.org), the Moth Photographers Group (mothphotographersgroup) and BugGuide (VanDyk) were used to collect occurrence records and contact individuals to obtain additional data and documentation. Many of the adult and larval photographs were obtained from a large number of individuals, most found through BugGuide. This paper would have not been possible without their contributions and their names are listed in the acknowledgements. Most larval photographs of wild individuals are last instar as this stage is most commonly discovered.

Overall range data for the species was estimated mainly from adult records in museum collections and larval photographic records as described above. Subdivision into the various populations based on last instar color was determined from photographic data. As with all range data, interpolation between individual record locations was used. Within each geographic

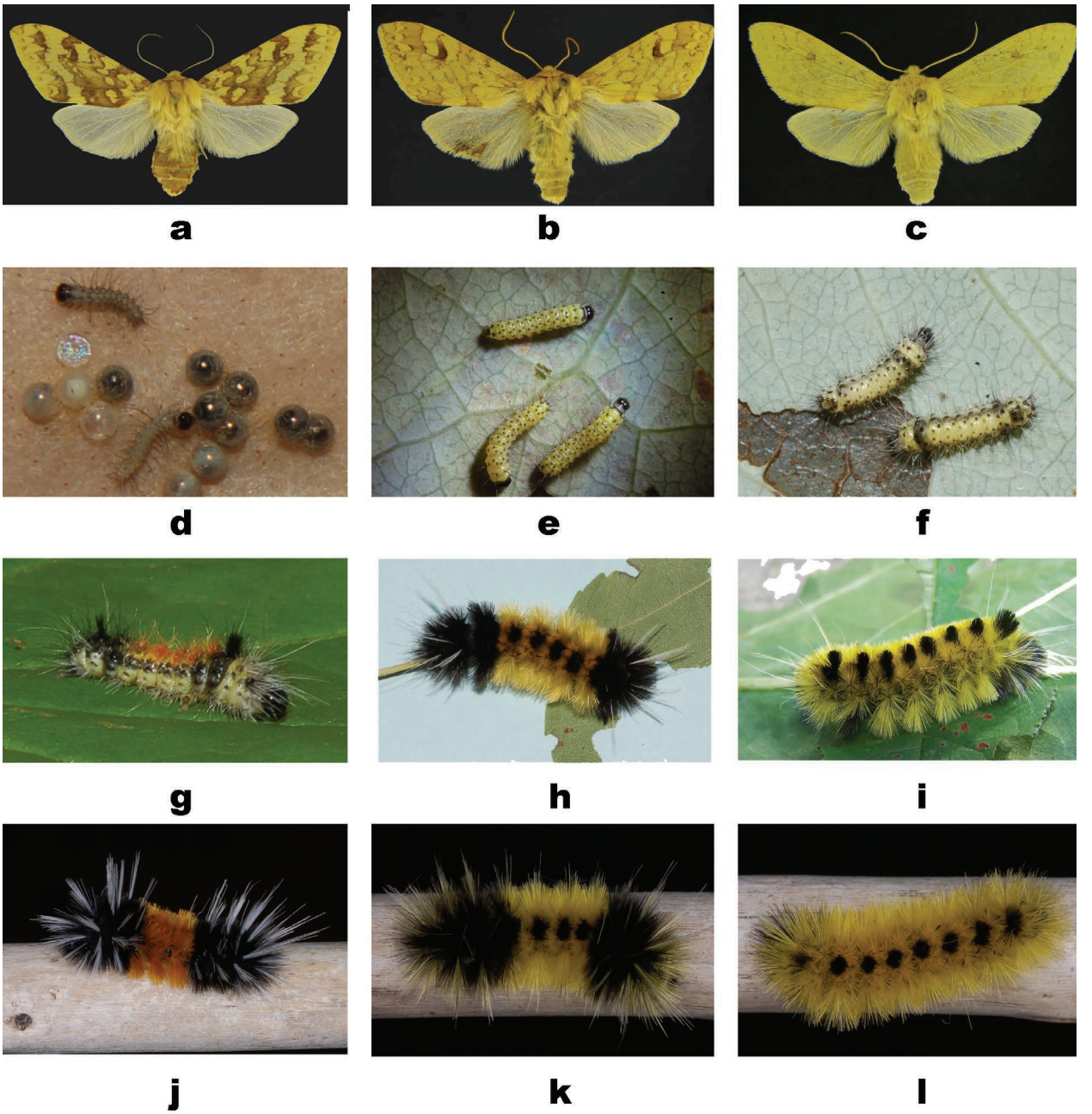

FIG. 1. Adult and larval forms of the geographic varieties of *Lophocampa maculata*. **a-c**, the high, intermediate, and low contrast forewing patterns (courtesy of Tom Dimock); **d**, eggs and newly hatched larvae; **e-g**, late first, second, and third instars; **h-i**, fifth instar Eastern form B/Y/B and Y varieties (courtesy of David Woo); **j**, fifth instar Western Interior form; **k-l**, fifth instar California Coastal form B/Y/B and Y varieties.

range, larval color patterns are consistent but occurrence of the species is restricted to suitable habitat.

Chemical Analysis of Pigments

Larval color variation is the most obvious difference between the various geographic populations. Pigments in setae were determined by chemical analysis. Analysis for eumelanin and pheomelanin was carried out by alkaline oxidation using hydrogen peroxide followed by high performance liquid chromatography (HPLC) analysis (Napolitano 2000). Pyrrole tricarboxylic acid served as the marker for eumelanin and benthiazole carboxylic acid for pheomelanin. A sample of synthetic pheomelanin, kindly provided by Dr. Napolitano, was used as a reference standard for that pigment and

eumelanin from black human hair was used as a eumelanin standard. Xanthophylls were extracted from setae and food plants, separated by HPLC (Thayer 1990), and characterized spectroscopically. The xanthophylls, zeaxanthin and lutein, were extracted from yellow corn (Moros 2002)), and used for comparison purposes.

Captive Rearing

Eggs from wild females, typically captured at outdoor lighting sites, were obtained from various geographic regions and raised in the laboratory to investigate the complete cycle of larval development. Eggs and early instars were kept in Petri dishes while the later instars were housed in plastic boxes with nylon mesh tops. Eggs were lightly misted at intervals of several days to prevent desiccation. All specimens were reared at ambient temperature (15–21°C) on a cycle of 16 hours light: 8 hours dark. Larvae were fed ad libitum with either vine maple (*Acer circinatum*), pacific willow (*Salix lasiandra*) or dune willow (*Salix hookeriana*), depending on food preference. Cocoons were stored refrigerated at 5°C as described (Winter 2000). Several cohorts from each geographic population were reared to facilitate comparison of larval and adult phenotypes. Captive pairing of adults was carried out in nylon mesh cages (75×38×38 cm; 0.11 m^3). Adults were kept on the same 16:8 light: dark cycle as larvae and were fed a diluted maple syrup solution placed on a banana slice.

Voltinism

Using museum data along with that from private collections, sufficient adult capture data were obtained from several narrowly defined geographic regions to assess voltinism. A kernel density calculation (MATLAB (www.mathworks.com)) was used to determine flight probability as a function of calendar date.

RESULTS

Larval and Adult Phenotypes

Eastern Population

Figure 1 shows typical examples of larval and adult individuals. The forewing pattern of the adult can be divided into three types. The high-contrast form, which is present in all the geographic regions, is shown in (a). The intermediate- (b) and low-contrast (c) forms are found, along with the high-contrast form, in the California Coastal population and in a small region of the central Appalachians. The early instars, one through three, are virtually identical in all populations. Figure 1 d–g shows the eggs and newly hatched larvae, late 1st instar, 2nd and 3rd instars of captive-reared individuals. The 4th instars have the same coloration as the 5th instars and are not depicted in Figure 1. Most of the available data on larvae of wild individuals are the 5th (and final) instar. Figure 1 h–l shows the coloration of the 5th instar from the different geographic populations. The appearance of individuals is determined by the pigmentation of the dense setae which cover the entire body. The Eastern population exhibits two larval varieties. These will be referred to as the black/yellow/black (B/Y/B) variety (Fig. 1h) and the all yellow variety (Y) (Fig. 1i). Both forms are characterized by a series of black middorsal setal tufts and longer, white setae at both ends of the body. These longer setae, which are white in all larval forms, are a hallmark of Lophocampa species. The Y form consistently exhibits eight black middorsal tufts. The number of visible spots is variable in the B/Y/B form because of variation in the extent of the black setae at both ends of the body. Based on available records of the two forms, the Y form is a minor component of the Eastern population. I have never observed it in several broods reared in captivity but photographic records of wild individuals suggest it occurs widely distributed within the Eastern populations, at low frequency (roughly estimated at <5% of individuals, based on relative numbers of the two phenotypes in photographic records).

The predominant adult form in the Eastern population has a strongly contrasting pattern of light and dark regions on the forewing (referred to as high contrast, HC, Fig. 1a). The hind wings of all *Lophocampa maculata* are cream-colored and are more opaque along the outer edge. Evidence from collections suggests that there is a variation in the adult phenotype found in the mid-Appalachian mountains (West Virginia and Kentucky) with much less contrast between the light and dark regions of the forewing. They can be divided into two types, intermediate contrast, IC and low contrast, LC, as previous defined (Fig. 1b and c). The IC and LC adult forms are only known from wild individuals. Thus, it is not known whether either of these adult phenotypes is connected to a particular larval phenotype.

Western Interior Population

Western interior populations of *L. maculata* are characterized, in the last instar, by a color pattern of black ends and an orange central region, referred to as B/O/B (Fig. 1j), but they lack the black middorsal setal tufts. The coloration of this form is constant over its entire range, based on all available data, which includes both wild individuals and captive reared broods. Adults of this population are consistently the HC phenotype (Fig. 1a).

California Coastal Population

Along the immediate coast of California there exists a population distinct from the Western Interior form in

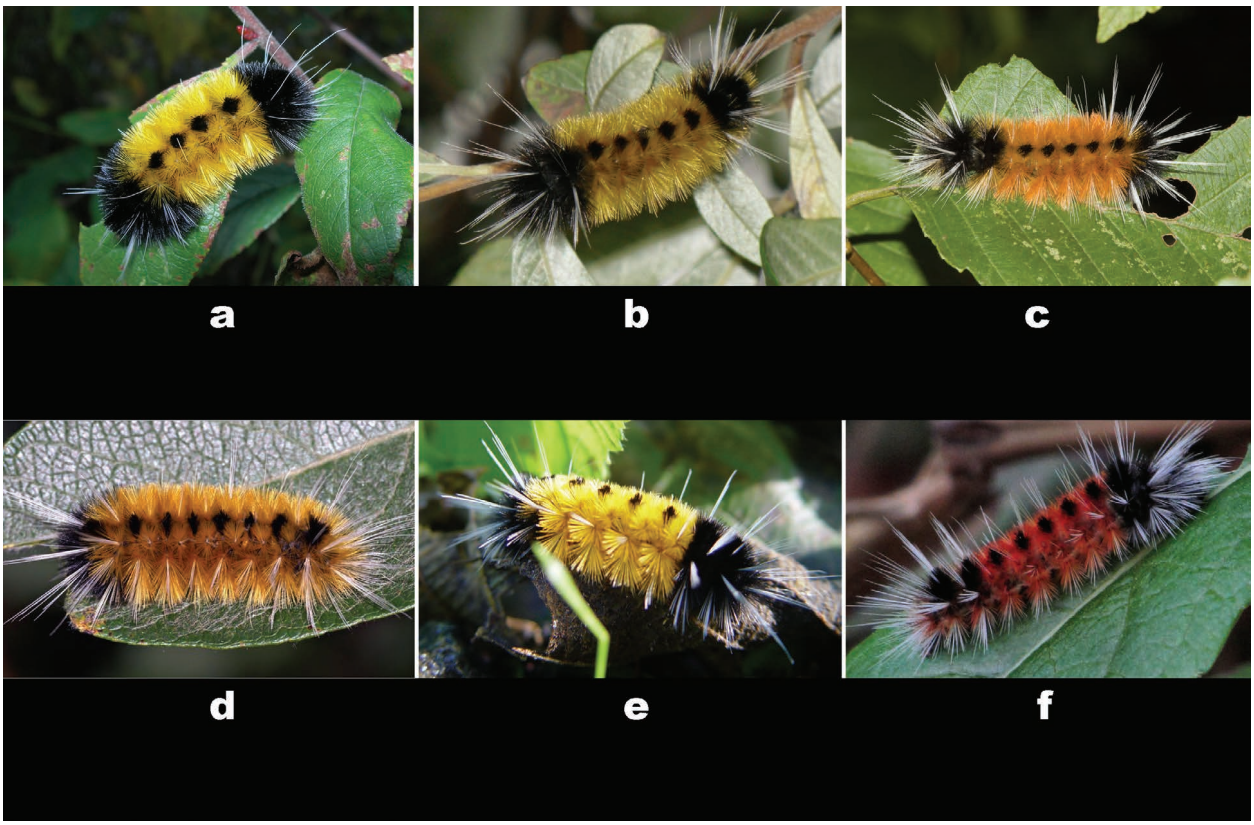

FIG. 2. Last instar larvae of *Lophocampa maculata* from the Pacific Northwest showing substantial variation in coloration. **a**, Sechet Peninsula, British Columbia, Canada (courtesy of Sylvia Moon); **b**, Olympic National Park, Washington, USA (courtesy of David Droopers); **c**, Trout Creek, Washington, USA (courtesy John Davis); **d**, Victoria, British Columbia, Canada (courtesy Ken Vaughn); **e**, N. Vancouver, British Columbia, Canada (courtesy of Eric Johnson); **f**, Gifford Pinchot N. F., Washington, USA (courtesy of Hoppingcrow)

many ways and referred to as the CA Coastal form. This population has been studied both in the wild and in captive-reared broods, most from Aptos, CA. The appearance of last instar larvae is like that of the Eastern population. Individuals can be either the B/Y/B or Y phenotype (Fig. 1k and l). The number of black dorsal tufts is eight in the Y phenotype, like the Eastern counterpart. The B/Y/B phenotype has variable numbers of black middorsal tufts, as does the Eastern phenotype, dependent on the extent of the black regions at both ends of the body. Based on the frequency of the two color patterns in wild individuals and observations of captive reared broods, there is a roughly 50:50 ratio of the two phenotypes. This is in contrast to the Eastern form in which the B/Y/B phenotype predominates. Egg clusters obtained from wild females and raised in captivity produce broods with either all Y, or all B/Y/B forms or a mixture of the two (Table I). Mating of a captive female with a single captive male produced a brood with the mixed larval color phenotypes, indicating that mixed broods from wild females are not the result of their mating with two different males. The data in Table I show that when a brood is the mixed variety, the number of B/Y/B and Y individuals is always about equal.

Adults of this population occur in the HC, IC, and LC forewing patterns as shown previously (Fig. 1a–c). The frequency of the different adult forms appears to be uniform over the geographic range of the CA Coastal population unlike the Eastern population, where the LC variety appears to be confined to a small geographic area. Captive rearing of many CA Coastal broods does not indicate a correlation between the larval and adult phenotypes.

Pacific Northwest Population

Unlike the other populations of *L. maculata*, in which the larval color phenotype is uniform (Western Interior) or a mixture of just two forms (Eastern and CA Coastal), the Pacific Northwest population (PNW) shows dramatic variation in the coloration of the last instar (Fig. 2). The extent of the black regions at either end of the body varies from none at one or both ends to the maximum extent for the species. Color of the central region can

FIG. 3. Geographic range of *Lophocampa maculata* based on currently available data. Color coding shows the approximate range of the Eastern Form (yellow); Western Interior Form (orange); California Coastal Form (green) and Pacific Northwest Form (purple).

range from red-orange through the typical rusty orange to an orange-brown or bright yellow. Black middorsal setal tufts are always present, with the number being variable, depending on the extent of the black regions at both the anterior and posterior ends of the body, a feature similar to the Eastern and CA coastal forms. In individuals lacking the black end regions, the number of black dorsal tufts is eight, as in the Y form of the Eastern and CA Coastal populations. Captive rearing of egg clusters from single females indicate that considerable variation in coloration of 3rd and 4th instar larvae also occurs in the PNW population and that variation in early instars does not necessarily result in variation in the final instar. The variability of the PNW population is in stark contrast to the Eastern, Western Interior and CA Coastal populations where there is uniformity of coloration within and between broods raised in captivity, except for the two major phenotypes, B/Y/B and Y, of the Eastern and CA Coastal populations. Adults of the PNW population consistently show the HC forewing pattern.

Geographic Range

The geographic range for *Lophocampa maculata*, is divided into four regions defined by the coloration of last instar larvae (Fig. 3). These are referred to as the Eastern (E), Western Interior (WI), California Coastal (CA Coast) and Pacific Northwest (PNW) populations.

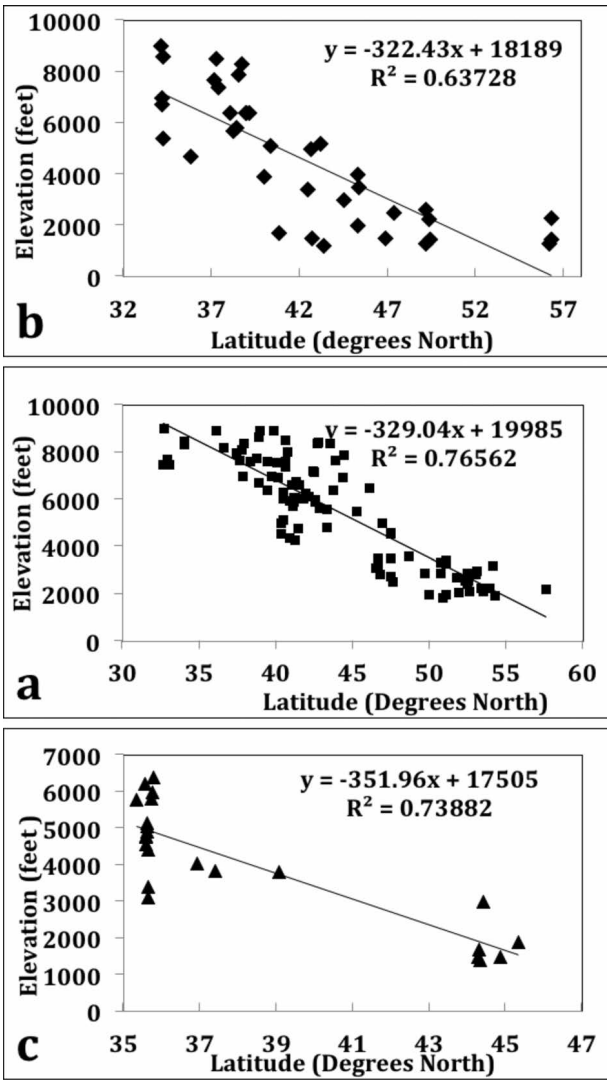

FIG. 4. Plots of elevation vs. latitude for confirmed observations of *Lophocampa maculata* in: **a**, Pacific Mountains (Sierra Nevada, Cascade and British Columbia Coastal ranges) based on 37 observations; **b**, Rocky Mountains based on 95 observations; and **c**, Appalachian Mountains based on 22 observations. The trend line on each plot is the best linear fit. The equation of each trendline is given along with the R^2 value.

Within each region, there is great consistency of the larval coloration, as described above (see Fig. 1), except for the PNW population (Fig. 2). Adult coloration is considerably less variable than larval coloration within a region.

There remains considerable uncertainty about the complete range of this species, particularly the northern limit. The northernmost data point is Yellowknife, NWT, Canada at 62.5°N while the southernmost is Mt. Graham, Arizona, USA at about 32.5°N, giving the species a latitude range of 30°. Within the various regions, distribution of the species may be spotty and dependent on suitable habitat. This appears to be especially true of the Great Basin region between the Pacific and Rocky Mountain ranges and the southern Appalachian Mountains. These areas are represented by little data.

The moth's range southward in the Pacific Mountains, Rocky Mountains, and Appalachian Mountains is possible because the species lives at progressively higher elevation (Fig. 4). Data on elevation vs. latitude were obtained from verified specimens or photographic data. The random nature of the observations, results in a scattering of data but the increasing elevation with more southerly latitude is obvious. The R^2 values provide an indication of the closeness of fit of the data to a linear relationship. It is interesting to note that for all three mountain ranges the slope of the best fit line is about the same. A shift southward of 1° of latitude results in an average increase in elevation of 300–350 feet.

Because suitable high elevation habitat is very fragmented in the southern part of its range, especially in the southern Rocky Mountains, the species exists in isolated "sky islands." Similar fragmentation may occur in the southern Appalachians. This geographic fragmentation coupled with the expected limited migration ability suggests these southern populations may exhibit greater genetic variation than the more contiguous northern populations. Efforts to explore this are underway, looking particularly at Arizona and New Mexico populations. The current period of global warming is expected to further minimize suitable habitat in the southern part of the species range and may eventually lead to local extirpations at the southern ends of its geographic range. In many places the species is now found at the highest possible elevation, making further increases in elevation in response to global warming impossible. In contrast, warming in the subarctic areas of northern Canada may allow the species to extend its range north as the range of willows and other deciduous host trees expand northward.

Chemical Basis of Pigmentation

Melanin is a widespread pigment responsible for pigmentation in animals, the browning reaction of damaged plant tissue (Riley 1997; Sugumaran 2002) and sclerotization of the insect cuticle (Nelson 2008). Melanins are complex polymeric oxidation products of the amino acid tyrosine resulting from the action of the enzyme tyrosinase and, possibly, other enzymes as well. Melanin occurs in two forms, brown to black eumelanin and orange to red pheomelanin. Pheomelanin results from condensation of the initial oxidation product of

TABLE 1. Phenotypes Resulting from Captive Rearing of Eggs from Wild Females of the California Coastal Form of *Lophocampa maculata**

Brood Type+	All B/Y/B Individuals	Mix of B/Y/B and Y Individuals	All Y Individuals
Number of Broods	1	4	6
Number of B/Y/B : Y		17:15 35:29 28:45 11:9	
Overall Ratio of B/Y/B : Y		91:98	

*All broods were from Aptos, California. Gravid females were collected at a light and allowed to ovipost in paper bags. Each brood represents the egg production from a single female. Larvae were raised under identical conditions of temperature and light (16 L:8 D) on Pacific Willow (Silax lasiandra). For the mixed phenotype broods, the ratio of the two color forms are given along with the overall ratio for all four broods.

+B/Y/B stands for the black/yellow/black color pattern (see text); Y stands for the yellow color pattern (see text)

TABLE 2. Phenotypic Characteristics of the Various Geographic Populations of Lophocampa maculata

Population	Last Instar Larval Coloration	Presence of Black Middorsal Tufts	Voltinism	Food preference
Eastern	black/yellow/black (majority form) all yellow (minority form)	yes	univoltine	Wide range of deciduous trees *Silax, Alnus, Acer, Betula*, etc.
Western Interior	black/orange/black	no	univoltine	*Silax, Alnus, Acer*, etc.
California Coastal	black/yellow/black (~ 50%) all yellow (-50%)	yes	bivoltine	Appears to feed exclusively on *Silax*
Pacific Northwest	Extremely variable	yes	univoltine	Wide range of deciduous trees: *Silax, Alnus, Acer*, etc.

tyrosine with thiols, such as cysteine. Because of their lack of solubility in almost all solvents and their polymeric, heterogenic nature, melanins are usually identified by characteristic products formed via oxidative degradation.

Analysis of the pigments in 5th instar setae by alkaline hydrogen peroxide degradation followed by HPLC analysis (Napolitano 2000) reveals the presence of pyrrole tricarboxylic acid (PTCA). PTCA is a recognized product of eumelanin degradation. Thus, in all geographic populations, the black pigment in setae is eumelanin. The orange pigment characteristic of last instar larvae of the Western Interior form has solubility and spectroscopic characteristics consistent with pheomelanin, however further work is needed to confirm this result.

Both eumelanin and pheomelanin are produced endogenously from the common precursor tyrosine. In contrast, the yellow pigment characteristic of both the Eastern and CA Coastal populations is exogenous in origin. Extraction followed by HPLC analysis, along with solubility and spectroscopic characterization, indicate it is a xanthophyll obtained from the larval diet. HPLC analysis of extracts of pacific willow (Silax lasiandra), a common host plant for the CA Coastal and PNW populations, indicates two predominant xanthophylls,

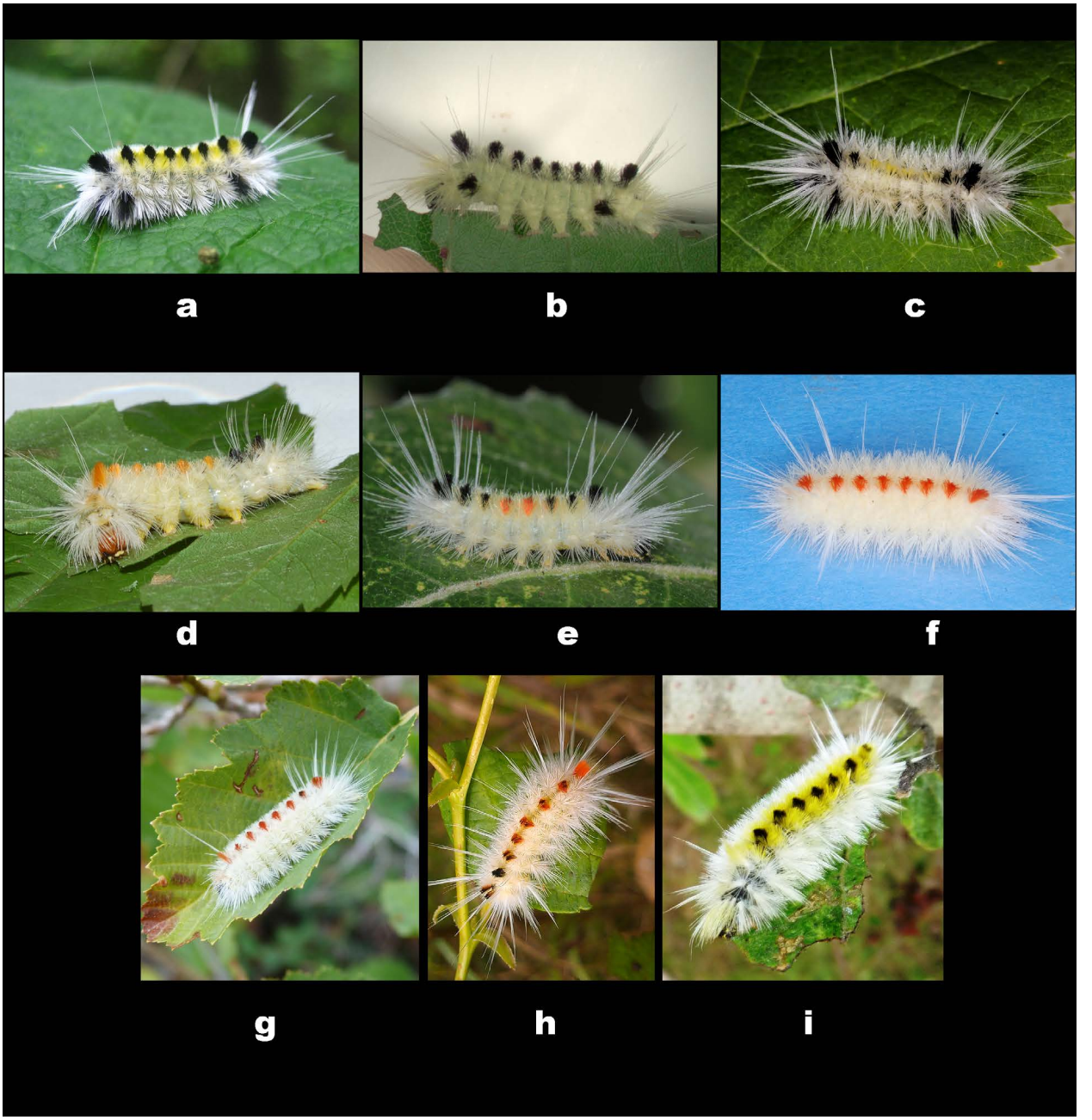

FIG. 5. Geographic variation in the "white" phenotype of *Lophocampa maculata*. All are 4th instars. **a**, Eastern form (courtesy Becca Walling); **b**, Eastern form (courtesy Rebekah Keating); **c**, Western Interior form (courtesy Harvey Schmidt); **d**, Pacific Northwest form; **e**, Pacific Northwest form (courtesy Peter Wood); **f**, Pacific Northwest form; **g**, Western Interior form (courtesy Dwaine Keuter); **h**, Western Interior form (courtesy David Droppers); **i**, CA coastal form (courtesy Brooke Sheridan).

lutein and zeaxanthin. Zeaxanthin appears to be the major component of the yellow setae pigment in *L. maculata* based on retention time in HPLC and visible absorption spectrum. Extraction of different colored setae indicates that the yellow pigment is not confined exclusively to the yellow setae but is found in both the black setae, found in all populations, and the orange setae in the Western Interior population, in roughly the same amount per mg of setae regardless of setae color. The yellow color is masked in black and orange setae by the more intense melanin color. In addition, a pigment with identical spectroscopic characteristics is obtained from the forewing of adults. It appears that the yellow-brown color of at least the lighter areas of the forewing is

due in part to xanthophyll retained from the larval stage. Admixture of varying amounts of eumelanin may be responsible for at least part of the pattern of maculation on the wing.

Newly hatched larvae of *L. maculata* from all populations are gray in color with dark black heads, indicative of eumelanin (Figure 1d). They have no yellow color. As shown in Figure 1e, after several days of feeding the integument turns a bright yellow, resulting presumably from ingestion of xanthophylls. Newly molted larvae have a yellow head capsule, which darkens over a period of several hours to its normal black color. This must result from synthesis of eumelanin, which then obscures the yellow color. Synthesis of black eumelanin eventually results in the typical black head capsule. This phenomenon has been observed in all *L. maculata* populations.

Partially Depigmented Forms of *L. maculata*

A rare and interesting variant in the larval coloration of this species involves individuals that exhibit a partial loss of setae pigmentation in the 4th or occasionally the 3rd and 4th instars, followed by return to normal pigmentation in the final, 5th instar. These individuals are referred to as partially depigmented since they lack pigmentation of the setae except for the middorsal tufts, for which the coloration can be black or orange or a combination of the two colors (Fig. 5). On occasion, a single whorl can have a mixture of differently colored setae and, in some cases, there is a yellow fringe around the black tuft.

The partial depigmentation phenomenon was first observed in Wisconsin, involving the Eastern population. Since then, it has been observed in all geographic populations, although there are differences between populations. A preliminary report of this phenomenon has been published (Strothkamp 2011). Since that publication considerable additional information has been obtained. The phenomenon has been observed in both wild populations and captive reared broods of the WI population from two widely separated locations in the San Bernardino and Sierra Nevada mountains of California, a single individual out of broods of 62 and 41 individuals, respectively, was observed to exhibit the partial loss of pigmentation. One case involved both the 3rd and 4th instars while in the other, only the 4th instar was involved. In both cases the 5th instar and the resulting adults had normal coloration.

Initial observations of wild individuals had involved single individuals, consistent with the captive reared observation of a single individual in two large broods. More recently, several reports have been obtained of multiple individuals found in the same immediate area at

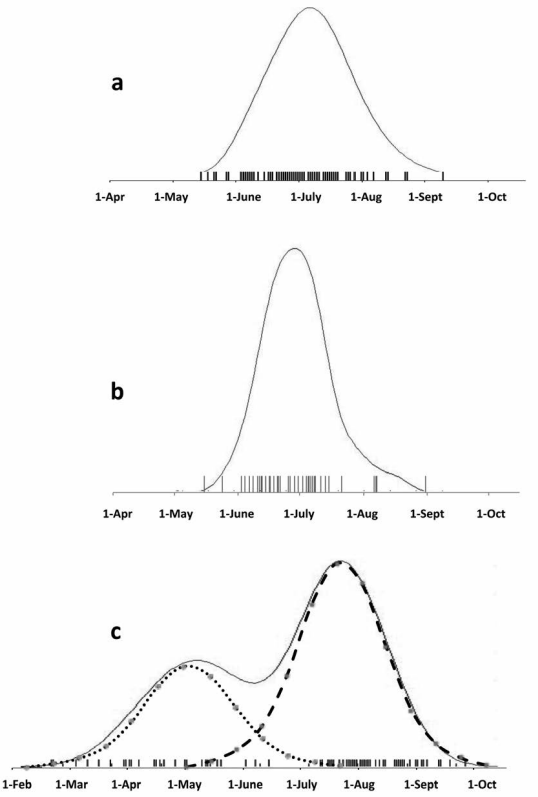

Fig. 6. Voltinism in *Lophocampa maculata* populations. Kernel density plots of flight data for three geographic populations of *Lophocampa maculata*. Data from: **a**, the state of Utah, USA (Western Interior population); **b**, the province of Quebec, Canada (Eastern population); **c**, California Coastal Population (from San Francisco to Los Angeles, CA, USA). In **c**, the solid line is the actual data showing a bivoltine pattern. The dotted and dashed curves show an approximate resolution of the data into a spring flight (dotted line) and a late summer flight (dashed line). Below each kernel density plot is a rug plot showing the actual dates of observations.

the same time, all at the same stage of development. This suggests that there is a variety of this phenomenon that may involve multiple individuals of a brood, or perhaps an entire brood. Unfortunately, in all cases except one of multiple, wild individuals, specimens were not collected and so our knowledge of the duration of the depigmented state and the appearance of normal adults rests on four instances of captive reared individuals. All of these involved single individuals except for a group of three collected in Portland, OR and provided to me. These individuals, obtained at the 4th instar, were almost certainly from the same brood and all

exhibited the reversion to normal pigmentation in the 5th instar and production of normal adults. This multi-individual phenomenon has been reported from Eastern and PNW populations. The paucity of simultaneously emerging adults from the depigmented larvae raised in captivity has prevented breeding. Thus, to date, it has not been possible to obtain broods from the phenotypically characterized larval forms to further investigate the inheritance of this trait. However, the limited data available suggests that the occurrence of the depigmented form can involve either a single individual, or perhaps an entire brood and does not have an obvious environmental explanation.

The presence of pigmentation in some regions of setae in these depigmented individuals is consistent with a complex mechanism of control of pigmentation involving a large number of gene products. The requirement for several alleles, each occurring with low frequency, to produce this phenotype could account for its rarity. *L. maculata* exhibits both spatial variation in pigment production within an instar and temporal variation between instars. The depigmentation phenomenon must involve control elements of pigmentation expressed differently in the different instars. Depigmentation involves both the endogenously synthesized melanin pigment and the exogenous xanthophyll pigment. In addition, loss of pigmentation in setae is only partial since all individuals exhibiting this phenomenon have either black or orange middorsal tufts and some have a surrounding ring of yellow pigment as well (Figure 5). Thus, even in the depigmented individuals there is some synthesis of pigment as well as absorption of pigment from food. This suggests that both the enzymes necessary for melanin biosynthesis and the proteins involved in absorption and transport of xanthophylls are intact. The genetic defect must be in one or several of the genes that regulate the expression of pigmentation in the various regions of the body. To my knowledge, no comparable phenomenon has been reported in other species of Lepidoptera. However, many mammals show a similar spatial variation in melanin pigmentation in their fur, which can result in striking patterns of coloration, such as in the Bengal tiger, *Panthera tigris tigris*. Many mammalian examples of genetic control of fur pigmentation with a spatial variation, like the tiger, have been studied (Eizirik 2010). The situation in the larval forms of *L. maculata* may be similar

The Bengal tiger provides an interesting parallel to the partially depigmented phenotype of *L. maculata*. Rare "white" tigers occur in the wild and have been bred in captivity (Thornton 1967; Thornton 1978). These individuals either lack completely or have reduced pheomelanin production but do produce eumelanin, giving them a pattern of black or brown stripes on a white background, and occasionally some regions of orange fur (Xu 2013). The net result is excess white fur relative to the wild type (analogous to the situation in *L. maculata*). Mating of two white tigers results in exclusively white offspring, consistent with inheritance as a recessive trait. Broods of *L. maculata* composed entirely of individuals possessing the "white" phenotype might have similar genetic underpinnings. However, the observation of single depigmented individuals in broods raised in captivity suggests that expression of this phenotype in *L. maculata* is not simply a case of dominant and recessive traits, as it appears to be in the Bengal tiger.

Voltinism

L. maculata is univoltine across most of its range. Records from geographically restricted populations were analyzed by calculation of kernel density functions (Fig. 6). The data for a typical Western population (a) and Eastern (b) show a single, reasonably symmetrical curve. In contrast, the CA coastal population is clearly bivoltine. The spring flight along the CA coast is not as large as the late summer flight. The two flights of the CA coastal population occur on either side of the early summer, univoltine flight of other *L. maculata* populations, although there is some overlap. The bivoltinism of the CA coastal population is likely a consequence of the longer growing season along the immediate coast. Populations in the Sierra Nevada Mountains, living at the same latitude, are univoltine. The moderating effect of the Pacific Ocean along the immediate coast results in a Mediterranean climate from southern CA to just north of San Francisco, which coincides with the known region of bivoltinism. The ancestral phenotype of *L. maculata* was likely univoltine, with a switch to bivoltinism along the immediate Pacific coast of California resulting from the development of a suitable climate during the warming period after the last glacial maximum. The current period of global warming is causing a similar switch from uni to bivoltinism in a number of central European butterfly and moth species (Altermatt 2010) and Scandinavian moths (Poyry 2011).

DISCUSSION

Division of the total range of *L. maculata* into four regions is warranted by the phenotypic characteristics summarized in Table 2. Last instar coloration, along with the other phenotypic characteristics given in the table, provide a clear distinction among the four populations. Given the size of the geographic areas, it seems likely

that there are additional, as yet unresolved, distinctions within each of the four regions that may, in the future, require further subdivision.

It is not known if the differences in larval color have a significant adaptive value. Because all the larval color patterns found in *L. maculata* are visually obvious on a leaf surface, perhaps all are adequate to warn would be predators that it is not a suitable target. The wide, and apparently stable, variation in coloration in the PNW populations is consistent with a lack of strong survival advantage to one color pattern in this region. For the other populations, the consistency over a wide geographic range suggests that some other factor that does have a survival advantage may be responsible. For example, genetic factors responsible for differences in larval coloration may be closely linked to another trait which is selected for in each region.

Most of the present range of *L. maculata* was not habitable by the organism during the last glacial maximum (LGM) because the area was either buried under ice or, in the regions immediately south of the ice mass, the climate was too severe to support either the necessary broadleaf trees or the moth itself. As the climate gradually warmed after LGM about 20,000 years ago, the present day geographic variants must have developed as the organism started to move north from a refugium (perhaps in northern Mexico) where it had uniform phenotypic features. The consequences of the repopulation of northern European biota after LGM from several southern refugia has been documented (Hewitt 1996, 1999) and some features may be applicable to North America.

Xanthophylls appear in all larval stages as integument or setae pigmentation in all populations of this species and in adults as well. This suggests that they were present in the ancestor of the species (and likely genus or even higher level taxon). Eumelanin also plays a major role in pigmentation in all populations and was likely part of the ancestral pigmentation. In contrast, the orange pigment has a minor role in the pigmentation of early instars of the Eastern and CA Coastal populations, where it is not present in the final instar. The major role of the orange pigment in the last instar of the WI population must result from a genetic alteration in its expression rather than a newly acquired biosynthetic capability, since all populations of *L. maculata* have the ability to synthesize the orange pigment. In the Western Interior form, production of orange pigment in the last instar larvae is also linked to a lack of black dorsal tufts.

All the univoltine populations of *L. maculata* show a peak flight at about the same time of year. This at first may seem unusual given the wide range of latitude but actually is consistent with the organism living in very similar climatic zones regardless of latitude, as noted earlier. The environmental cues used by this species to time the various stages of its lifecycle such as pupal development and continued larval development to the adult vs. diapause, likely involve a combination of hours of daylight and degree days, as observed for other moth species (Nagarkatti 2001; Tobin 2008). Detection of the genetic variation responsible for the phenotypic characteristics described in this paper is a major focus of future work. A complete understanding of both the phenotypic and genotypic variations within this small creature may potentially shed light on fundamental mechanisms of evolution as well as the postglacial environmental changes that occurred in North America.

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