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A COMPREHENSIVE NATURAL HISTORY REVIEW OF *CHLOSYNE LACINIA* (GEYER, 1837; LEPIDOPTERA: NYMPHALIDAE): PATTERNS OF PHENOTYPIC VARIATION AND GEOGRAPHIC DISTRIBUTION

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ABSTRACT. We conducted a literature review and added some novel observations of the natural history of the bordered patch butterfly, *Chlosyne lacinia* (Nymphalidae). Regarding color and patterning, *C. lacinia* is considered one of the most variable butterflies in the Western Hemisphere, with phenotypic variation occurring in larvae, pupae, and adults. Several studies have been conducted on *C. lacinia*, partly due to its notable phenotypic variation and status as a pest species of domestic sunflowers (*Helianthus annuus*). Even so, the origins, development, and maintenance of phenotypic variation remain poorly known. Having the most extensive geographic range of any species in its genus, *C. lacinia* ranges from Argentina to the mid-latitude midwestern United States. Moreover, *C. lacinia* displays six distinct adult morphs across its geographic range. Morphologically continuous, relatively geographically narrow gradients between adjacent morphs have given rise to alternative interpretations about subspecies. By providing the first comprehensive maps of adult morphs, including data collected via citizen science in iNaturalist, we provide directions for further research into the species' biology.

RESUMEN. Realizamos una revisión bibliográfica sobre la historia natural de la mariposa del parche bordeado, *Chlosyne lacinia* (Nymphalidae) que complementamos con observaciones nuevas. En cuanto al color y el patrón, *C. lacinia* se considera una de las mariposas más variables del hemisferio occidental, con variación fenotípica en larvas, pupas y adultos. Se han realizado numerosos estudios sobre *C. lacinia*, principalmente debido a su notable variación fenotípica y a su condición de especie plaga del girasol doméstico (*Helianthus annuus*). Aun así, el origen, desarrollo y mantenimiento de esta variación fenotípica siguen siendo poco estudiados. Teniendo la distribución geográfica más extensa de todas las especies de su género, *C. lacinia* se encuentra desde Argentina hasta latitudes medias del medio oeste de Estados Unidos. Además, *C. lacinia* presenta seis distintos morfos adultos a través de su distribución geográfica. Debido a que morfos geográficamente adyacentes presentan una morfología continua entre ellos, el reconocimiento de las subespecies es controversial. A través de mapas de distribución de cada morfo adulto con datos de iNaturalist, planteamos hipótesis y preguntas para futuras investigaciones sobre la biología de esta especie.

Additional key words: geographic range, intergrades, life history, plasticity, polymorphism

The bordered patch butterfly, *Chlosyne lacinia* (Geyer, 1837; Nymphalidae), is one of the most phenotypically variable butterflies in the Western Hemisphere, displaying striking color-and-pattern variation at larval, pupal, and adult stages (Kons 2000, Bonebrake et al. 2011, Santiago-Rosario 2021). It covers the most extensive geographic range among the 33 species in the genus, spanning from northern Argentina to midwestern and southwestern portions of the coterminous United States (Figure 1; Kons 2000). Despite its extensive range and unique color-and-pattern variation across larval, pupal, and adult life stages, the natural, ecological, and evolutionary history of *C. lacinia* remain poorly understood, offering ample opportunities for novel research. We herein provide a comprehensive literature review of the published natural history and ecology of *C. lacinia*, while also providing novel observations and hypotheses, especially regarding the geographic distribution of adult wing-color morphs.

Life Cycle

Chlosyne lacinia is a medium-sized nymphalid butterfly, known for its distinctive coloration across larval, pupal, and adult life stages. In natural conditions, *C. lacinia* has a relatively short generational time of 34-40 days (Stamp 1977). However, in laboratory settings, generational time tends to increase, with a reported average of 53 days, depending on environmental conditions (Drummond et al. 1970).

Females lay eggs in clutches, ranging from 25 to 450 eggs, on the undersides of host leaves, with an average of 139 larvae hatching per clutch, all hatching simultaneously (Drummond et al. 1970). The eggs are initially yellow-green and later turn reddish-brown 24-48 hr before hatching (Drummond et al. 1970). Egg desiccation is a frequent cause of mortality in *C. lacinia*. Both single layer and multilayer egg clusters have higher success with increased humidity; multilayer clusters in humid conditions have the highest success (Clark & Faeth 1998). Multilayer clusters also protect interior eggs from parasitoids, such as wasps that cannot insert their ovipositors between or through the layers to parasitize the eggs (Vane-Wright & Ackery 1984).

Upon emergence, the larvae first consume their chorions before feeding gregariously on the undersides of the host-plant leaves in a group-generated silk

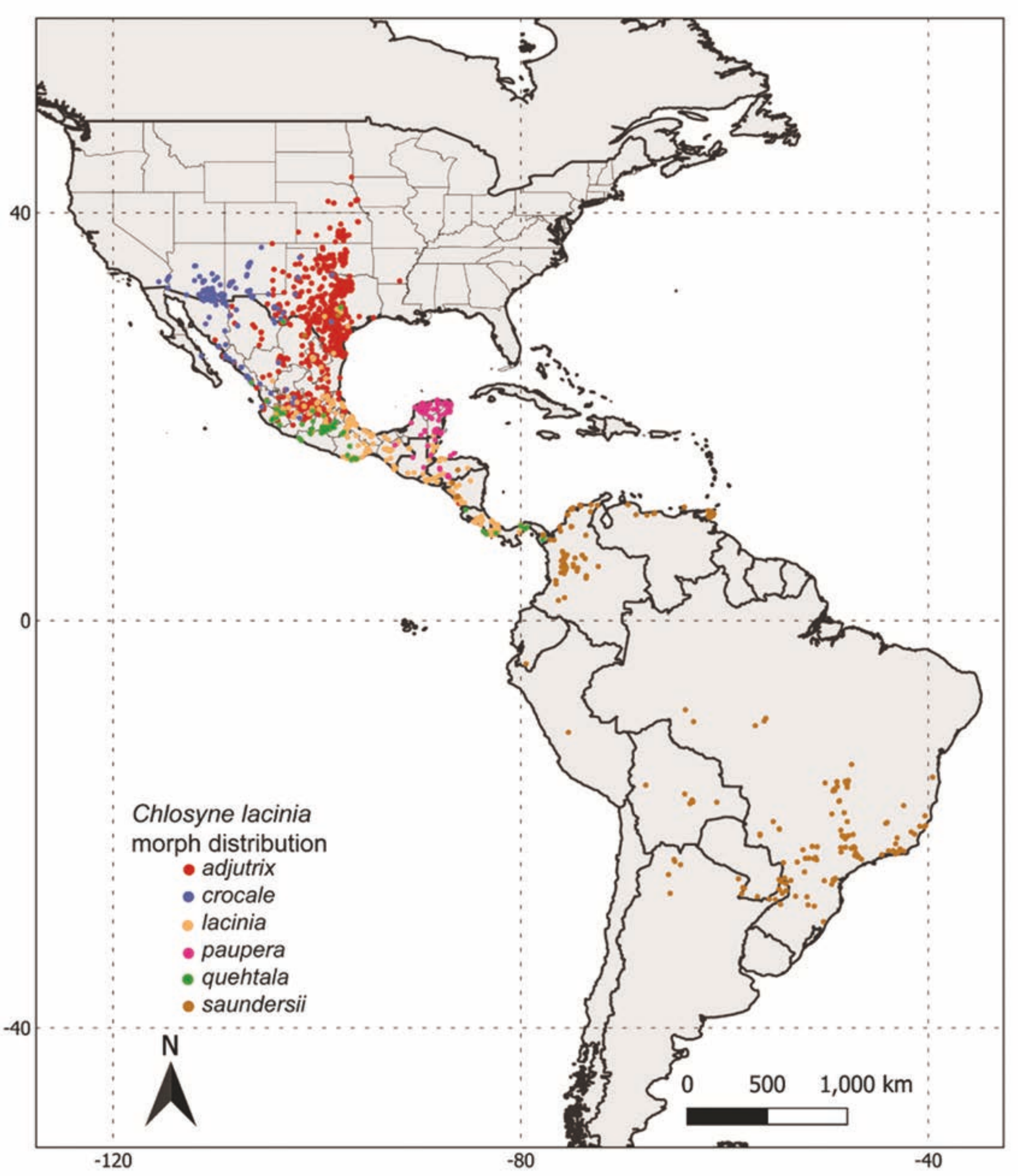

FIG. 1. Distribution of the adult morphs of *C. lacinia*. The taxonomy of the morphs follows the treatment proposed by Kons (2000).

webbing. As they feed, the larvae progress through five instars - each lasting three to seven days. Varying developmental times depend on environmental factors such as temperature, humidity, resource availability, or presumed competition (Drummond et al. 1970, Stamp 1977, LYS-R pers. obs.). Notably, larvae on their 3rd to 5th instar may enter an aestivation period when temperatures are high and food plants are scarce. In the laboratory, unfed larvae of the 3rd and 4th instar will contract their bodies, shrinking in length while expanding in girth. They can remain quiescent for multiple months, to then resume feeding and complete development when food is available again (Drummond et al. 1970). Temperate-zone populations of *C. lacinia* enter diapause around the 3rd instar, which appears to be linked to photoperiod (Drummond et al. 1970). However, in tropical populations, the cue for diapause is still unknown (Moreira et al. 2021). Diapausing tropical *C. lacinia* larvae also contract in length while increasing body fat stores and have a brown exoskeleton instead of

Table 1. Host plants of *Chlosyne lacinia* larvae, with reported regions of occurrence (Kendall 1959, Neck 1977, Gallon et al. 2019). All larval host plants are in the family Asteraceae.

Host plant	Region or country reported
Ambrosia artemisiifolia	Nearctic
Ambrosia trifida	Nearctic
Gaillardia puchella	South and Central United States
Helianthus annuus	United States, cultivated in Central and South America
Helianthus debilis spp. curcumerifolius	South Central United States
Silphium integrifolium	Midwestern and Southern United States
Silphium radula	South Central United States
Thithonia diversifolia	Mexico, Central, and South America
Verbesina encelioides	Central and Western United States

black. These morphological and physiological changes are thought to be cued in preparation for oxidative stress during diapause (Moreira et al. 2021).

The larvae disassociate from one another around the 4th to 5th instar and often crawl away from the larval host plant to pupate. Common pupation sites include host-plant stems, the undersides of host-plant leaves, fence posts, or any other nearby object (Drummond et al. 1970). Male pupae are smaller than females and adult males of a particular age-cohort are reported to emerge approximately 24-36 hr before females, though this can also vary with environmental conditions (Drummond et al. 1970).

After they eclose, adults fly off in search of a mate. Females take one to two days after emergence to fully mature, creating a time lag with the males who are fully mature and ready to mate immediately after they eclose (Drummond et al. 1970).

Host Plants

Host plants for *C. lacinia* larvae are in Asteraceae (Table 1). *Helianthus* (sunflowers) are the most common host plants, but *C. lacinia* can also be found feeding on other host plants including, but not limited to, species within the genera: *Ambrosia* (ragweed), *Echinacea* (cone flowers), *Gaillardia* (blanket flowers), *Silphium* (rosinweeds), *Tithonia* (Mexican sunflowers), and *Verbesina* (crownbeard) (Kendall 1959, Neck 1977, Gallon et al. 2019). *Helianthus annuus* (common sunflower) is the preferred host species in the United States. Drummond et al. (1970) reported larvae occasionally feeding on *Ambrosia trifida*, *Helianthus debilis*, *Silphium,* and *Verbesina virginica* in the United States. Also in the United States, larvae sometimes switch from *H. annuus* to *Verbesina encelioides* when *H. annuus* plants start to senesce in late summer and early fall.

C. lacinia adults have much broader food-plant choices than the larvae. Adults feed on the nectar of *Gaillardia aristata* (great blanketflower) and *Wedelia texana* (creeping oxeye) but would not oviposit on these plants in captivity (JMP, pers. obs.). In anthropogenically modified landscapes with heavy human land-use, adults will feed from flowers of many "weedy" plant species. Furthermore, *C. lacinia* apparently does not imprint on its larval host (Ting & Hanson 2002).

Predators and Parasites

There is currently only one documented endoparasite of *C. lacinia* eggs. *Trichogramma fasciatum* (Trichogrammatidae: Hymenoptera) has been reared in laboratory settings from *C. lacinia* larvae collected in the field (Drummond et al. 1970).

Chlosyne lacinia larvae are eaten by ground- and plant-dwelling invertebrate predators. Fire ants, *Solenopsis xyloni* (Formicidae: Hymenoptera), are common predators of *C. lacinia* in pre-adult life stages (Clark & Faeth 2003). Predatory stink bugs (Pentatomidae: Hemiptera), including *Alcaeorrhynchus grandis* and *Podisus nigrispinus* have also been documented as larval predators of *C. lacinia* (Malaguido & Panizzi 1998, Dominguez da Silva et al. 2009). *C. lacinia* is not preferred by *A. grandis,* but *A. grandis* will consume *C. lacinia* if other food sources are unavailable (Malaguido & Panizzi 1998). *P. nigrispinus* has been used for biological control for *C. lacinia* in agricultural sunflower fields in Brazil (Dominguez da Silva et al. 2009). A metallic-blue pentatomid, *Stiretrus anchorago*, commonly predates 3rd and 4th instar larvae, sometimes even destroying whole broods (Drummond et al. 1970). Also, members of the wasp genus *Polistes* (Vespidae: Hymenoptera) commonly predate primarily 4th and 5th instar *C. lacinia* larvae (Drummond et al. 1970).

Apanteles lunatus (Brachonidae: Hymenoptera) have been found to parasitize all larval stages. Drummond et al. (1970) found 48.6% of all 5th instar larvae parasitized in the field. Two fly species, *Eupharocera daripennis*

FIG. 2. **A**) From left to right, *nigra*, *bicolor*, and *rufa C. lacinia* larval color morphs. **B**) *C. lacinia* larvae have created a silk trail while traversing between leaves. **C**) A *C. lacinia* larva cannibalizing a pupa. Photos taken by Jamie M. Phelps.

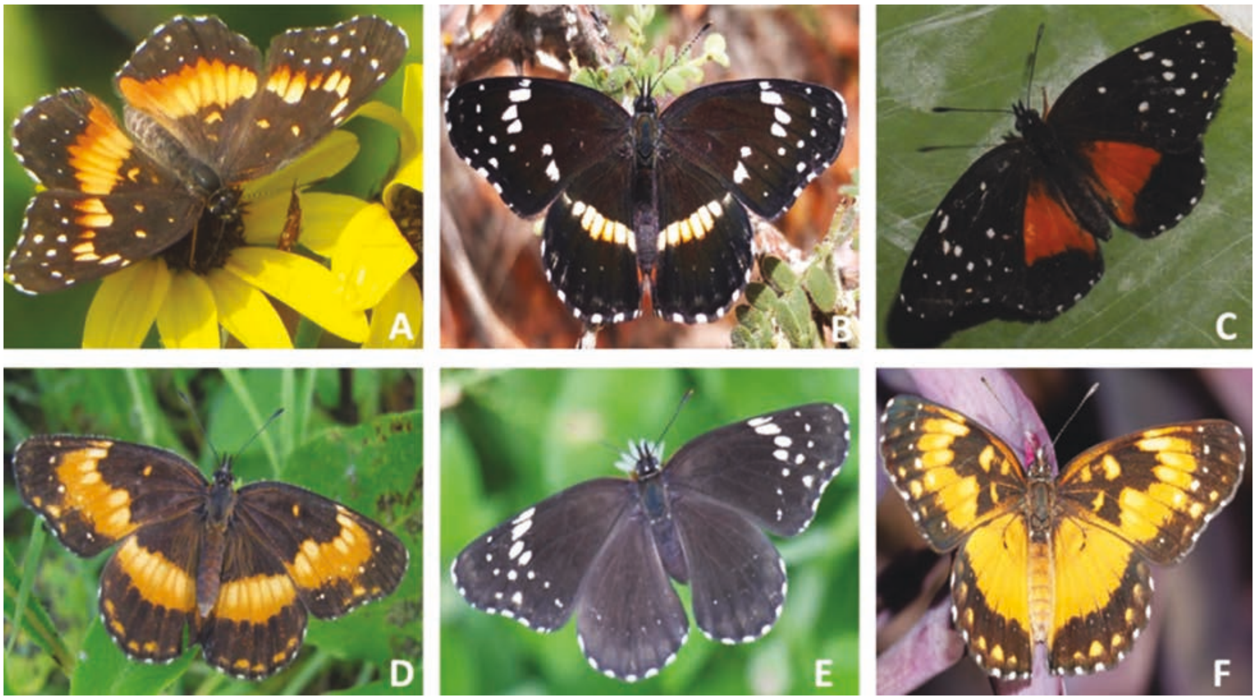

FIG. 3. Adult morphs from the dorsal side of *C. lacinia*. **A**) *adjutrix*, **B**) *crocale*, **C**) *lacinia*, **D**) *paupera*, **E**) *quehtala*, **F**) *saundersii*. Images provided with permission in order by iNaturalist users: coeller, tedmacrae (Ted C. MacRae), delmer, adorantes (Alfredo Dorantes Euan), sandino (Sandino García-Vega), and isismedri (Isis Medri).

and *Siphosturmia melitaeae* (Tachinidae: Diptera), are also common parasites; however, these species appear to only oviposit on 4th and 5th instar larvae. (Drummond et al. 1970). There is also some evidence that different larval morphs are parasitized at different rates (see Drummond et al. (1970) for additional information on parasitism in *C. lacinia* larvae).

There are few documented predators of *C. lacinia* pupae. A small pteromalid, *Pteromalus archippi* (Pteromalidae: Hymenoptera), is a common parasite of fully formed *C. lacinia* pupae, as well as two other Hymenoptera, *Cratichneumon vinnulus* (Ichneumonidae: Hymenoptera) and *Spilochalcis phoenica* (Chalcididae: Hymenoptera) (Drummond et al. 1970).

Arachnids are common predators of adults. Unidentified jumping spiders (Salticidae) have been observed to eat adults (Drummond et al. 1970). *C. lacinia* adults have also been found in the webs of the orb spiders *Argiope aurantia* and *A. trifasciata* (Argiopidae) (Drummond et al. 1970). Additionally, crab spiders (Thomisidae) are predators in Arizona (T. C. Bonebrake, pers. comm.). No avian nor reptilian predators have yet been reported to predate on adults, even though they are commonly present in shared habitats.

Color Pattern Variation Across the Life Stages

Larvae

As previously mentioned, *C. lacinia* displays extensive color-and-pattern variation across larval, pupal, and adult life stages. Upon hatching, *C. lacinia* larvae are small and yellow-brown with black head capsules. Head capsules can remain black or become red as the larvae develop. All larval morphs appear capable of developing either head capsule color (JMP, pers. obs.). As the larvae develop into their 3rd instars, they develop into one of three primary color morphs: *bicolor*, *nigra,* or *rufa* (Figure 2A) (Edwards 1893, Gorodenski 1969, Neck et al. 1971). The *bicolor* morph is primarily black or dark gray with a yellow-orange to an orange-red dorsal band that extends from the prothoracic shield to the anal plate, along with some white spotting between body segments, a spiracular white/gray band, and a subspiracular white/gray band. The *nigra* morph is generally entirely black but can have a white/gray dorsal band and can display a white/gray spiracular band and a white/gray subspiracular band across the body. The *rufa* morph is orange-red to orange. Setae in all morphs are black and start to become prominent in the 3rd instar.

The basic genetics of the larval color polymorphism, which is entirely distinguishable at the 4th/5th instar, have been interpreted from laboratory crosses. The *bicolor* and *nigra* morphs are apparently determined by a simple, one-locus, two-allele pair, with *bicolor* being dominant to *nigra* (Gorodenski 1969, Neck et al. 1971). Crosses involving individuals of the *rufa* morph indicate an epistatic locus to the *bicolor-nigra* locus that determines the *rufa* morph, with *rufa* being dominant to non-*rufa*. *Bicolor* is the most commonly occurring morph in sampled field conditions with frequencies as high as 70-80%. The *nigra* morph is generally more common than the *rufa* morph in sampled natural populations. The morphs are also present in equal proportions in males and females (Neck et al. 1971). There is currently no evidence suggesting that larval coloration has geographic patterning, but this detail has not been extensively investigated.

Pupae

This species' pupal coloration can range from predominantly whitish to predominantly black (Santiago-Rosario 2021). There is an intermediate checkered morph that features a whitish background with varying amounts of black markings (Santiago-Rosario 2021). Additionally, all morphs display orange circular markings around the spikes on the pupa.

The factors that cause the development of different pupal morphs are currently unknown. However, laboratory observations suggest that the amount of black pigmentation may be a developmentally or phenotypically plastic trait influenced by substrate coloration or background matching. This could be a cue detected by the 5th instar larvae when searching for a pupation substrate (Santiago-Rosario 2021). Further research is needed into the mechanisms behind pupal colors for this species.

Adults

Within its broad range, the species is reported to display six distinct adult morphs based on wing color-and-pattern: *adjutrix* (orange and brown/black with white markings), *crocale* (black with white markings and a cream band in the hindwing), *lacinia* (black with red-orange patches on the hindwings), *paupera* (orange and brown/black with some orange markings), *quehtala* (black with some white markings), and *saundersii* (orange with black markings), each occupying its own range with intergrades between adjacent morphs (Table 2, Figure 1, Figure 3; Kons 2000). Disagreement remains on the number of subspecies within *C. lacinia*, with some arguing for none (Kons 2000), and others arguing for four subspecies (Edwards 1893, Scott 1986).

For all morphs, the primary pigmentation on the dorsal side is black. All morphs have a red spot near the hindwing tornus, which is a diagnostic characteristic of the species (Scott 1986, Kons 2000). There is usually at least one white spot on the dorsal side of the forewing. Notably, the dorsal side of the hindwing is more variable

TABLE 2. The geographic distribution of *Chlosyne lacinia* morphs adapted from information in Kons Jr. (2000), along with descriptions of the six morphs.

Morph	Geographic Region	Coloration
adjutrix	**USA:** CA, NM, OK, TX **Mexico:** Chihuahua, Hidalgo, Jalisco, Nuevo León, Oaxaca, San Luis Potosí, Sinaloa, Tamaulipas, Veracruz, Yucatán	**Forewing:** dorsal median band with cream scales basally and orange scales distally, few cream to pale orange markings distal to postmedian dots **Hindwing:** dorsal median band with cream scales basally and orange scales distally
crocale	**USA:** AZ, CA, NM, NV, TX, UT **Mexico:** Chihuahua, Colima, Jalisco, Sinaloa	**Forewing:** narrow cream dorsal median band with patches appearing almost white **Hindwing:** narrow cream median band and cream patches in discal cells
lacinia	**Mexico:** Chiapas, Morelos, Oaxaca, San Luis Potosí, Veracruz **Central America:** Costa Rica, El Salvador, Guatemala, Honduras, Panama	**Forewing:** black with white markings, can have a narrow median band **Hindwing:** large orange patch, anal cells are black
paupera	**South America:** Bolivia, Colombia, Ecuador, Venezuela	**Forewing:** dorsal area distal to the position of the median band is black, orange discal cell patch with diffuse border **Hindwing:** black with orange scaling in the basal and median areas, with orange patches distal to the postmedian dots
quehtala	**Mexico:** Colima, Guerrero, Jalisco, Michoacan, Morelos, Sinaloa, Veracruz **Central America:** Costa Rica, Panama	Only black with white markings on dorsal and ventral surfaces **Forewing:** white median band, few distal white patches **Hindwing:** few distal white dots
saundersii	**Central America:** Extreme E. Panama (Darien Gap) **South America:** N. Argentina, W. and extreme S. Brazil, Bolivia, Colombia, Ecuador, Paraguay, Peru, Trinidad, Venezuela	**Forewing:** black areas basal to the median band are generally diffused with orange **Hindwing:** broad dorsal orange patch, orange and cream scales are not differentiated into separate patches

than the forewing. The dorsal hindwing can display some red-orange postmedian spots, an all red-orange discal area, a few rows of white postmedian spots, or a yellow-orange postmedian band of varying width. Alternatively, the dorsal hindwing coloration can be almost entirely black.

Furthermore, the ventral side of the wing is also variable; it can be primarily golden-yellow with large yellow-orange spots and a thick golden-yellow median band, can have a few rows of red-orange and white spots, or can have a yellow-white hindwing median band of varying width (Higgins 1960, Scott 1986, Brock & Kaufmann 2003, Glassberg 2017). Adult coloration is geographically patterned but is apparently not associated with larval coloration (Edwards 1893). In his 1893 letter, W. H. Edwards stated that he found 19 *crocale* and nine *adjutrix* morphs from a single brood (Higgins 1960).

Bonebrake et al. (2011) sought to determine whether there were cryptic species within *C. lacinia* populations. Using mitochondrial DNA, they specifically investigated mitochondrial sequence variation in *C. lacinia* among populations by sampling in temperate and tropical locations. They found a subclade structure that does not correspond to geography nor to subspecific designations. In other words, Bonebrake et al. (2011) found no evidence of cryptic species. Since using a few mitochondrial-genetic regions may not provide enough resolution at the population level, this finding needs to be examined further with broader genomic sampling to test whether divergence among adult morphs follows their geographic structure. Multiple studies have provided observational evidence linking color morphs to geographic regions using museum collections and observational records (Higgins 1960, Kons 2000).

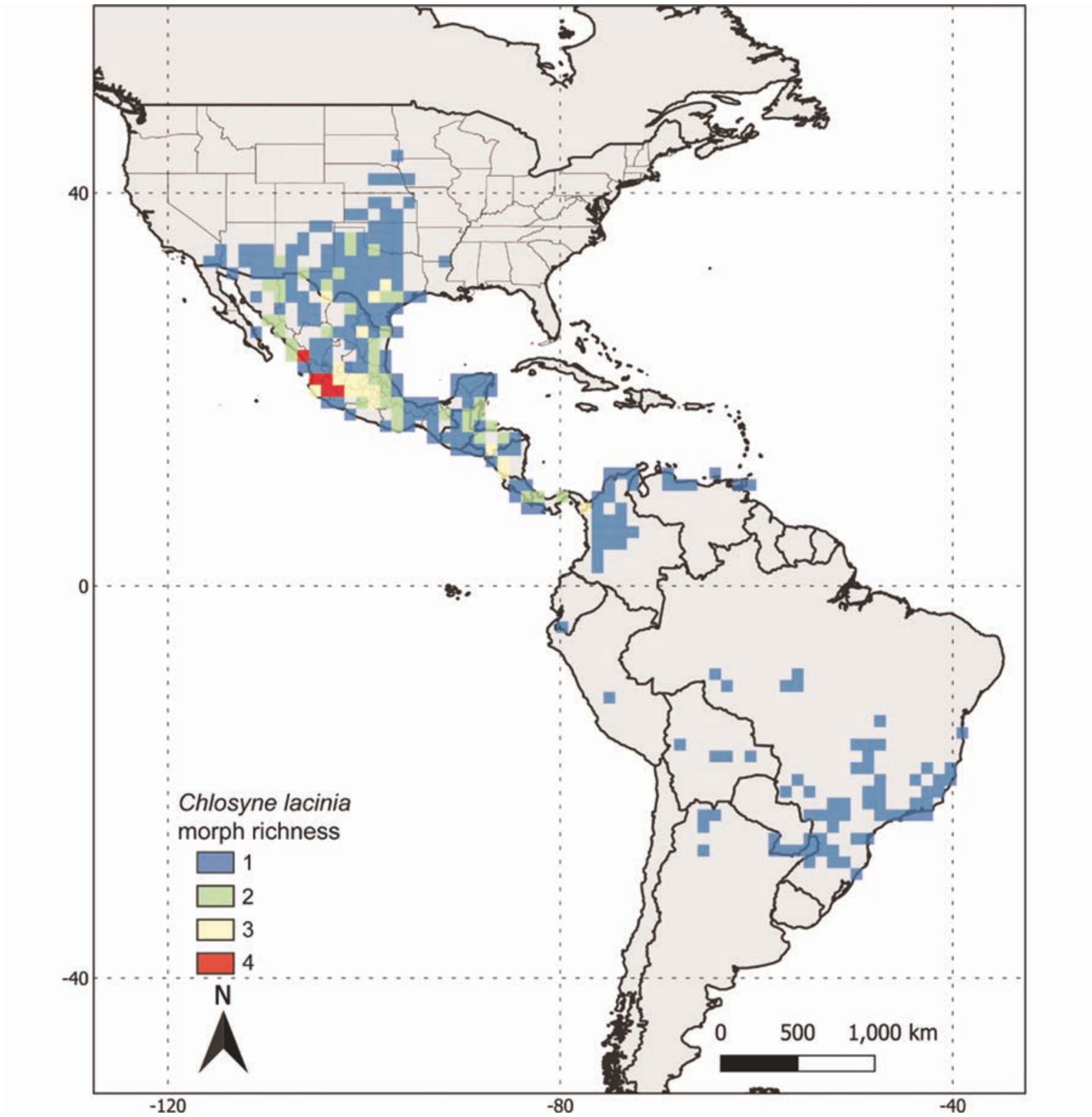

FIG. 4. The richness of the morphs of *C. lacinia* in 1°x1° grid cells. The map was plotted in QGIS 3.0, where the grid analysis was performed with the FSC plugin for biological records, version 3.4 (Burkmar 2023). The records were retrieved from the GBIF repository, including including iNaturalist photographic records (GBIF 2022).

Adult Color Pattern Variation Across the Geographic Range

The geographic range of *Chlosyne lacinia* extends from Argentina to the midwestern and southwestern United States (Figure 1 and Figure 4). Accordingly, this species has a tropical, subtropical, and partially temperate-zone distribution, where it covers a broad range of habitats: tropical and subtropical moist broadleaf forests, tropical and subtropical broadleaf dry forests, tropical and subtropical savannas, grasslands, shrublands, and the Brazilian Atlantic lowlands (Scott 1986). In the United States, adults can be found from February to November in many parts of the range, and year-round southwards, in southern Texas. *C. lacinia*'s geographic range in the United States includes habitats such as desert hills, mesquite woodlands, oak woodlands, and pinyon woodlands (Scott 1986, Glassberg 2017). In Costa Rica, the abundance of *C. lacinia* increases during the rainy season (DeVries 1987). In these two countries, *C. lacinia* is commonly found in roadsides, pastures, and other disturbed habitats (DeVries 1987, Glassberg 2017). These disturbed places tend to be the prime habitat for *C. lacinia*'s food plants, particularly in the case of

Helianthus annuus L. (Asteraceae), the preferred food plant throughout its range in the United States.

Variation of the color-and-pattern in the wings has a geographic structure (Figure 1). Each of the six morphs from Kons (2000) occurs with little overlap of other morphs' ranges. The morph *saundersii* is the only one that occurs in South America but also has a few records in mid-Central America. The morph *quehtala* occurs west of the Darien region in Panama up to the western slopes of the Sierra Madre Occidental in Mexico. There is a clear gap between Nicaragua and Guatemala, presumably due to a lack of collected *quehtala* morph specimens in this region. The *lacinia* morph occurs continuously across Central America up to northeastern Mexico, with a few records in the southern US. The Mesoamerican endemic *paupera* morph is almost restricted to the Yucatán peninsula, with a few records southwards in mid-Central America. *Adjutrix* and *crocale* are the only morphs in the northernmost range of the species. *Crocale* occurs mostly on the western range of the species, east of the Sierra Madre Occidental. Likewise, *adjutrix* occurs mostly in the eastern range of the species and west of the Sierra Madre Oriental. There are a few records on the opposite slope of each morph's range, as well as on the Mexican Plateau (Figure 1, Figure 4).

Even though there is little overlap among many of the morphs' occurrence ranges, multi-morph overlap occurs in a few areas (Figure 4). Up to four morphs (*adjutrix*, *crocale*, *lacinia*, and *quehtala*) co-occur on the western slopes of the Sierra Madre in Mexico, where the Trans-Mexican belt, the Sierra Madre Occidental, and the Sierra Madre del Sur meet (Sosa et al. 2018). The Trans-Mexican belt is an important biogeographic region, acting as an elevational bridge between the western and the eastern slopes of the Sierra Madre cordilleras, which may explain the high variation of the adults' morphology in the area. Another biogeographically important area is the Pacific slopes in mid-Central America where up to three morphs (*lacinia*, *paupera*, and *saundersii*) co-occur (Gutiérrez-García & Vásquez-Domínguez 2013). *C. lacinia* morph-level richness is geographically patterned similar to species-level richness in the genus (Figure 4, Figure 5). The role of abiotic factors, such as geological history and topography (factors important in the origin of mountainous biota; Rahbek et al 2019), in the evolution of the different morphs remains unknown.

Thermoregulatory Behaviors

Temperature influences thermoregulatory behavioral ecology of *C. lacinia* (Bonebrake et al. 2014). Adult individuals in temperate and tropical populations of *C. lacinia* exhibit thermoregulation behaviors such as basking and avoidance posturing. Adults sampled in temperate Arizona and tropical El Salvador maintained similar body temperatures throughout the day. Global warming is predicted to have a positive effect on relative fitness in temperate populations whereas some tropical populations in South America may experience negative relative fitness impacts (Bonebrake et al. 2014).

Mating and Oviposition

In the field, male *C. lacinia* adults typically wait for females on hilltops to mate, with males often patrolling and guarding territories (Brock & Kaufmann 2003). In captivity, we have observed males conducting courtship displays by rapidly beating their wings while "dancing" around the female. However, a female can decline the advance by turning her abdomen upwards or to the side - a behavior noted in other nymphalid species (Pliske 1975). Rapid fluttering of the wings by the female may indicate either acceptance or rejection of the male's advances (JMP, pers. obs.). Copulation usually occurs mid-morning and lasts for approximately 45 minutes (Drummond et al. 1970). In laboratory settings, humidity levels can influence mating behavior, with adults more likely to mate in more humid conditions indoors (JMP, pers. obs.). Therefore, access to areas with preferred levels of humidity is crucial for maintaining a *C. lacinia* laboratory colony.

After successful copulation, the female butterfly searches for a suitable host plant to lay her eggs (Table 1 provides a list of potential host plants). Females use specific behaviors during this process, such as drumming and antennal dipping. Drumming involves rapid tapping of the leaf alternately with the foretarsi, whereas antennal dipping refers to the repeated lowering of the antennae down to the drummed area of the substrate. These behaviors presumably help the female verify the suitability of the host plant using the foretarsal contact chemoreceptors, particularly in environments where airborne olfaction is unreliable (Calvert & Hanson 1982).

Additional Larval Behavioral Ecology

Chlosyne lacinia larvae are gregarious at least until their 3rd instar when they start to become solitary. As many as 800 larvae have been observed on a single host plant (Rasmussen 1979). Upon hatching, larvae gregariously consume and breakdown leaf material, creating a distinctive skeletonized leaf. Group feeding apparently allows the small instars to consume the leaves more easily (Clark & Faeth 2003). Larvae have been experimentally shown to have increased survival rates in groups (Clark & Faeth 2003). The aggregations also may serve as a defensive strategy against predation. We have observed larvae in laboratory settings to have a semi-synchronized movement of rearing back on their prolegs

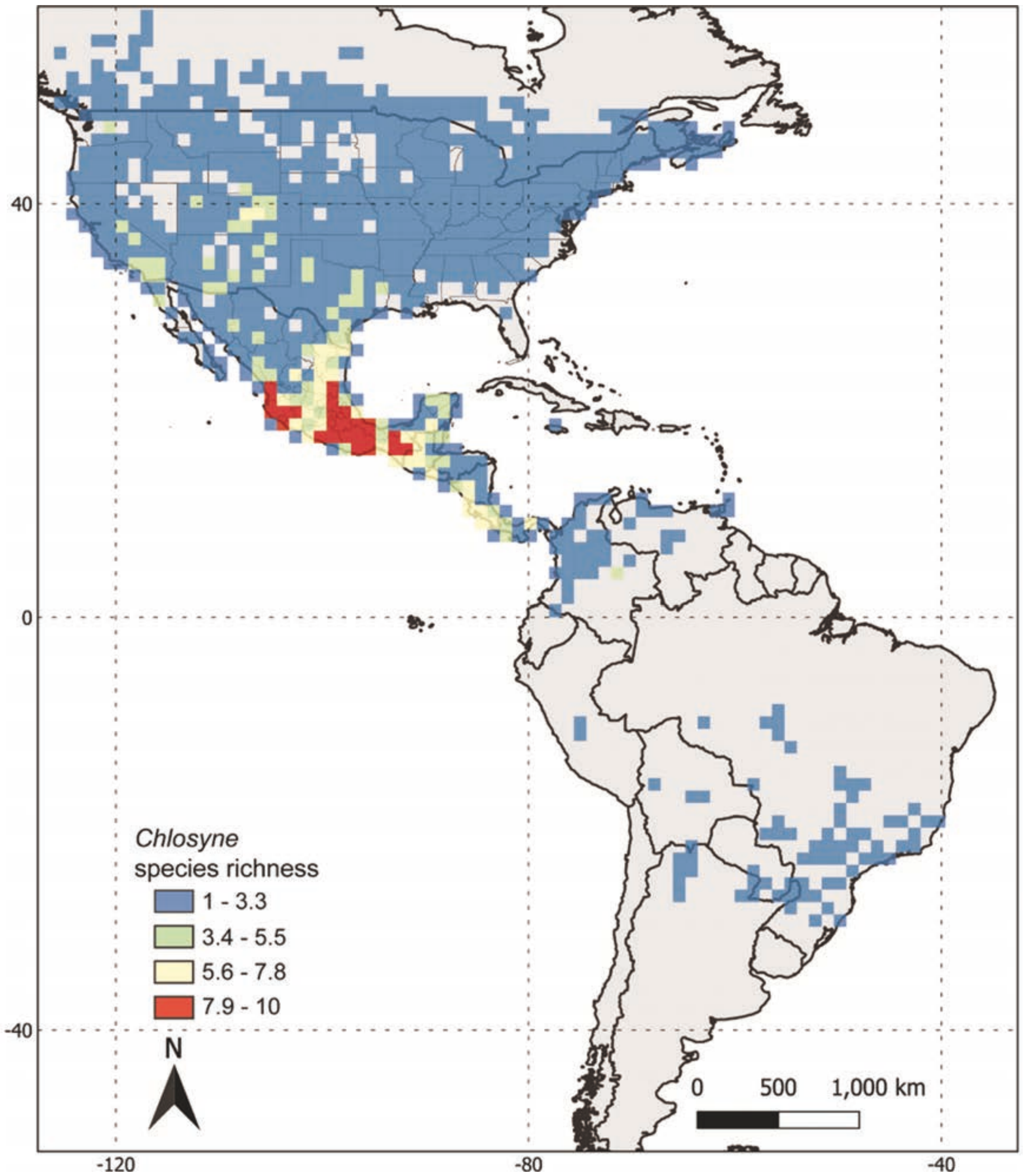

FIG. 5. The richness of all species of *Chlosyne* in 1°x1° grid cells. The maps were plotted in QGIS 3.0, where the grid analysis was performed with the FSC plugin for biological records, version 3.4 (Burkmar 2023). The records were retrieved from the GBIF repository, including iNaturalist photographic records (GBIF 2022).

and waving the raised part of the body back and forth. Even so, this potential group defense and the presumed aposematism, especially of the red-morphed (*bicolor* and *rufa*) larvae, are probably ineffective against predators that attack en masse, such as fire ants (*Solenopsis xyloni*) (Clark & Faeth 2003). As the larvae grow to larger instars (4th and 5th), they can coexist if resources are ample; however, when resources are scarce, we have observed cannibalistic behaviors toward other larvae and pupae in the laboratory (Figure 2C). Egg cannibalism has also been reported (Clark & Faeth 1998).

The larvae use silk to traverse between leaves. When moving from a consumed leaf to a fresh one, one or two larvae will move off the leaf along the stem followed in single file by the rest of the brood. Each larva lays down a silken thread which is reinforced by subsequent larvae. Thus, the group constructs a distinct silken trail (Figure

2B). The feeding aggregation is reformed as larvae arrive onto the new leaf (Bush 1969).

The time of day can also influence aggregation behaviors. Stamp (1977) reported that larvae tended to form smaller groups immediately after 1200 h. Aggregations increased to larger sizes later in the day, with the largest groups occurring between 1600 and 1800 h. The number of single larvae decreased after 1600 h (Stamp 1977).

Future Directions

Variation in *Chlosyne lacinia*'s larval, pupal, and adult morphological traits presents many areas for further study. The proximal and ultimate causes for color variation remain unknown in the pupal stage and understudied in both larvae and adults. For example, whether geographic variation exists in larval-morph frequencies among populations is not known. To further study pupal coloration, we recommend both crossing experiments to investigate a potential genetic component to pigmentation in pupae and experiments to assess developmental or phenotypic plasticity in larvae exposed to different environmental cues such as temperature, humidity, background color, and pathogens. The ultimate evolutionary explanations for larval variation and for pupae that differ in color from nearly entirely black to nearly entirely whitish could begin to be tested with field experiments, especially those in which each morph is exposed to a variety of conditions.

Because most aspects of *C. lacinia*'s behavior, life cycle, and basic biology have been studied in a piecemeal fashion, often consisting of single studies of single populations, much additional variation is likely to be exposed through further investigations throughout the species' geographic range. For example, *C. lacinia* displays interesting courtship behaviors in the laboratory, which may differ among individuals and populations. Further observations and experiments regarding both male "dances" and female acceptance or rejection would be informative. Additionally, although at least one publication suggests that females do not use male wing color to choose mates, this has not been tested in any population of *C. lacinia* (Vane-Wright & Ackery 1984).

The geographic patterning of adult wing-color-and-pattern morphs is a particularly striking phenomenon ripe with opportunity for further study. For example, multivariate statistical analyses of character traits among morphs and intergrades, as well as transcriptomic approaches to adult color variation would aid in our understanding of the different proximal developmental mechanisms that give rise to adult wing-patterning. Because to our knowledge no attempts have been made to determine whether wing colors and patterns provide any aposematic, mimicry, or intraspecific signaling advantages to individuals, various types of experiments with adults that differ in wing color and patterning would be especially informative. For example, mate-choice experiments to test for assortative mating among different morphs of *C. lacinia* could help better understand the evolution of the distinct phenotypes. These experiments could also include other *Chlosyne* species, such as *C. californica* and *C. rosita,* which have strikingly similar dorsal patterning to *C. lacinia*. Further genetic studies, coupled with spatial patterning of biotic and abiotic factors throughout the species' range, could help better understand divergence among the potential lineages and sort among the alternative interpretations (morphs, subspecies, etc.; Edwards 1893, Higgins 1960, Kons 2000, Bonebrake et al. 2011).

While much of the rapid speciation of butterflies took place in the tropics, the Melitaeini tribe, which includes the genus *Chlosyne*, is thought to have originated in the Nearctic (Wahlberg & Zimmermann 2000, Kawahara et al. 2023). To further resolve whether *Chlosyne*, and *Chlosyne lacinia* in particular, also have a Nearctic origin, followed by geographic expansion into Mexico, Central and South America, would substantially advance our understanding of the evolutionary histories of these groups. In addition, understanding whether the evolutionary history of the morphs follows a geographic structure will clarify their origin.

Key questions concern the intergrades identified by Kons (2000): are these hybrids that occupy hybrid zones between populations? If so, have they been stable over the long term, or are they recent phenomena resulting from geographic range expansion by the various morphs, perhaps aided by anthropogenic proliferation of "weedy" food plants? In any case, the geographic distributions of *C. lacinia* or any of its morphs may have changed substantially upon European colonization of the Western Hemisphere. A useful comparison concerns the eastward expansion of *Colias eurytheme* (Pieridae), whose dramatic range expansion was facilitated by deforestation and the widespread agricultural proliferation of its host-plant, alfalfa (Hovanitz 1944). Sunflower domestication is thought to have originated approximately 4000 years ago in North America and spread to El Salvador by the first millennium B.C.E. (Lentz et al. 2001). A combined biogeographic study on the anthropogenic range expansion of domestic sunflowers and the relatively recent biogeographic history of *C. lacinia* could be incredibly useful for understanding the biology of the bordered patch butterfly.

Additional research on larval behaviors would be beneficial in this highly gregarious species. Field and laboratory studies on larval displays, shared use of silk, and preferences for group sizes, would further our understanding of potential larval communication, conflict, and cooperation.

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