Scientific Note: Observations of Queen butterfly (*Danaus gilippus*) larvae with unusual pigmentation in south Texas, USA

Ridlon J. Kiphart[1] and Andrew K. Davis[2,3]

[1]Cibilo Nature Center, 140 City Park Rd., Boerne, TX, USA 78006
[2]Odum School of Ecology, University of Georgia, Athens, GA, USA 30602
[3]Corresponding author: akdavis@uga.edu

Abstract: Lepidopteran larvae are often brightly colored to signal their distastefulness to predators. Within most species, there is usually a degree of individual variation in color patterns that has rarely been studied. Here we report observations, across several years, of larval Queen butterflies (*Danaus gilippus*) in the state of Texas, USA that appeared highly unusual with respect to their color pattern. Queen larvae normally have alternating bands of dark and light stripes and yellow spots, but the Texas caterpillars had patterns of violet-red bands alternating with white bands and minimal yellow spots, giving them an overall red and white appearance. To put these observations into context, we conducted a survey of color patterns of this species by evaluating postings of online photographs from around the country (n=57). In online images, the base color (non-white stripes) of the species was predominantly black (80% of cases), but varied from dark purple to violet. The survey results suggested that the observations of unusual larvae in Texas may represent an extreme form from a naturally occurring, wide spectrum of color patterns in this species.

Key words: Lepidopteran larvae, pigmentation, Queen caterpillar, *Danaus gilippus*, Texas

INTRODUCTION

Many lepidopteran larvae show a degree of intraspecific variation in cuticular pigmentation that is associated with, or caused by, seasonal variation (Hazel, 2002), temperature (Solensky & Larkin, 2003, Davis *et al.,* 2005), differences in host-plants (Yamasaki *et al.*, 2009) or density effects (Anazonwu & Johnson, 1986; Gotthard *et al.*, 2009). For certain species, there is little knowledge of color variation other than descriptions in general field guides and anecdotal reports. The Queen (*Danaus gilippus* (Cramer, 1776)) is one such species. Like its relative the Monarch (*D. plexippus* (Linnaeus, 1758)), its larvae have alternating transverse bands or stripes of black and white, and the Queen usually has dorsal yellow spots. However, official descriptions of the larvae can vary. They have been described as "bluish-white, reddish-brown beneath, with narrow to wide transverse stripes of reddish-black" (Scott, 1986: 231), or as having a "whitish violet body with adjacent stripes purple, reddish brown and yellow" (Devries, 1987: 212). Wagner (2005) stated that they have a "bold combination of yellow and white over reddish brown to jet black ground" (Wagner, 2005: 137). One publication from the scientific literature anecdotally indicated the larvae can have two general 'forms', the typical form and a rarer, 'dark' form, in which the yellow spots are reduced or absent (Calhoun, 1996). Collectively, these reports suggest there is a degree of variation in larval appearance in this species, although it has yet to be fully described.

The current project came about when Queen larvae with unusual color characteristics were discovered in an area in south Texas in the summer of 2010 and then again in summer 2013 and summer 2016. Here we describe these unusual larvae and also explain how we attempted to put these observations into context by examining online photographs of Queen larvae from across the country, to better understand the most frequent color pattern of the larvae.

OBSERVATIONS AND DISCUSSION

Field observations in south Texas

The first observation was on June 26, 2010, at the Cibilo Nature Center (29°46'53.78", 98°42'32.78"W) (www.cibilo.org) in Boerne, Texas, where the lead author volunteers as a naturalist. There is a 600m^2 patch of native milkweed (*Asclepias asperula*) at this location that is regularly monitored by trained volunteers at the nature center for eggs and larvae of monarchs and queens. On this day, one of the volunteers (Lee Morris) observed and photographed what appeared to be a 'red-striped' Queen larva (Fig. 1A). There were no other larvae present on that day. Then on June 29, 2010, the lead author observed a Queen larva similar to this in a milkweed patch in his backyard near Bergheim, TX (Fig. 1B). This site is in a rural subdivision with small patches of *A. asperula*, and it is regularly monitored for larvae by the lead author. To his knowledge, larvae such as these had never before been seen at either location, despite many years of milkweed monitoring throughout the summer months (i.e. 5+ years). Moreover, since these initial observations, there have been additional sighting of 'red-striped queens' at one of these locations. Two more red-striped, 5th instar queens were observed at the Cibilo Nature Center on June 15, 2013 (by Linda Plevak and Antonio Martinez); another was seen on July 6, 2013, and the lead author observed one on June 23, 2016 (Fig. 1C).

The Queen larvae in question each appeared to have white transverse stripes that are typical in color and extent, but the alternating dark bands (stripes) were dark purple or violet (as opposed to black, which is typical) and their yellow dorsal spots were nearly absent (Figs. 1A, B, C). This combination of characteristics resulted in what appeared to be 'red-and-white' striped caterpillars, which starkly contrasts with the usual pigmentation of queens, which consists of alternating white and black stripes with yellow spots (see survey results below).

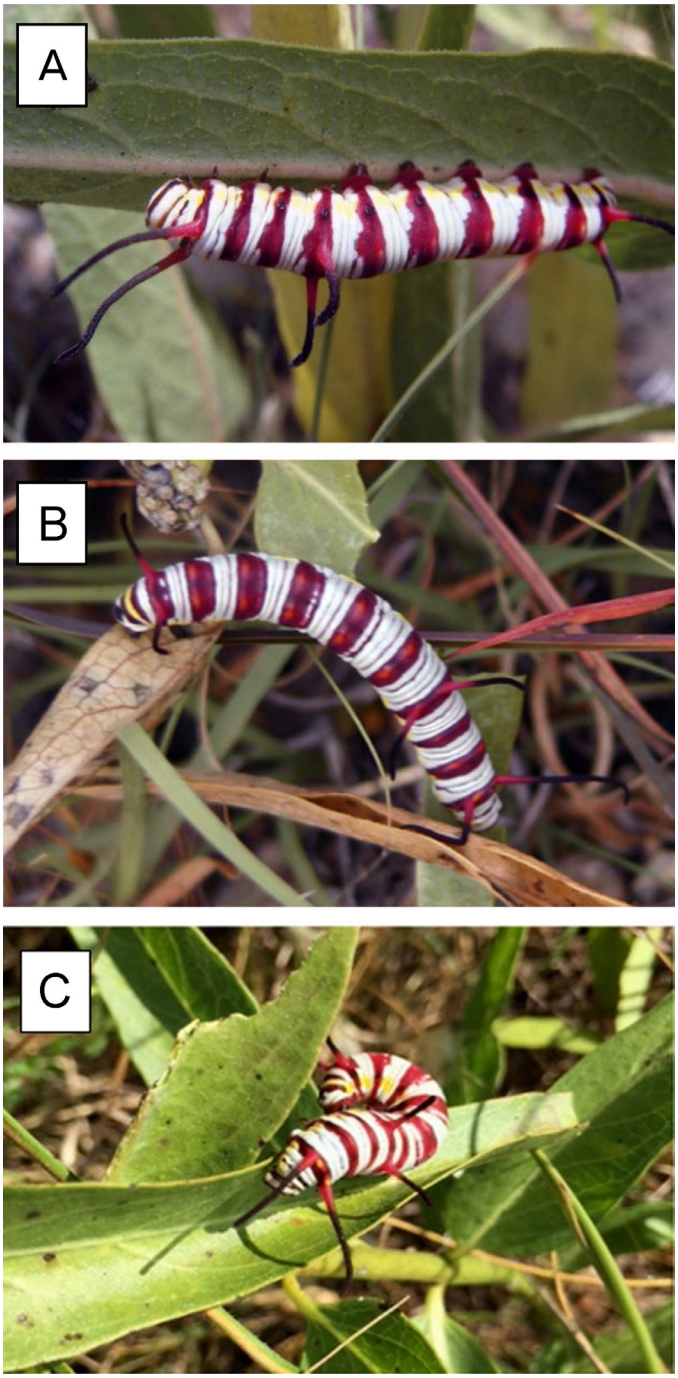

Fig. 1. Photographs of unusual Queen larvae from Texas. Shown are queens from: **A**. Boerne, TX on June 26, 2010. **B**. Bergheim, TX, on June 29, 2010. **C.** Boerne, TX, on June 23, 2016. Photographs were taken by Lee Morris (A), and Skip Kiphart (B, C).

Survey of online photographs

The observations in south Texas prompted us to conduct an exploratory survey of Queen larvae photographs on the internet. The goal of this exercise was to document the variation in Queen larvae characteristics. We used Google as a search engine (www.google.com), and searched for images using the text strings "queen butterfly caterpillar" and "queen butterfly larva". We attempted to limit the search to only photographs of wild larvae (i.e. we did not use images from websites of butterfly breeders) and we only used photographs of late-stage larvae (4th and 5th instar). We also did not use more than one image from any website unless it was clear that different caterpillars were photographed (this only occurred once). Finally, we only used pictures where the caterpillar (photograph) location was specified. This resulted in a total of 57 images of Queen caterpillars from throughout its range (though most images [n=54] were from locations within the United States). The majority of images were posted on personal blogs and on the photo-sharing site, Flickr (www.flickr.com), which Google also searches.

For each photograph, one of us (AKD) recorded basic characteristics of the larval pigmentation. Specifically, the color of the non-white stripes was visually gauged, and we recorded the dominant color of the entire larva (dominantly black, white or intermediate). We also recorded whether the yellow dorsal spots were present or absent (or barely visible). These survey data were compiled in a spreadsheet for elucidating general patterns.

Survey results

The dark (i.e. non-white) stripes of Queen larvae were classified as being black in the majority of photographs (80.7%, Table 1). Example images of larvae with black stripes are shown in Fig. 2 (A, D, and E). The dark stripes of the remaining larvae (19.6%) were various shades of purple, although there were no larvae with the bright violet color of the south Texas individuals (Fig. 1). The purple color can be seen in Figs. 2B, C, and H. There was no obvious geographic pattern to these individuals, based on the locations of the photographs. The lack, or reduction of yellow dorsal spots (Figs. 2D, E) was even rarer among Queen larvae, occurring in only 11% of larvae. Interestingly, most of these (5 out of 6) were only found in the state of Texas, with one observed in Florida (Fig. 2D).

Table 1. Results of a survey of Queen larvae pigmentation based on online photographs. For each photograph, we classified the color of the non-white stripes of the larva. The majority of photographs were of larvae within their range in the United States. There was no obvious geographic pattern to the distribution of colors in this table.

Non-white Stripe Color	# Larvae	%
Black	46	80.7
Dark purple	4	7.0
Purple	2	3.5
Reddish-purple	4	7.0
Violet	1	1.8
TOTAL	57	100

The results of this exploratory survey allow us to put the unusual observations of larvae in south Texas into context. These data indicated there is a non-trivial degree of natural variation in Queen larvae pigmentation across its range, as anecdotally suggested by the varying descriptions from field guides (Scott, 1986; Devries, 1987; Wagner, 2005). About 20% of all Queen larvae appear to have purple or reddish-purple stripes, although none as bright as those in South Texas (Figs.

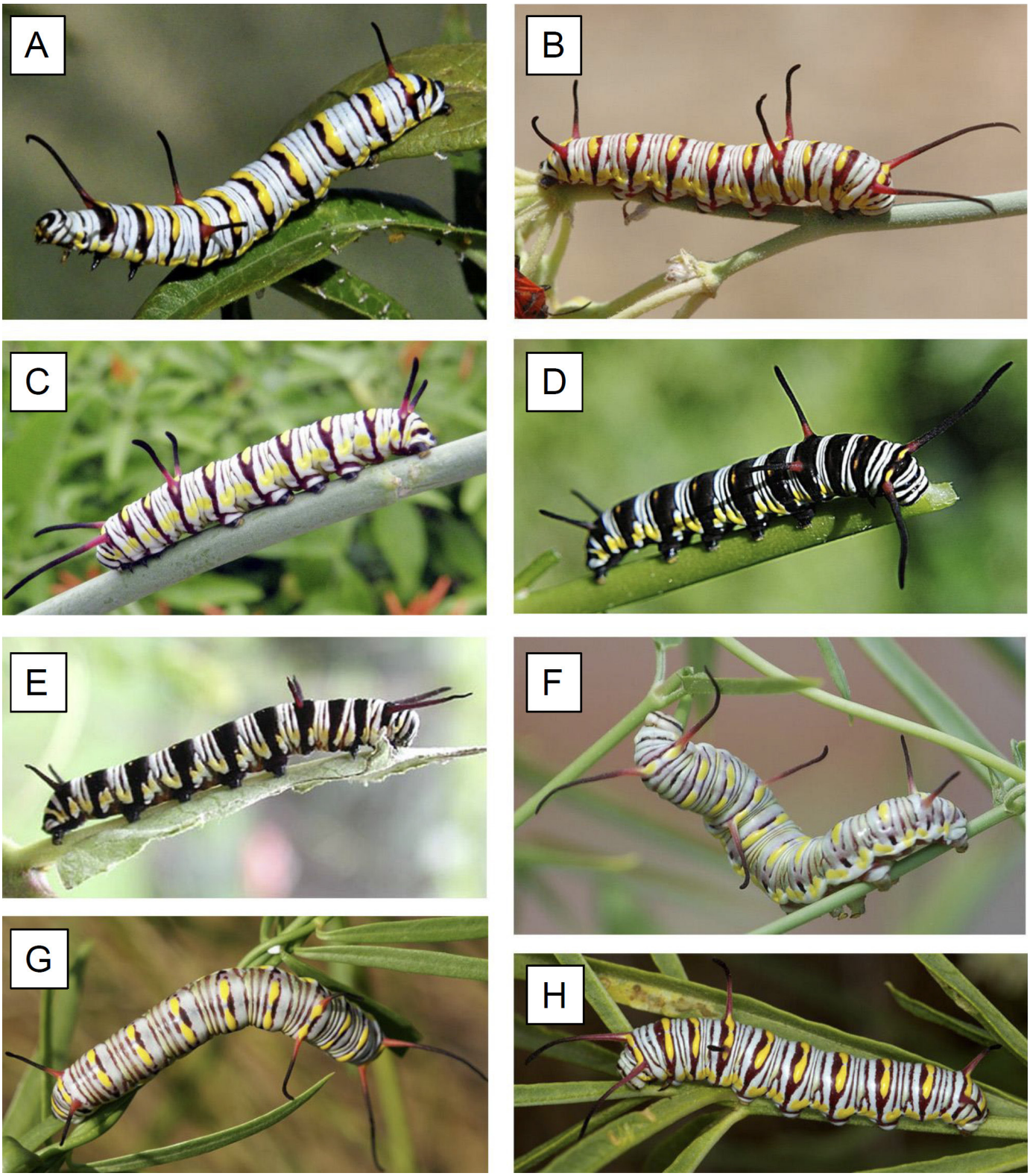

Fig. 2. Photographs of 'typical' Queen caterpillars throughout the distribution of the species, obtained during a survey of online images. Photo locations (photographers) are listed below. Note: while these specimens were photographed throughout the range of the Queen butterfly, the pictures do not necessarily reflect geographic variation in pigmentation, since multiple larval forms can be present in one region. All photos were used here with permission. **A**. Round Rock, Texas (Joseph Kilgus). **B**. Scottsdale, Arizona (Gene Hanson). **C**. Pima, Arizona (David Bygott). **D**. Biscayne Park, Miami, Florida (Susan Ford Collins). **E.** Dallas, Texas (Dale Clark). **F.** Pima, Arizona (Michael J. Skinner). **G**. St. Catherines Island, Georgia (Christa Hayes). **H**. Thomas F. Riley Wilderness Park, Orange County, California (Jay Cossey).

1A, B, C). Moreover, the lack of yellow dorsal spots, such as seen in the Texas sightings, is even rarer. In fact, besides the Texas sightings, we found no other photographs of larvae with both these characteristics.

The biological significance for the unusual pigmentation of the Texas Queen larvae is unclear, and will require further field and laboratory investigation. In some parts of their range, Queen larvae may mimic larvae of other co-occurring danaine species (Willmott *et al.*, 2011), although that explanation seems unlikely for the current observation in Texas given the lack of other butterflies with these characteristic larval markings. A more plausible explanation is that the observed pattern is a result of the high temperatures typical of the south Texas region. In the Boerne, TX location, the average maximum temperature for the month of June ranged from 29-35ºC between 2010 and 2016 (www.wunderground.com). Prior research on another lepidopteran larvae with a reddish-purple background color, the pipevine swallowtail (*Battus philenor* (Linnaeus, 1771)), showed how these larvae can look more reddish in color when reared under high temperature (36ºC), and they become dark purple at 30ºC (Nice & Fordyce, 2006). This is thought to be an adaptive response to avoid overheating, since darker pigmentation absorbs more solar radiation. While *B. philenor* and *D. gilippus* are unrelated, they may share this same adaptation given the similarities of the larval base color.

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