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Tri-trophic movement of carotenoid pigments from host plant to the parasitoid of a caterpillar

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ABSTRACT

Insect parasitoids normally produce white colored eggs. *Habrobracon gelechiae* (Hymenoptera: Braconidae) is a gregarious ectoparasitoid of various caterpillars. We found that adult female *H. gelechiae* lays yellow colored eggs when its larvae developed from host larvae of *Choristoneura rosaceana* and *Epiphyas postvittana* (both Lepidoptera: Tortricidae) that were fed green plant leaves, but white colored eggs when these same host larvae species were fed non-plant diets. This study investigated the causes of egg color in *H. gelechiae* and the possible consequences in terms of parasitoid fitness resulting from differential egg color. Using high-performance liquid chromatography we demonstrated that the yellow coloration resulted from the uptake of carotenoid plant pigments (mainly lutein and β-carotene) that were initially ingested by the caterpillar larvae from plant leaves, later absorbed by the parasitoid larvae (F_0) feeding on the host and carried over to the adult parasitoids, and finally translocated to the eggs (F_1) of the parasitoids. The amount of plant pigments consumed by the parasitoid larvae (F_0) affected the intensity of the yellow color of the parasitoid's eggs (F_1). Similarly, egg color was affected by the adult female parasitoids lifetime egg production and deposition rate. Further tests suggest that the observed differences in egg color did not have a genetic basis and did not affect egg viability or fitness. To our best knowledge, this is the first report of a tri-trophic and multi-stage translocation of carotenoid plant pigments in parasitoids. We discuss possible evolutionary significance and putative functions of the absorption of plant pigments by parasitoid species.

1. Introduction

Many insects exhibit great plasticity in color morphs or patterns in response to environmental conditions such as temperature, diet, or environment (Greene, 1989; Bond, 2007; Tanaka et al., 2012). Color polyphenisms in caterpillars, for instance, can be induced by rearing temperature, seasonal differences in diets such as from ingested leaves or background colors (i.e., aposematism) (Hudson, 1966; Losey et al., 1997; Nijhout, 2003; Sandre et al., 2007; Noor et al., 2008). Many insects sequester plant pigments that function in pigmentation for protection by means of camouflage, mimicry, or warning coloration to avoid predators (Bohm, 1998; Barbosa and Caldas, 2007; Sandre et al., 2007) or sexual attraction (Harborne and Grayer, 1994; Carroll et al., 1997), and as antioxidants to enhance physiological functions (e.g., immunity, nutrition, or photo-protection) (Hinton, 1981; Eichenseer et al., 2002; Ojala et al., 2005; Cazzonelli, 2011).

Color polyphenism of insect eggs, however, has rarely been observed (Hinton, 1981), especially for parasitoid eggs, which are normally white colored. One exception of egg color polyphenism in parasitoids, as reported briefly by Gupta (1951), was for the parasitoid *Bracon* (= *Habrobracon*) *gelechiae* Ashmead (Hymenoptera: Braconidae). Gupta (1951) reported that adult female *H. gelechiae* laid white colored eggs when, as larvae, they were reared from moth larvae of *Gnorimoschena operculella* (Zell.) (Lepidoptera: Gelechiidae), *Scirpophaga nivella* Fabricius, *Argyria sticticraspis* Hampson, *Chilo zonellus* Swinhoe (all Lepidoptera: Pyralidae), and *Sesamia inferens* Walker (Lepidoptera: Noctuidae); whereas yellow colored eggs were laid by *H. gelechiae* that as larvae were reared from *Plusia orichalcea* Fabricius or *Earisa insulana* Boisduval (all Lepidoptera: Noctuidae). This led the author to conclude that the moth host species induced the parasitoid's color polyphenism, although critical information, such as the caterpillars' food (plants or artificial diets), was not given in this early report (Gupta, 1951).

Habrobracon gelechiae is a gregarious ectoparasitoid, attacking various host caterpillar species (Clausen, 1978; Daane et al., 2013). The adult female first stings and paralyzes a host larva and then lays a clutch of eggs onto the host's external surface or nearby the host larva; the developing parasitoid larvae consume

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the host larva before they pupate (Daane et al., 2013). *Habrobracon gelechiae* is native to North America and commonly attacks the obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) in California (Daane et al., 2013), a polyphagous moth pest that is native to and distributed throughout North America (Chapman et al., 1968). *Choristoneura rosaceana* larvae feed on various fruit crops including apple, pistachio, peach, pear, plum, and cherry (Chapman et al., 1968). The parasitoid was also found to readily attack the light brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Wang et al., 2013a,b), an introduced polyphagous pest of various fruit and horticultural plants in California (Wang et al., 2012). Recently, we noticed that adult female *H. gelechiae* produced yellow eggs when, as larvae, the parasitoids were reared on *C. rosaceana* or *E. postvittana* caterpillars feeding on leaves of green host plants; whereas this parasitoid laid white eggs when, as larvae, they were reared on the same caterpillar species that had been fed non-plant artificial diets.

We suspect that the observed egg color polyphenism in *H. gelechiae* may be induced not by host species, as reported by Gupta (1951), but rather from the absorption and translocation of yellow plant pigments in the food chain, successively through three trophic levels. Pigments are synthesized by plants and algae, as well as some bacteria and fungi (Armstrong and Hearst, 1996; Hirschberg, 2001). In plants the pigments are found primarily in the leaves and flowers and include chlorophyll, which gives plants their green color, and carotenoids, which gives plants various other colors (yellow, red, or orange) (Goodwin, 1988; Bohm, 1998). The most abundant carotenoid is lutein (a yellow pigment), which is often not obvious because of the presence of chlorophyll. However, when chlorophyll is not present, the carotenoids are dominant (Goodwin, 1988). Carotenoids constitute a large group of compounds and some can be modified after their absorption by animals (Armstrong and Hearst, 1996; Rodriguez-Amaya, 2001; Maoka, 2009). However, this is rare and most insects are incapable of synthesizing carotenoids, with two known notable exceptions – the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) (Moran and Jarvik, 2010) and the two-spotted spider mite *Tetranychus urticae* Koch (Arachinida: Tetranychidae) (Altincicek et al., 2012) – which have each uniquely acquired the carotenoid biosynthesis genes from fungi via horizontal gene transfer to synthesize carotenoids. Many animals absorb, but do not synthesize, plant pigments through dietary intake from plants or algae (Armstrong and Hearst, 1996; Hirschberg, 2001; Cazzonelli, 2011). In fact, the sequestration of plant pigments by insects is common and the presence of plant pigments is often responsible for the yellow–green color of many caterpillars, with the ingested plant pigments accumulating in the hemolymph and tissues (Cromartie, 1959; Feltwell and Valadon, 1972; Carroll et al., 1997; Eichenseer et al., 2002; Sandre et al., 2007). For example, the parsnip webworm *Depressaria pastinacella* Stainton (Lepidoptera: Oecophoridae) selectively acquires lutein and other xanthophylls from flowers of *Pastinaca sativa* (Apiaceae) (Carroll et al., 1997).

Tri-trophic transfer of plant pigments is reported only in plant–insect–bird food chains, where some insectivorous birds accumulate pigments from leaf chewing insects (e.g., Partali et al., 1985). However, a tri-trophic movement or multi-stage translocation of plant pigments has not yet been reported in plant–insect–parasitoid food chain. To this aim, we investigated egg color polyphenism in *H. gelechiae* and its possible consequences in terms of parasitoid fitness. First, we demonstrated how the caterpillar host food (green plants vs. non-plant artificial diet), rather than the caterpillar species itself, caused the observed egg color polyphenism in *H. gelechiae*. In this experiment, we used two common host species, *C. rosaceana* and *E. postvittana*, and fed these caterpillar larvae either leaves from different host plant species or a dry-bean based artificial diets. Second, we performed high-performance liquid chromatography to identify yellow plant pigments in the hemolymph of host larvae fed green plants and in the egg of the resulting adult female parasitoids that developed from the plant-fed host larvae. Third, we confirmed that the yellow pigmentation of the parasitoid's eggs was not present or detectable in the subsequent generation (i.e., no inheritance). Fourth, we showed that the intensity of the yellow color of the parasitoid's egg decreased with the increasing number of oviposition events, which indicates that the color intensity may depend on the amount of yellow plant pigments absorbed by the parasitoid larvae and carried over to the adult females. Finally, we observed whether or not yellow pigments affected egg viability or fitness.

2. Materials and methods

2.1. Insects

Studies were conducted under controlled conditions (24 ± 1 °C, 16L:8D, 40–60% RH) at the University of California's Kearney Agricultural Research and Education Center (Kearney) in Parlier, California (CA), and the University of California's Insectary and Quarantine Facility in Berkeley, CA.

The *C. rosaceana* colony was established with field-collections from pistachio orchards in Fresno County, CA, in 2006, and then maintained at Kearney on an artificial diet (water, dry lima bean, wheat germ, torula yeast, agar, fructose, alphacel, ascorbic acid, wheat germ oil, sorbic acid, formalin, methyl p-hydroxybenzoate, and cholesterol; Krugner et al., 2005) using the rearing methods as described by Daane et al. (2013). The *E. postvittana* colony was established with field-collections from Santa Cruz County, CA, in 2007, and then maintained at Berkeley on an artificial diet (water, dry lima bean, brewer's yeast, agar, ascorbic acid, Vanderzant vitamins, sorbic acid, formalin, and methyl p-hydroxybenzoate; Cunninghan, 2007) using the rearing methods as described by Bürgi and Mills (2010).

A laboratory colony of *H. gelechiae* was established from parasitized *C. rosaceana* larvae that were field-collected from pistachio orchards in Fresno County, in 2010. The parasitoid was then maintained on *C. rosaceana* larvae from the Kearney colony, or *E. postvittana* larvae from the Berkeley colony. Rearing methods of the parasitoid on both host species were similar. Adult parasitoids were held in large (61 × 61 × 61 cm) organdy cages (Bug Dorm2, BioQuip, Rancho Dominguez, CA) with water and honey provided for the wasps. Since the parasitoid prefers larger larvae (Wang et al., 2013b), 5th instar hosts were used for the culture. Host larvae were collected from diet cups, and exposed to adult parasitoids either in folded tissue paper (*C. rosaceana*) or 150 ml plastic cups (*E. postvittana*). The exposure period was generally 1–2 d, which resulted in >80% parasitism. Parasitized larvae were then transferred to clean Bug Dorm2 cages and held for the emergence of adult wasps. Experiments using *C. rosaceana* were conducted at Kearney while those using *E. postvittana* were conducted at Berkeley (*E. postvittana* is a newly invasive pest that could not be brought to Kearney (Fresno County), where it has not yet been found). All experiments were conducted under the same room conditions as described previously.

2.2. Egg color of the parasitoid

To determine if host food or host species would induce egg color polyphenism in *H. gelechiae*, the parasitoid larvae were reared on *C. rosaceana* or *E. postvittana* larvae fed green plants or non-plant artificial diet, respectively. Early instars of *C. rosaceana* or *E. postvittana* were first reared on artificial diets, as described previously. After