Scientific Note: Baculovirus infection may affect wing color of the Zebra Longwing butterfly

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Abstract: While melanization in insects is a well-documented physiological response to wounds, bacterial, fungal and viral infections, and parasites, the potential influence of the process on the wing color of Lepidoptera has not received as much attention. Here we report a case in which the response to melanization influenced the wing color of a subgroup of *Heliconius charithonia* (Nymphalidae) infected with baculovirus. The individuals that survived the infection had dark-orange rather than light yellow stripes. This observation may help to explain dusky-winged melanic variants in this and other species that are known to be occasionally collected in the wild. The next logical step will be to culture the virus and to conduct a set of controlled experiments infecting mature larvae at different stages of development, in order to document the precise moment at which the infection transitions from being lethal to becoming color-altering.

Key words: aberration, melanism, phenotypic plasticity, variation, wing coloration

Melanization in insects as a physiological response to wounds, bacterial, fungal and viral infections is well-documented and has received considerable attention in recent years (e.g., Nakhleh *et al*., 2017, and references therein). In Lepidoptera, as in many other insects, melanin is also very important for forming wing pattern, and, in butterflies, the genetics of melanism has been explored by different research groups using different models and from different perspectives (e.g., Wittkopp & Beldade, 2009; Monteiro *et al*., 2013; Zhang *et al*., 2017 and references therein). Frequently, melanic polymorphisms are under genetic control, as for instance in the polymorphic Tiger Swallowtail butterfly, where females can be striped yellow or mimetic black (Scriber *et al*., 1996). Variation in melanism in Lepidoptera and other insects can also be environmentally induced through heat shock, slight variation in rearing temperature, and by chemicals (e.g., Otaki, 2008; Mongiardino *et al*., 2012; Sourakov, 2015, 2018, and references therein).

The influence of infection and injury on melanization of Lepidoptera wings is less understood. Here we report melanization likely due to immune response in a subgroup of the Zebra Longwing butterfly, *Heliconius charithonia* (Nymphalidae), which, while reared in captivity, was accidentally infected with a baculovirus. Six perfectly formed surviving butterflies from this subgroup produced dark-orange phenotypes sharply contrasting with the normal yellow striped ones.

On July 2, 2017, a single batch of 16 presumably sibling eggs was collected by AS on a shoot of *Passiflora* 'incense' (*coccinata* x *incarnata*) (Passifloraceae) in Gainesville, Florida (Fig. 1A). The resulting caterpillars were reared in a single container until the last instar, when two caterpillars were randomly isolated into individual containers to complete their development, while the rest continued to be reared gregariously, gradually removed one-by-one as they entered the prepupal stage. The 14 caterpillars that were kept together were presumably infected with baculovirus ingested with the plant. Ten caterpillars that were ahead in development pupated, while the remaining four died with symptoms corresponding to a typical baculovirus infection, which leads to cellular lysis "liquefying" the hosts. Among the ten gregariously-reared individuals that pupated, six emerged into aberrant, dusky-winged, but otherwise normal adult butterflies (Fig. 1B), three emerged but died immediately upon emergence unable to spread their wings, and one died fully formed inside the pupa, all with altered wing colors. The two larvae that were removed into the separate containers and reared in isolation resulted into normal adults (Fig. 1C). Microscopic examination of the ethanol-preserved dead insects by JM confirmed the presence of occlusion bodies diagnostic of baculovirus infection, supporting not only the hypothesis that the death occurred as a result of the infection (that has very characteristic symptoms and is highly contagious), but also leading to a strong supposition that the wing color change in the surviving gregariously-reared individuals was induced by baculovirus.

The conclusions based on the observations reported above are obviously preliminary and are contingent on conducting controlled experiments. The next logical step will be to culture the virus, which is likely to be host-specific and probably undescribed, and to conduct a set of controlled experiments,

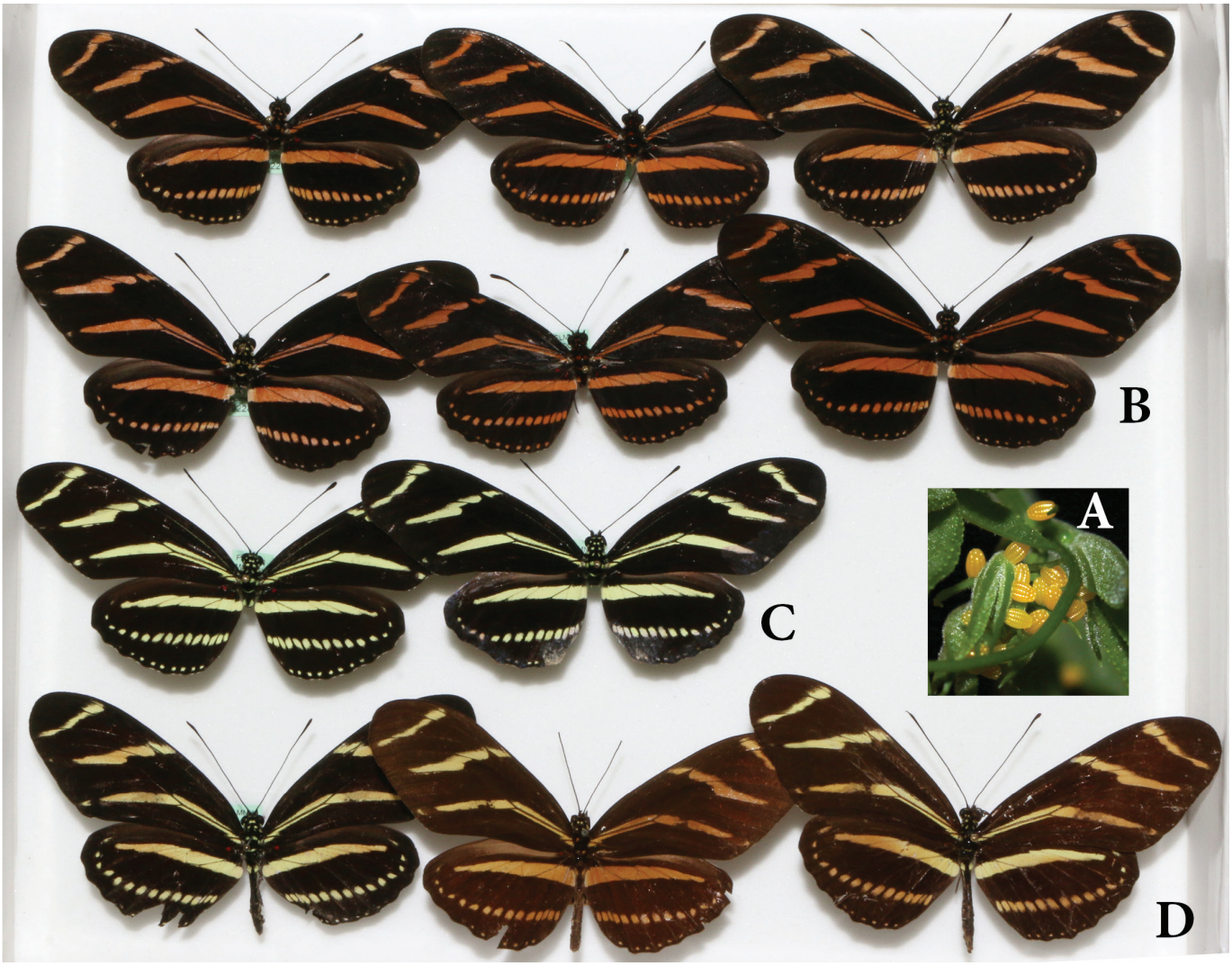

Figure 1. *Heliconius charithonia*: (A) an egg batch from which individuals figured in (B) and (C) were reared; (D) wild-caught specimens exhibiting different degrees of melanization.

Figure 2. Sample *Heliconius charithonia* individuals reared from a single egg batch in Gainesville, Florida in July 2017: (A) presumably infected with baculovirus during development, (B) one that avoided infection. (i) dorsal, (ii) ventral.

infecting mature larvae at different stages of development and analyzing the resulting wing color. We decided to report our observations at the present stage since other *Heliconius* research groups may be better equipped to follow up with the necessary experiments, especially those where the corresponding melanin-related molecular pathways could be simultaneously explored.

While rare aberrations are frequently interpreted by taxonomists as genetic (e.g., as representing a new rare taxon or an unusual mutation), they are as likely to result from environmental factors such as temperature shocks and infections. For instance, the first author (pers. obs.) obtained occasional dusky-winged individuals among several hundred *Utetheisa ornatrix* (Lepidoptera, Erebidae) that he reared in the lab, and dusky-winged and/or otherwise melanized individuals of *Hypercompe scribonia* (Lepidoptera, Erebidae) from pupae that were accidentally dropped and injured at the early stages of pupal development. It is very likely that the occasional more or less orange-striped wild-caught aberrations of *H. charithonia* (e.g., Fig. 1D) are results of infections that the insects were able to overcome during development. These aberrations have been occasionally seen and documented photographically among commercially reared individuals (e.g., by Chin-Lee (2007) in the Butterfly Rainforest of the Florida Museum of Natural History), but they are too rare and too sporadic to represent genetic mutations. Nevertheless they are striking, and when, as in our case, the melanization is perfectly symmetrical, aberrant individuals placed side by side with the typical form (Fig. 2) may be easily mistaken for a different species or a case of polymorphism. This note therefore also demonstrates some of the difficulties in interpreting naturally found variations in Lepidoptera wing color.

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