Experimental evidence for polyphenism in *Automeris io* (Lepidoptera: Saturniidae) in north Florida

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Abstract: We tested anecdotal evidence that *Automeris io* is a polyphenic species in the southeastern United States by using a split-brood design experiment involving five broods and over 150 individuals. We split each brood into two groups, one that underwent diapause and one that did not. We achieved 100% diapause in the diapausing group by rearing larvae under the ambient light and temperature conditions of October-November in Gainesville, Florida. We eliminated diapause in the second experimental group by rearing larvae in the lab under 24 hr-light conditions at 25°C. The yellow ground color of the forewings and the corresponding hind wing margin colors were displayed by 100% of the males in the non-diapausing group (N=34). In contrast, the orange or tawny color on the forewing and the corresponding hind wing margin was obtained in 90% of the diapausing males (N=50), which emerged throughout the following summer. There were no obvious differences between diapausing and non-diapausing females. Thus we established a correlation between diapause and sex-limited polyphenism, which appear to have similar or perhaps identical initiating trigger(s). However, this experiment and other evidence suggest that the diapause is not the trigger for the expression of polyphenism.

Key words: aberration, bivoltine, environment, genetics, inbreeding, organismal ecology, phenotypic plasticity, univoltine

INTRODUCTION

The possibility of polyphenism in the southeastern United States populations of *Automeris io* has been previously suggested (Manley, 1990; Sourakov, 2014). It has been suggested that the males of this species are more likely to display a rose, orange, or tawny forewing ground color in the spring when they have undergone diapause as pupae, whereas their normally yellower siblings skipped diapause or fly later in the year. These observations, however, based on either seasonality of these morphs or on lab rearings with diapausing and non-diapausing groups resulting from similar conditions, required experimental evidence. Heretofore it remained unclear as to what degree environmental conditions influence diapause and coloration in this species, and how much of either have a genetic basis.

Sourakov (2014) concluded that the diapause could be easily bypassed by raising *A. io* caterpillars under a 24 hr-light regime, and that diapause correlates with darker wing color in males. In order to confirm the presence of this correlation, we divided caterpillars from five broods into two groups and raised one group under artificial lab conditions, which led to non-diapausing specimens, and one under conditions found naturally during October-November, which resulted in diapausing pupae. The results concerning observed polyphenism are reported below.

MATERIALS AND METHODS

A total of five broods (2016-4, 2016-5, 2016-6, 2016-7 and 2016-10, initially ca. 400-500 first instar larvae) were used in the split-brood design experiment conducted between September 2016 and August 2017. The origins of these broods are listed in Table 1. Eggs from each brood were divided into two groups: one reared in a lab at 24 hr-light and 25ºC, and another reared indoors, but in ambient temperature and light conditions. With a few exceptions of caterpillars raised on black oak (*Quercus nigra*), the majority were fed sugarberry (*Celtis laevigata*). The group of brood 2016-5 that was reared under ambient conditions was brought into the lab during the fourth-fifth instar and were reared to pupation there. Plant material was replaced with freshly cut branches three times a week. Pupae were monitored daily for emergence.

Additionally, the first author reared four broods (2017-4XW and 2017-7XW, 2017-7X7 and 2017-7X10, see Table 1 for their origins) on various oaks under ambient conditions outdoors during September-October 2017 starting at 3rd-4th instars (prior to that the immature stages were kept in the lab under a 24 hr-light regime).

Table 1. The origins of *Automeris io* broods used in the present study.

Brood name	Male parent source	Female parent source
2016-1	wild	wild, Gainesville, FL
2016-4, 2016-6, 2016-7	Williston, FL, 3 different wild males	offspring of 2016-1
2016-5	2016-1	2016-1
2016-10	wild, Gainesville, FL	wild, Williston, FL
2017-4XW	wild, Williston, FL	2016-4
2017-7XW	wild, Williston, FL	2016-7
2017-7X7	2016-7	2016-7
2017-7X10	2016-7	2016-10

RESULTS

With the exception of a single individual, all pupae reared under 24 hr-light conditions, including brood 2016-5 which was under these conditions only from the fourth instar, skipped diapause and emerged 25-30 days following the spinning of the cocoon. There were 62 such individuals in total, with 34 males (55%). All typical non-diapausing males in this group can be described as having predominantly yellow forewings

Brood #2016-4. Emerged 29.XI.2016

Brood #2016-4. Emerged 08.VI.2017

Brood #2016-6. Emerged 30.XI.2016

Brood #2016-6. Emerged 22.VI.2017

Brood #2016-7. Emerged 29.XI.2016

Brood #2016-6. Emerged 23.V.2017

Brood #2016-10. Emerged 29.XI.2016

Brood #2016-10. Emerged 16.VI.2017

Fig. 1. Sibling non-diapausing (left) and diapausing (right) males of *Automeris io*, raised under different conditions representing four different broods (see text for details).

with varying amounts of brown or pink basally over the yellow ground color and with a yellow hind wing margin (Fig. 1, left).

In contrast, 100% of their siblings, reared under October-November 2016 ambient conditions, went into diapause, and 90% of males in this group can be described as having either orange or tawny forewings and a beige, rose or orange hind wing margin (Fig. 1, right). There were a total of 46 such males, with four diapausing males exhibiting a color pattern characteristic of non-diapausing individuals (predominantly yellow).

The group of larvae from brood 2016-5 that were reared under ambient conditions up to the 4^{th}-5^{th} instar, which were then brought into the lab and reared to pupation under a 24 hr-light regime, resulted in 100% of males displaying yellow ground color typical of non-diapausing individuals.

DISCUSSION

While *Automeris io* is highly polymorphic in the southeastern United States, polyphenism related to diapause has been thus confirmed beyond a doubt by the present study. The 10% of the individuals that did not exhibit orange color despite exhibiting diapausing behavior suggested that while the correlation between the diapause and orange color has been shown, the diapause is not a trigger for the expression of polyphenism.

This conclusion was further confirmed by additional evidence described below. On two occasions, the late instar caterpillars of *A. io* that were found in nature in September and reared through in the lab emerged without having undergone diapause, resulting in orange individuals. The four broods reared outdoors in August-September 2017 resulted in many non-diapausing males which, as expected, displayed a yellow forewing ground color, but with several exceptions of orange forewings typical of the diapausing males in the experiment described above.

In addition to the obvious differences in photoperiod between the 2016 and 2017 ambient rearing conditions, the temperature differed according to normal seasonal changes in Gainesville, FL: average low/highs 20/32ºC in September; 12/24ºC in November (weather.com). These temperature differences were reflected by differences in development of *A. io*. For instance, while a typical prepupal stage (time from cocoon-spinning to pupation) in November and in the lab takes 7-8 days, under the ambient conditions of September-October, the same stage takes as little as 3-4 days. Proportionately, pupal development took 25-30 days under lab conditions of *c.* 25ºC, but occurred in as little as 16 days under September-October ambient temperatures.

Based on the phenology of *Automeris io* in Louisiana, Brou (2003) put into question the traditional view of the bivoltine nature of the species in which the overwintering generation results in a spring flight, which lays eggs in May-June and gives rise to caterpillars that develop over the summer and result into the second (September) generation whose offspring undergo a diapause. He suggested that the species flies continuously throughout the summer. The emergence of the diapausing group in our experiment supports Brou's view, as it occurred throughout the summer. Thus, while *A. io* in north-central Florida undoubtedly is capable of producing more than one generation per year as shown in our rearing, the May-June and August-September flight peaks may result from several generations of moths whose emergence from pupae is stimulated by natural conditions.

Based on the fact that transferring larvae from diapause-causing conditions to non-diapausing conditions as late as 4^{th}-5^{th} instar caused a non-diapausing pupae and phenotype suggests that diapause in *A. io* is activated by stimuli experienced in the final instars (6^{th} in males, 7^{th} in females) or in the prepupal stage.

Recent experimental wing pattern manipulations of *A. io* using sulfated polysaccharide injections (Sourakov, 2017) suggest that wing pattern determination occurs during the prepupal stage or earlier. More experimental work under manipulated but controlled laboratory conditions is required to pinpoint the exact moments in determination of wing pattern related to polyphenism and the exact environmental conditions involved. It would also be interesting to explore the molecular basis of the observed phenomenon.

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LITERATURE CITED

Brou, V. A. 2003. *Automeris io lilith* (Strecker) in Louisiana. *News of the Southern Lepidopterists' Society* 25: 44-45.

Manley T. R. 1990 (1991). Heritable color variants in *Automeris io* (Saturniidae). *Journal of Research on the Lepidoptera* 29: 37–53.

Sourakov, A. 2014. On the polymorphism and polyphenism of *Automeris io* (Lepidoptera: Saturniidae) in north Florida. *Tropical Lepidoptera Research* 24: 52-59.

Sourakov, A. 2017. Giving eyespots a shiner: Pharmacologic manipulation of the Io moth wing pattern. *F1000Research* 6:1319 (doi: 10.12688/f1000research.12258.2).