

ON THE POLYMORPHISM AND POLYPHENISM OF *AUTOMERIS IO* (LEPIDOPTERA: SATURNIIDAE) IN NORTH FLORIDA

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Abstract – Raising field-collected batches of *Automeris io* eggs and larvae in north central Florida and further breeding the resulting lines in the lab for over three years yielded over 200 highly variable specimens. The polymorphism and seasonal polyphenism in male coloration are illustrated and analyzed. Expression of the rose or tawny-orange ground color of the forewing is shown to be provoked by the diapause, as was previously suggested by others. However, it was found that this expression is not clear-cut and is determined by the genetics of the parental stock and of the individual moth, and seems to be linked to the propensity for diapause and to the diapause duration. Gainesville, north-central Florida, where the study was conducted, appears to be in a transitional zone between the more northern subspecies *A. io io* and the more tropical *A. io lilith*, and both are present here, expressing not only different phenotypes, but also different phenotypic response to diapause. During the breeding experiments, *A. io* underwent two generations per year when kept at the natural light cycle, with two emergences, one in April-June and another in September-November, followed by a winter diapause. Many of the pupae from the summer generation broods went into diapause instead of emerging in the fall, which, in nature, should reduce the risk of mortality for the whole brood. The diapause can be eliminated in the fall generation in most (though not all) individuals by raising larvae at 24 hour-light cycle.

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

INTRODUCTION

Thomas Manley, who bred thousands of *Automeris io* from stocks originating around the United States, suggested that any large sample of *A. io io* in the north-east of the country contains males with brown scales on the dorsal forewing and that selective breeding can quickly increase the number of these scales (Manley 1990). He contrasted these basal patches with the rose, tawny, or brown all-wing diffused coloration of diapausing generations from coastal Georgia and peninsular Florida, the populations of which he referred to as *A. io lilith*. Manley (1990) suggested that this diffused coloration is determined by seasonal polyphenism. Although his discussion of male phenotypes and their variability is extensive, his illustrations of the upper forewing surface are limited to seven individuals, and none is featured from Florida. He intended to discuss the status of *A. io lilith* from Florida “in a later paper” (Manley 1990, p. 44). A year later, he stated in his paper on diapause of *A. io*, in which no moths were illustrated, that “Males of diapausing generation of *A. io lilith* in Florida are phenotypically distinct from those of the non-diapausing generation(s). Males from diapausing pupae are tawny brown, whereas males from non-diapausing pupae are yellowish with a light suffusion of rosy-red scales” (Manley 1991, p. 309).

In the present note, I report some of my observations concerning polymorphism and polyphenism of *A. io* populations around Gainesville, Florida. Gainesville is uniquely located on the border between temperate and tropical regions. Its tropical climate and vegetation allow for many tropical insects to thrive there, yet the freezing temperatures that occur several times every year force most insects into diapause or some other form of hibernation. Data and illustrations presented below resulted from breeding local *A. io* for three years in the lab, which was conducted in the hope of understanding the extent and the origin of the phenotypic diversity in this variable species. For breeding methods, one is referred to Sourakov (2013).

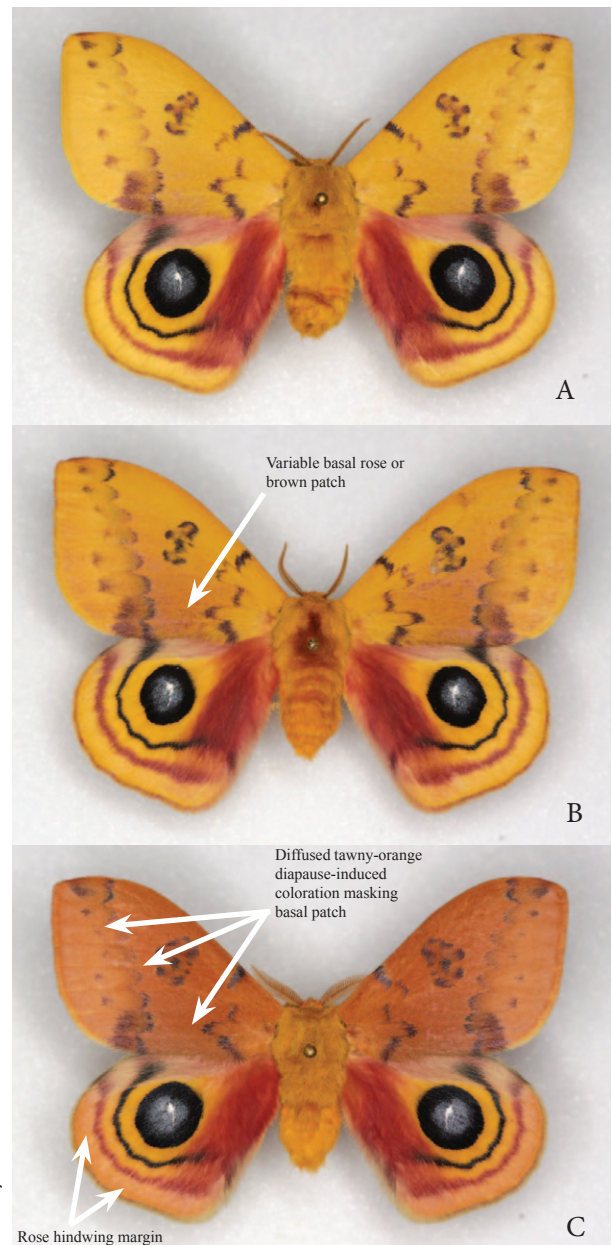


Fig. 1. Three among 9 sibling males of *Automeris io* nr. *io*, raised from wild collected egg batch, Gainesville, north-central Florida. (A, B) Two of the seven yellow males represent non-diapausing phenotypes which vary in the extent of basal forewing patch; (C) Male showing diffused tawny-orange coloration of the forewing and rose-orange hindwing margin represents a diapausing phenotype.



Fig. 2. Males of *Automeris io* nr. *lilith*, raised from sibling-to-sibling crosses (A, B, C – brothers; D – their cousin), Gainesville, north-central Florida.

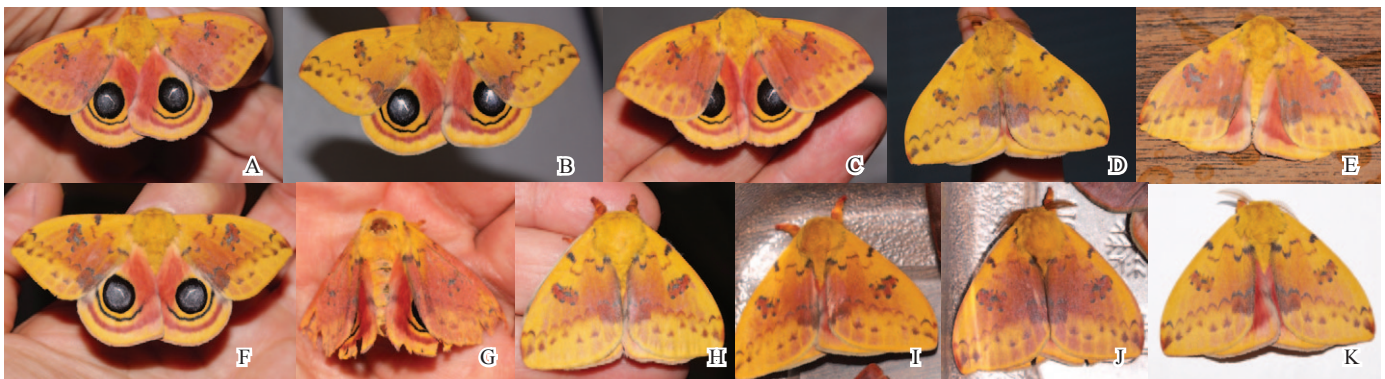


Fig. 3. Sibling non-diapausing males of *Automeris io* (Gainesville, FL), bred in captivity under 24-hour-light conditions, that did not go through a diapause. This sample shows the variable - from hardly any (B, D, K) to almost all-wing (A, C, G) - extent of basal forewing rose patch.

RESULTS AND DISCUSSION

Polymorphism in *Automeris io* males in north Florida

To start, I would like to draw attention to three sets of sibling *A. io* males (Figs 1-3). Males in Fig. 1 resulted from a batch of 19 eggs found in August 2013 and raised under 24-hour light conditions. Although the rearing took place in the fall, when *A. io* pupae normally go into diapause, seven of the males from this batch emerged in December 2013 - one month following pupation - hence bypassing the diapause. These males had coloration typical of *A. io io* from the northern U.S., with the exception that the brown scales at the base of forewing were rose in color. These rose, burgundy, tawny, or brown basal patches can be more or less pronounced, as can be seen from another batch of sibling males shown in Fig. 3, ranging from a few scales to large portions of the wing surface, but they never reach the costa or apex of the forewing, which is yellow or,

sometimes, orange.

Out of the nine siblings represented by Fig. 1, two males emerged after diapause in February and May, and one of them expressed a tawny-orange ground color of the wing, which is likely what Manley called the “tawny-brown” diapausing phenotype.

Another set of sibling *A. io* males is shown in Fig. 2 and illustrates a different type of diversity in color patterns that occurs within a single brood of *A. io* in Gainesville, FL. Three of the males (Figs 2A,B,C) are brothers and one (Fig. 2D) is their cousin, all resulted from sibling-to-sibling mating. According to Manley (1990), the dark-brown color as in Fig. 2A is a rare expression of a recessive gene that occurs due to inbreeding, and indeed, the male in Fig. 2A is one of its kind among ca. 100 males that I raised. These rare phenotypes, if they were collected in the wild, could prompt questions about the conspecificity of *Automeris* phenotypes found in Florida.

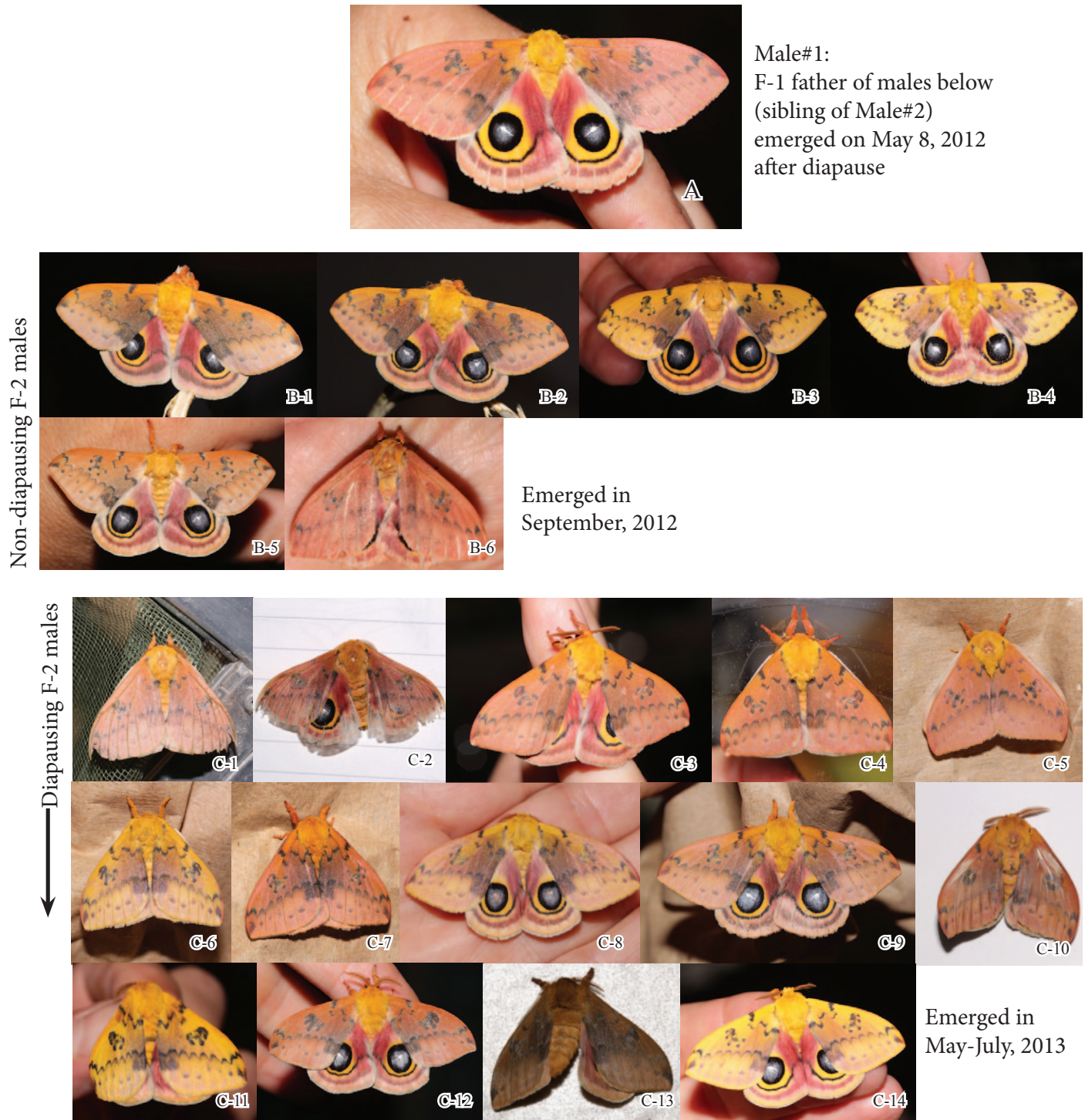


Fig. 4. (A) Male of *Automeris io* nr. *lilith*, raised from wild collected eggs in Gainesville, north-central Florida, exhibits diffused rose forewing coloration typical of diapausing individuals. Its sons (B, C), which resulted from sibling-to-sibling crossing, show lower frequency of this coloration in individuals that emerged in the fall without diapause - (B), than in individuals that went through the winter diapause - (C).

However, when reared from the same egg batch as more common *A. io* phenotypes, one can conclude that they certainly represent nothing more than a part of the phenotypic variability within *A. io*. Still, the variability featured in Fig. 2 appears to be of a different kind than that featured in Fig. 1, and perhaps is particular to *A. io lilith* – a more tropical subspecies that has limited genetic exchange with the northern *A. io io*.

Evidence of polyphenism in *Automeris io*

The purpose of this note, however, is not to analyze the genetics or taxonomy of *A. io* (which, who knows, may become a subject of my future papers), but to determine how the expression of phenotypic plasticity in male coloration relates to diapause. Analyzing the illustrations presented here, one can conclude that it indeed does so, supporting observations by Manley (1990, 1991). However, the results of my breeding

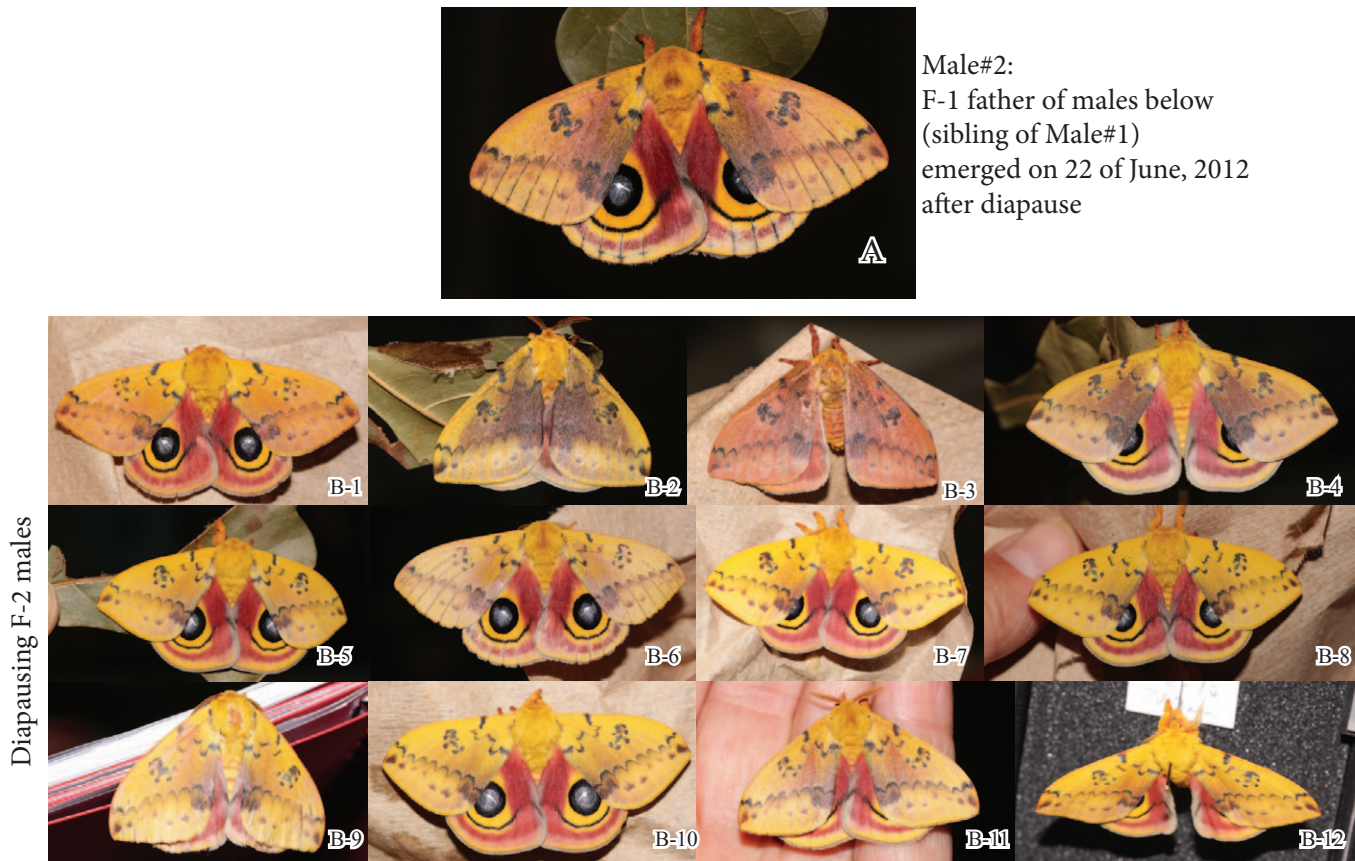


Fig. 5. (A) Male of *Automeris io* nr. *lilith*, raised from wild collected eggs in Gainesville, north-central Florida, that **does not** exhibit rose diffused forewing coloration even though it went through a diapause. Its sons (B) that resulted from sibling-to-sibling crossing, with the exception of one (B-3) individual, show no rose forewing coloration typical of diapausing individuals, even though they all went through diapause and emerged in May-July, 2013.

experiments are not as clear-cut as his statements, and here is why:

1. The set of *A. io io* males illustrated in Fig. 1 (as well as sibling-to-sibling crosses of this line not illustrated here) consisted of 12 non-diapausing males that were yellow (as in Fig. 1A,B) and two diapausing males, one of which was tawny-orange (Fig. 1C). Manley (1990) stated that in Louisiana this phenotype is found with a frequency of 6% in the spring generation. For the brood figured here, the probability of two events (the diapause and unique color) accidentally coinciding is quite small (less than 6%*), and hence, it is more prudent to suppose that the unique coloration of the male in Fig. 1C was caused by the diapause, even though the pupae were not subjected to any special treatment, but remained in the lab at ca. 20°C. Yet only one of the two diapausing males expressed that dark phenotype, and the other, though it diapaused longer, did not. Hence diapause alone is not sufficient for inducing this phenotype.

2. As far as *A. io lilith* is concerned in my breeding experiments, Male#1 (Fig. 4A) produced 2 out of 6 (33%) sons with rose diffused forewing coloration (Figs 4(B-5,6)), 2 yellow (B-3,4), and 2 intermediates (B-1,2) that emerged before the diapause. On the other hand, in the same brood but among diapausing males, 10 out of 14 (71%) had diffused rose

coloration similar to that of their father (Fig. 4C).

3. A sibling of Male#1, Male#2 (Fig. 5A), which did not express diffused rose color despite undergoing diapause, produced only one son with diffused rose coloration (Fig. 5(B-3)). The other 11 males in this entirely diapausing brood were yellow. Considering that Male#1 and Male#2 were siblings that were reared simultaneously under similar conditions but emerged 40 days apart and produced different phenotypes and propensities for diapausing in their sons, it is reasonable to hypothesize that the diffused rose ground color is genetically linked to diapausing habits.

4. The results of continuous breeding of lines from Male#1 and Male#2 support this hypothesis. Male#3 and Male#4 (Figs 6A,7A, respectively) are both sons of Male#1. Male#3 produced 5 out of 19 (26%) rose non-diapausing sons (Fig. 6B) and 8 (100%) rose diapausing sons (Fig. 6C). Male#4 produced 0% (N=9) rose non-diapausing sons (Fig. 7B) and 4 out of 5 (80%) rose diapausing sons (Fig. 7C). Hence, they both showed a hereditary propensity for producing more sons with diffused rose ground coloration among diapausing individuals than among non-diapausing ones.

5. Male#5 (Fig. 8A) is the son of Male#2 and neither he nor his father nor most of his siblings exhibited rose ground forewing coloration. However, when it was bred to a female that came from the "rose" line of Male#1, this female produced

* This calculation was made based on the 6% frequency of occurrence of this coloration in March-June diapausing generation in *A.io* males in Louisiana (Manley 1990). Probability of the appearance of this coloration in the line shown in Figs 9, 10 was calculated using Bernoulli trial ($n=14$, $p=0.06$, $q=0.94$).



Male#3
F-2 father of the males below
(son of Male#1/brother of Male#4)
emerged in May, 2013
after diapause

Non-diapausing F-3 males



Non-diapausing F-3 males



Emerged in
September-November, 2013

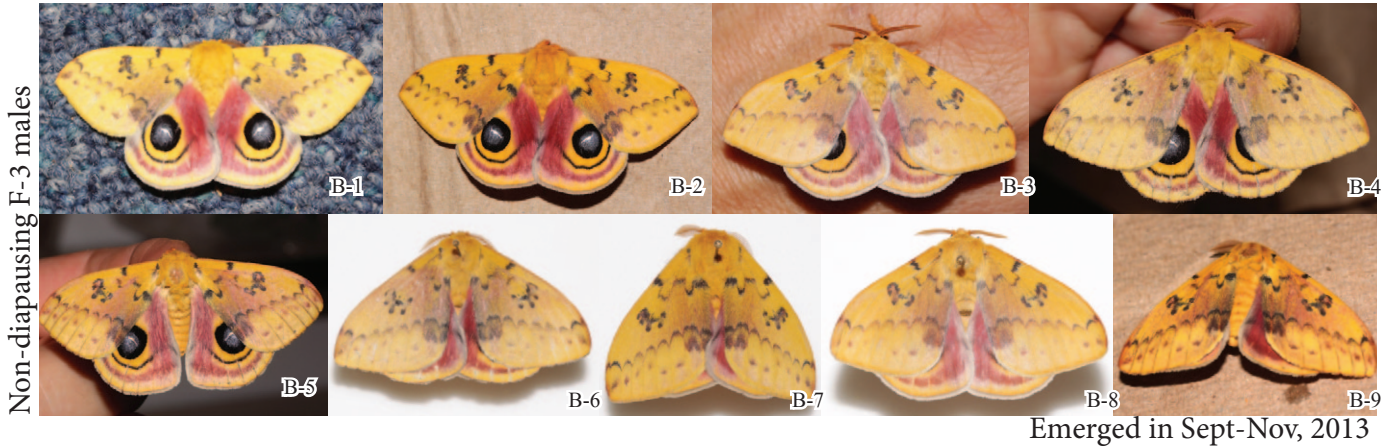
Diapausing F-3 males
Emerged in May-June, 2014



Fig. 6. (A) Diapausing male of *Automeris io* nr. *lilith* bred in captivity (same individual as in Fig. 4(C-7)) exhibits diffused rose forewing coloration typical of diapausing individuals. Its sons (B and C) that resulted from cousin-to-cousin crossing show lower frequency of this coloration in individuals that emerged in the fall without diapause - (B), than in individuals that went through the winter diapause - (C).

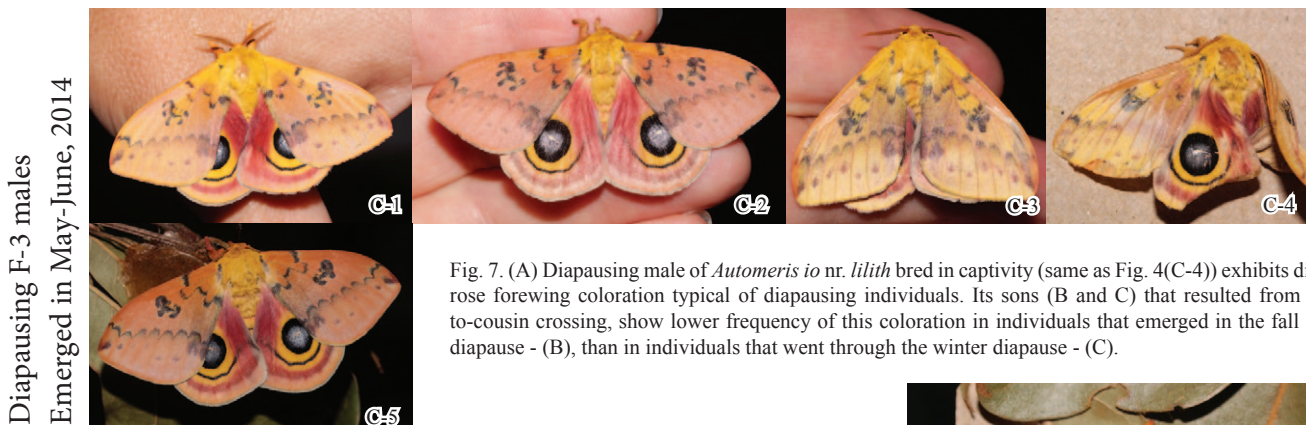


Male#4
F-2 father of the males below
(son of Male#1, brother of Male#3)
emerged in May, 2013
after diapause



Non-diapausing F-3 males

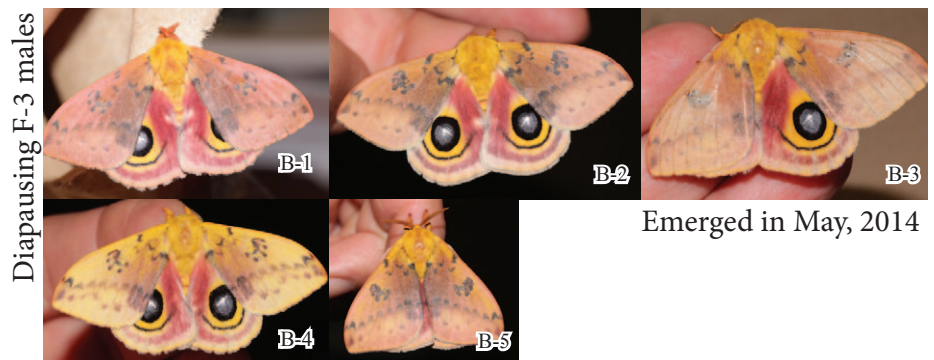
Emerged in Sept-Nov, 2013



Diapausing F-3 males
Emerged in May-June, 2014

Fig. 7. (A) Diapausing male of *Automeris io* nr. *lilith* bred in captivity (same as Fig. 4(C-4)) exhibits diffused rose forewing coloration typical of diapausing individuals. Its sons (B and C) that resulted from cousin-to-cousin crossing, show lower frequency of this coloration in individuals that emerged in the fall without diapause - (B), than in individuals that went through the winter diapause - (C).

Male#5
F-2 father of the F-3 males below,
son of Male 2, cousin of Males 3,4
emerged in May, 2013
after diapause



Diapausing F-3 males

Emerged in May, 2014

Fig. 8. (A) The diapausing male of *Automeris io* nr. *lilith* bred in captivity (same as Fig. 5(B-5)) **does not** exhibit diffused rose forewing coloration typical of diapausing individuals. However, its sons (B), all of which when through diapause, are almost exclusively rose-colored. This progeny resulted from cousin-to-cousin crossing to a female offspring of Male#1. Hence the propensity for diffused rose coloration of diapausing individuals, which was weakly exhibited in the parental male, was fortified by mating into a “rose” family.

4 out of 5 (80%) diapausing rose males (Fig. 8B).

Based on the data from six *A. io* broods presented above, it is logical to conclude that the diffused rose or tawny ground forewing coloration in males of *A. io* in Florida can be stimulated by diapause, supporting the hypothesis of seasonal polyphenism, but this characteristic can be variably expressed depending on the genetic predisposition.

One can also observe, starting with Males#1 and #2 and throughout breeding lines, that whenever rose coloration was absent in the diapausing part of the brood, it was mostly among males that emerged later than their rose diapausing siblings (Figs 4-8 - NB! Photos are posted in order of emergence). So, early emergence from diapause may be genetically linked to expression of rose ground forewing color.

Manley (1991) exposed his diapausing pupae to negative temperatures, and perhaps that is why, judging from his descriptions, he obtained darker-colored diapausing males. The diapausing pupae of the broods illustrated in the present paper were kept either in the lab at 20°C or in an unheated part of the house, where temperature fluctuated between ca. 10°C and 25°C. Hence it appears that though diapause stimulates the development of rose and tawny diapausing phenotypes, low temperatures are not required to induce them.

The diapause and voltinism of *Automeris io* in Florida

In addition to providing data on polymorphism and polyphenism, my breeding experiments have shown that, although *A. io* in north central Florida is capable of producing two generations per year (as suggested by Manley (1991), its bivoltine nature is not clear-cut. While there are *A. io* males present in the collection of the McGuire Center that were collected in Gainesville as early as mid-March, the emergence of moths from diapausing cocoons in the lab occurred in April-June. The second generation may emerge as early as late June, or as late as October, depending on how early its parents emerged after diapause in the spring, and on how long the larvae developed, which was shown in the lab to vary from two to three months with the hostplant choice (Sourakov 2013; Sourakov unpublished data), and may be even faster in nature. The larvae of next generation larvae that pupate in October-December are supposed to enter the winter diapause, based on Manley's description.

The above scenario, that indeed may occur, would make *A. io* in Gainesville, FL a bivoltine species. However, I found that a significant portion of pupae did not emerge in the fall, but instead enter diapause (Figs 4-8). The reason this occurred may have been determined by when the first generation laid eggs, the rate of development of the larvae, and the genetics of individual offspring.

Regardless of the cause, one can easily see that this strategy of dividing brood emergence between fall and spring can be very beneficial for the overall brood survival. A 100% emergence in the fall would be risky partly because the larvae from eggs laid by these moths may have insufficient time to develop; in north-central Florida, good nutritious foliage becomes hard to find late in the fall. Most of the common hostplants of *A. io* shed leaves, or the nitrogen in foliage becomes depleted, and hence moths may be forced to lay on

other, less conventional hostplants, which carries its own risks (Sourakov 2013 and references therewith). Additionally, there is a threat of freezing temperatures that can come as early as November, of parasitoids, whose populations tend to build up by the fall, and of inbreeding.

Manley (1991), who assessed the diapausing habits of *A. io* for the entire south-eastern U.S., placed Gainesville in his region "D", where, he suggested, there are two broods per year. Immediately south of Gainesville, according to his map, starts region "C", where, according to Manley, there are three broods per year, and in the lower half of peninsular Florida (region "B") there are four non-diapausing broods (continuous breeding).

The study presented here demonstrates that Manley's unambiguous assessments may have been based on samples with specific genetic compositions, but that in north-central Florida around Gainesville, the individual *A. io* may exhibit both bivoltine and univoltine (and perhaps even trivoltine) reproduction, depending on genetics and environmental conditions. The start of *A. io* diapause is determined by the photoperiod, and the end by temperature and humidity (Manly 1991). I found that one can bypass the diapause of entire or partial broods by raising larvae in 24 hour-light conditions. This plasticity explains why *A. io* was able to colonize such vast territories with different climates, from Canada and the northern United States, where diapause is critical for survival of the species, to South Florida, where the photoperiod must no longer produce diapause, or is counteracted by other factors.

More breeding of various populations and genetic strains of *A. io* combined with careful record keeping would contribute to the ongoing quest to understand the biology of widespread polyphagous species.

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