

EFFECT OF LARVAL PHOTOPERIOD ON MATING AND
REPRODUCTIVE DIAPAUSE IN SEASONAL FORMS OF
ANAEA ANDRIA (NYMPHALIDAE)

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ABSTRACT. In experiments conducted under simulated field conditions in Baton Rouge, Louisiana, laboratory-reared summer-form female *Anaea andria* Scudder from 13- and 14-h larval photoperiods underwent oocyte maturation and mated within two days of eclosion. Laboratory-reared winter-form females from 13-h larval photoperiods did not mate, and showed little oogenesis 20 days after eclosion in simulated field conditions. Data from field-collected specimens numbering 55 winter forms and 26 summer forms support the experimental results, and indicate that female winter forms remain unmated and in reproductive diapause in the fall. Results suggest that larval daylength, by determining adult seasonal form, also regulates reproductive diapause and mating in *A. andria*.

Additional key words: Charaxinae, *Croton capitatus*, seasonal dimorphism.

Anaea andria Scudder (Nymphalidae: Charaxinae) is distributed from Texas to Nebraska, E to West Virginia, Georgia, and the Florida panhandle (Opler & Kriezek 1984). It is common in the southern Mississippi basin and Gulf Coast where it occurs with its primary host plant, *Croton capitatus* Michx. (Euphorbiaceae), an annual herb.

Adult *Anaea andria* are characterized by distinct seasonal wing dimorphism induced by larval photoperiod (Riley 1980, 1988). Winter-form butterflies emerging in fall and surviving until the following spring are characterized by apically acute and falcate forewings, well developed hindwing tails and anal angle projections, and brighter and more contrasting coloration than summer-form butterflies. Summer forms have non-falcate forewing apices, reduced tails and anal-angle projections on hindwings, and lighter overall coloration. Photoperiods of 14 h or more result primarily in summer-form adults. Decreasing photoperiods result in a greater percentage of winter-form individuals (Riley 1988).

In Louisiana, summer-form *A. andria* occurs from May to September when actively growing host plants are available. The winter form begins to appear in late August, and survives until June of the following year. Its appearance in the fall is followed shortly by the beginning of *Croton capitatus* senescence.

The occurrence of two distinct seasonal forms, one when food plants are abundant, and another when they are in decline, suggests that a corresponding difference in female reproductive status may also occur. In this paper, effects of larval daylength on reproductive diapause and

mating in laboratory-reared seasonal forms of *A. andria*, and the mating and reproductive status of field-collected seasonal forms are reported.

METHODS

Experimental insects were collected in East Baton Rouge and East Feliciana parishes, Louisiana. Larvae were collected from host plants in the field, or reared from eggs deposited on container-grown host plants by reared and field-collected butterflies confined in 2-m³ Saran® screen outdoor cages (Chicopee Manufacturing Co., Cornelia, Georgia 30531). Adults were collected using traps similar to those described by Rydon (1964) baited with bananas.

Larvae were reared in clear plastic 26 × 20 × 10-cm boxes containing a raised 3-mm mesh metal grid to prevent undue larval contact with feces. Larvae were fed fresh *Croton capitatus* leaves, and the boxes cleaned daily or as needed. Pupation occurred on tops and sides of the boxes and on plant material.

Seasonal forms of *A. andria* were produced by rearing the third instar in environmental chambers with controllable photoperiods. To obtain summer-form butterflies, 14 h of light was used. Winter and summer forms were obtained using a 13-h photoperiod. Temperature in the chambers was 27°C during the experiments.

Laboratory-reared males and females of each seasonal form were maintained in separate outdoor cages. Eight hours after eclosion, females were numbered with permanent ink on the underside of a hindwing and released into the cages. Males were also caged eight hours after eclosion, and remained in the cages until death. Virgin females were always caged with males of equal or greater age.

Well-ripened bananas were provided for adult food. Cage location provided exposure to full sun 6 h/day. One cage corner was covered with plywood to provide a shaded resting area for the butterflies. The experiment was conducted from 1 June to 31 October 1986; consequently, insects were exposed to a changing natural photoperiod. Each seasonal form was caged during the time of year when each can be found in the field, summer forms from 1 June to 23 September, winter forms from 6 August to 31 October.

Stage of oogenesis, number of mature eggs, and mating status were determined by dissection and examination of the female reproductive system. Summer forms were dissected 2 and 3 days after eclosion, and winter forms 10 and 20 days after eclosion. Stage of oogenesis is described using a scale of 0–5, 0 denoting no evident oocyte development, and 5 the presence of mature eggs (further explained in Table 1). Insects were judged to be in reproductive diapause if oocyte development 10

TABLE 1. Percentage mated, stage of oogenesis, and number of mature eggs/female in 2- and 3-day-old mated and unmated laboratory-reared summer-form *A. andria* under simulated field conditions.

Age (days)	N	Percent mated ¹	Mean stage of oogenesis ^{2,3}	No. mature eggs/female	
				Mean ³	Range
2	18	83.3a	4.1a	0.6a	0-4
3	44	90.9a	4.9b	44.2b	0-82

¹ Not significant according to Fisher's Exact Test.

² 0-5 scale. 0 = no visible oocyte formation; 1 = beginnings of oogenesis; 2 = slight enlargement of oocytes; 3 = some oocytes 50% mature; 4 = greater oocyte enlargement, no oocytes at median oviduct; 5 = mature oocytes at median oviduct.

³ Means in columns followed by the same letter do not differ significantly according to *F*-Test ($P < 0.01$).

days after eclosion was rated < 2.0 . Mated status of females was determined by spermatophore presence in the bursa copulatrix.

Percentage mating was analyzed using Fisher's Exact Test; all other variables were subjected to analysis of variance (SAS Institute 1985).

Voucher specimens are in the Louisiana State University Entomology Museum.

RESULTS

Laboratory-reared butterflies. Winter-form butterflies resulted only from the 13-h photoperiod. Twenty-seven females and 30 males were reared. Twelve females were dissected after 10 days, and 15 dissected after 20 days of caging with winter-form males. None of the female winter forms had mated, and none of their ovarioles showed any sign of oogenesis. No courtship behavior or mating attempts were seen. It was therefore concluded that winter-form females remain in reproductive diapause for at least 20 days after eclosion. Male mating behavior and female attractiveness may also be inhibited in winter forms.

Summer-form butterflies resulted from both photoperiods, 40 females and 41 males from the 14-h, and 22 females and 25 males from the 13-h. Comparison of summer-form data from both photoperiods indicates that oogenesis, number of mature eggs/female, and mating were not significantly affected by these larval photoperiods.

Age was the most important factor affecting stage of oogenesis and number of mature eggs per female in recently eclosed summer forms. Two-day-old females showed significantly less oogenesis ($F = 31.51$; $df = 1, 58$; $P < 0.01$), and carried fewer mature eggs ($F = 59.18$; $df = 1, 58$; $P < 0.01$) than 3-day-olds (Table 1). The age \times photoperiod interaction was not significant.

Percentage of mated 2- and 3-day-olds did not differ (Table 1). In several instances, courtship of virgin females was observed within hours of their release into the outdoor cage. Although age of the males involved

was not known, it was concluded that mating behavior of male and female summer forms is not suppressed in recently eclosed butterflies. These observations suggest that most female summer forms are mated within a few days of emergence.

Among unmated summer forms, four were three days old, and three were two days old. Mean number of mature eggs/female for the 3-day-olds was 23.5 (range 0–48) while none of the 2-day-olds contained mature eggs, evidence that oogenesis in summer forms progresses with age, independent of mating.

Field-collected butterflies. Forty-two winter-form females were trapped during September–November 1984 and September 1985. They showed little evidence of oogenesis, and all were unmated (Table 2). Thirteen overwintered winter forms trapped in April and May 1985 were mostly mated and contained fully mature eggs (Table 2).

Summer-form females, collected in September 1984 and June to mid-September 1985, all carried fully mature eggs and all were mated (Table 2).

DISCUSSION

Laboratory results and field observations indicate that newly eclosed summer-form females undergo rapid oogenesis and are mated within two days of emergence. This enables summer forms to immediately begin ovipositing and larvae to complete development before onset of unsuitable environmental conditions. Conversely, winter-form females remain unmated and in reproductive diapause for a considerable time after adult eclosion. They are thus relieved of the physiological burden of producing and carrying mature or maturing eggs when environmental conditions are not favorable for oviposition and larval development. Reproductive status appears to be linked to adult seasonal form since summer forms from both 13- and 14-h photoperiods underwent rapid oogenesis while winter forms from the 13-h photoperiod remained in diapause.

Field observations of courtship, mating, and feeding behavior in *A. andria* support these conclusions (unpubl. data). No courtship activity has been seen in winter forms during fall. However, newly eclosed male and female winter forms are readily attracted to fermented fruit baits, indicating a possible feeding response linked to preparation for overwintering. In spring, winter-form males have been observed exhibiting strong territorial behavior, chasing other males, patrolling along forest edges and then returning to the same perch, engaging in courtship behavior, and attempting to mate with females. Baits placed near male territories and perches in spring have proven relatively unattractive and trapping ineffective. These observations lend support to the ex-

TABLE 2. Collection month, percentage mated, and stage of oogenesis in female *A. andria* collected 1984-85 in East Baton Rouge and East Feliciana parishes, Louisiana.

Month collected	Seasonal form	N	Percent mated	Mean stage of oogenesis ¹
September	Winter	18	0.0	0.0
October	Winter	23	0.0	0.5
November	Winter	1	0.0	1.5
April	Winter ²	5	80.0	5.0
May	Winter ²	8	100.0	5.0
June though September	Summer	26	100.0	5.0

¹ Same scale as in Table 1.

² Overwintered butterflies.

perimental results and suggest different behavioral priorities in winter forms before and after overwintering. Factors initiating oogenesis and mating in winter forms that have overwintered are unknown.

My experience with bait traps during summer in Louisiana and Missouri, and that of Vernon Brou, Abita Springs, Louisiana, who operates bait traps year-round, indicate that summer forms are both trapped and collected less frequently than winter forms. They are also less common in collections. The total number of field-collected summer forms in collections of the author, V. Brou, and the Louisiana State University Entomology Museum is 77 compared to 241 winter forms (Riley 1988). This discrepancy may be due to collecting bias, but may also indicate greater behavioral priority for reproduction vs. feeding in summer forms, similar to winter-form behavior in the spring. These observations along with the experimental results suggest that larval daylength, by determining adult seasonal form, is also a major factor regulating mating and reproduction in recently emerged *A. andria*.

Photoperiod is well documented as a diapause inducing and regulating stimulus for insects (Beck 1980, Danilevsky et al. 1970, Tauber et al. 1986). It is an ideal environmental cue for *A. andria*. The nymphalid *Polygona c-aureum* L. is very similar in its response to daylength (Hidaka & Aida 1963, Fukuda & Endo 1966, Endo 1970, 1972). In *P. c-aureum*, reproductive diapause and seasonal wing dimorphism are determined by photoperiod but are under independent neuroendocrine control. Pheromone production and mating receptivity of female *P. c-aureum* are also hormonally regulated and under photoperiodic control (Endo 1973). The results presented here suggest that a similar interaction between daylength and neuroendocrine system could be controlling wing dimorphism, diapause, and mating in *A. andria*.

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