

THE BIOLOGY AND LABORATORY CULTURE OF  
*CHLOSYNE LACINIA* GEYER (NYMPHALIDAE)

BOYCE A. DRUMMOND III, GUY L. BUSH, AND THOMAS C. EMMEL,<sup>1</sup>

Department of Zoology, University of Texas, Austin

The nymphalid butterfly, *Chlosyne lacinia* Geyer, is the most widely distributed species of its genus, ranging from Argentina northward into Texas, New Mexico, Arizona, and the Imperial Valley and adjacent desert areas of California (Comstock, 1927; Ehrlich and Ehrlich, 1961). Occasionally it may penetrate as far north as Kansas and Nebraska (Klots, 1951).

The objective of this paper is to present an outline of the life history and laboratory rearing techniques established as a result of an investigation recently initiated on the ecological genetics of the various color morphs present in the Texas populations. Details concerning the inheritance and fitness in natural populations of certain color morphs will be published later.

#### Rearing

Larvae of *Chlosyne lacinia* were reared in the laboratory from egg masses collected in the field or laid in the laboratory by wild-caught females. Individual 4 and 6 quart polyethylene tubs with tight-sealing lids were used to hold the larvae of separate broods in early rearing procedures. Both ventilated and unventilated lids were tried. The larvae were supplied daily with fresh leaves of *Helianthus annuus* and the containers cleaned of frass.

No attempt was made to regulate humidity, but larvae in unventilated tubs in which the humidity was high (90% or more) developed more rapidly than those in the ventilated tubs. However, at high humidity the larvae were much more susceptible to disease; mortality was particularly high in the fourth and fifth instars. Most containers were kept at a constant 21° C under artificial light of 16 hour daylength. It was noted during the early attempts at rearing that larvae developed more rapidly at higher temperatures but were more subject to disease. Although the use of ventilated lids reduced mortality of larvae reared at high temperature, the *Helianthus* leaves dried out within a matter of hours, rendering them inedible to the larvae and thus lengthening developmental time.

The feeding and disease problem was satisfactorily solved by employing the Special Vanderzant-Adkisson Modified Wheat Germ Diet (Adkisson et al., 1960) as an artificial medium (available commercially from Nutritional

<sup>1</sup> Present address: Department of Zoology, University of Florida, Gainesville 32601.

Biochemical Company, Cleveland, Ohio 44128). The medium was supplemented with the antibiotics aureomycin and acromycin, as well as methyl parasept; these were added at the concentration of 0.001% by weight to help check disease. Finely ground dried *Helianthus* leaves (2.5 to 10% by weight) were normally included as a feeding stimulant. Although larvae have been raised on media lacking the *Helianthus* additives, they developed more rapidly and only acquired full coloration on the diet supplemented with leaves, particularly at the higher concentrations.

Larvae raised on the artificial medium were normally kept at 24°–26° C under a 16 hour daylength or under continuous lighting; the latter condition caused some larvae to enter diapause. At this temperature the medium, which was placed in strips on the bottom of unventilated polyethylene containers, required changing only once a week for the first three instars, and only slightly more often as the larvae increased in size.

Mortality of medium-raised larvae was low through the first four instars, but the fifth instar larvae were quite susceptible to infection. The overall incidence of infection, however, was much lower than in larvae reared on leaves alone. The medium apparently fulfilled most of the nutritional requirements of this butterfly since it supported three generations of insects in our laboratories. The effects of the diet on fecundity and viability were not established but are now under study.

Adults were collected by placing pupae in one-pint paper ice cream cartons, the solid tops of which were replaced with gauze. Upon emergence, the adults were maintained under an artificial 16 hour day and fed once or twice a day on a solution of one-third honey (or sugar) and water (1:3 by volume) soaked into pieces of sponge or commercially available pressed cotton (Coets, Quilted Squares; Personal Products Company, Milltown, New Jersey) placed on the gauze.

Mating was accomplished either in small wire cages under the partial shade of a tree or in large indoor mating cages artificially lighted with a bank of four Gro-Lux fluorescent lights. High frequencies of mating occurred when these cages contained several adults. In these same large cages small *Helianthus* plants were available for the females to oviposit upon, following mating.

#### Life History

*Chlosyne lacinia* in central Texas is multiple brooded; a generation occurs about every 35–45 days under field conditions. Adults fly from March to November with individuals apparently passing the winter as diapausing third instar larvae (there are five larval instars). Diapause appears to be a photoperiodic response because larvae frequently entered diapause in

the third instar when they were held in the laboratory under light conditions of short day length (i.e. 8–12 hours).

Larvae in central Texas appear to feed primarily on species of the tribe Heliantheae (Compositae). The primary larval foodplant from March through July is the sunflower, *Helianthus annuus* L. It may feed occasionally on *H. cucumerifolius* Torr. and Gray, *Ambrosia trifida* var. *texana* Scheele, *Verbesina virginica* L. and *Silphium* sp. (Kendall, 1959, and personal communication). We have also found a few broods on *Viguiera dentata* (Cav.) Spreng. and *Simsia calva* (Engelm. and Gray) Gray. As *H. annuus* dies out in August, females begin to oviposit on *Ximenesia encelioides* Cav. During this period larvae can be found feeding on both host plants.

Mating usually occurs in midmorning with copulation lasting about forty-five minutes. Up to 500 eggs may be deposited in one or two clusters by a female on the underside of a host plant leaf. Counts made of day-old larvae from 75 collected wild egg masses revealed an average of 139 ( $Se = \pm 11$ ) hatched eggs per mass, with a range of 22 to 480.

The initial pale greenish-yellow egg color changes to dark brown 24–48 hours before hatching. The pale yellow larvae of a single egg mass hatch simultaneously. After consuming the empty eggs, they begin feeding gregariously on a thin silken web in a tightly packed mass on the underside of the host plant leaf. When disturbed, first and second instar larvae frequently will begin a synchronous jerking movement of the body. The function of the web is not known, but it may possibly help to maintain colony unity and protect the larvae from some predators and parasites.

When a leaf has been consumed, one or two larvae will move off the leaf along the stem followed in single file by the rest of the brood. Each larva lays down a silken thread which is reinforced by the larvae following it. A distinct silken trail is thus constructed by the group. Upon arrival at a new leaf, the feeding aggregation is reformed (Bush, 1969).

Third to fifth instar larvae may enter a quiescent period during the month of August in Texas when the temperature is high and food plants are scarce. In the laboratory, unfed larvae in the later stages (primarily third and fourth instar) will contract and remain quiescent for up to two months or more and resume feeding and complete development when food is provided. Mr. Roy Kendall has informed us that he has been able to maintain unfed larvae of *C. lacinia* for over a year. Thus it appears that this species is well adapted to the xeric conditions of central Texas.

Usually by the fourth instar, the larvae within a brood begin dispersing singly or in small groups and may move to adjacent plants, becoming well scattered by the fifth instar. Dispersal appears to coincide with the ap-

TABLE 1. Life table of *Chlosyne lacinia* reared on sunflower leaves in the laboratory at 21° Centigrade.

	1st instar larvae per egg mass	Incubation period (days)	Instar 1 (days)	Instar 2 (days)	Instar 3 (days)	Instar 4 (days)	Instar 5 (days)	Pupa (days)	Total time egg-adult (days)
Mean	138.8	9.10	7.8	4.5	3.7	7.2	12.0	9.0	53.3
Range	22-480	6-15	7-8	4-5	3-5	5-9	4-22	7-11	— <sup>2</sup>
No. of broods	75	13	6	6	6	6	5	21 <sup>1</sup>	

<sup>1</sup> Number of individuals.

<sup>2</sup> The range of the total developmental time could not be determined with accuracy because the egg masses used to calculate the incubation period and the broods used to obtain data on the length of the larval instars were not necessarily the same.

pearance of larval color patterns which usually begin to manifest themselves in the third instar and are quite well developed by the fifth. In one color phase, the ground color is black, in another the ground color is almost solid orange, and in a third the ground color is black with an orange dorsal stripe. More detailed descriptions of the immature stages of this butterfly may be found in Edwards (1893).

The function of dispersal is not clear, though perhaps it is primarily the result of food competition. It may also serve to reduce the incidence of disease, parasitism, and predation among individual larvae of the same brood. This interpretation is supported by the fact that fourth and fifth larval instars under crowded laboratory rearing conditions were much more susceptible to disease, which is apparently quiet contagious, than were the earlier stages under the same conditions. As will be discussed in more detail later, mature larvae were also more susceptible to parasitism than earlier stages and predation appeared to be greatest among third to fifth instar larvae.

Fifth instar *Chlosyne* larvae frequently wander a considerable distance from their feeding site prior to pupation, which may occur on sheltered sites on a wide variety of surfaces such as *Helianthus* stems, fence posts, or leaves. The base color of the pupal case changes after 24 hours from pinkish-white to ivory or yellow. It is often speckled with highly variable black markings, producing intergrades between pure white and almost black pupal cases. Male pupae are slightly smaller than females and within a given brood emerge approximately 24-36 hours before the females.

Laboratory breeding experience revealed that emerged females must usually mature for one or two days prior to mating, thus producing a 2 to 3 day lag between the sexual maturity of the male, which may mate the same day of emergence, and the female. A delay in the emergence and

TABLE 2. Life table of *Chlosyne lacinia* reared individually on standard laboratory diet with ground sunflower leaves at 22°–23° C.

	Incu- bation period	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Pupa	Total time egg–adult
Mean (days)	10.76	7.56	4.87	6.54	8.97	9.21	7.32	53.67
S.E.	±.09	±.13	±.27	±.37	±.73	±.44	±.18	±1.12
Range	10–14	5–10	3–12	2–13	5–21	5–14	6–9	45–64
No. of individuals	98	85	70	57	34	29	28	28
died	—	15	16	11	1	0	1	43
diapause	—	0	0	2	23	4	0	29

mating of females would tend to reduce inbreeding by encouraging dispersal among individuals within a brood prior to mating. This is the opposite of maturation in *Euphydryas editha* and *Cercyonis* species, where females can and do mate immediately upon emergence but males must mature at least 24 hours before mating attempts are made (Emmel, 1969).

Two life tables for *Chlosyne lacinia* are presented in Tables 1 and 2. Table 1 was compiled from data collected from mass rearings of broods at 21° C on *H. annuus* leaves. When at least 50% of the larvae had molted the date was recorded. As all individuals in a brood usually develop synchronously, this instar molting time involved little intrabrood variance. Those that failed to molt were usually diseased or parasitized.

Table 2 represents the time required for individual larvae to reach the adult stage. These larvae were selected at random from different broods and reared in isolation on laboratory media at 22°–23° C and constant light. The results obtained from the two methods of rearing appear to be in fairly close agreement. It is apparent, however, that the life cycle takes considerably longer in the laboratory than under field conditions, though accurate life tables have not been compiled for wild broods. The principal reason for faster development in the field may be the higher diurnal temperatures (to 39° C) which increase larval feeding and hence growth rates.

### Parasitism and Predation

#### *Parasitism*

It is clear the parasitism accounts for a considerable portion of the mortality occurring from the egg to the adult stage in natural populations. However, because of the sampling methods employed and the increased rate of parasitism with larval maturity, it was not possible to obtain an accurate overall estimate of the rate of parasitism and its effects on natural populations.

TABLE 3. Rates of parasitism by the braconids *Apanteles lunatus* and *A. rufocoxalis*. Larvae were collected at the instar indicated and held in the laboratory until the parasites emerged. Fourth and fifth instar broods were collected on plants isolated from other broods to insure that all individuals came from the same brood.

Stadium	No. of Broods	Broods Parasitized	No. of Larvae	Larvae Parasitized	% Broods Parasitized	% Larvae Parasitized	% Larvae Parasitized per Parasitized Brood
Instar I	6	2	518	3	33.3	0.6	1.2
Instar II	66	35	7846	359	53.0	4.6	8.2
Instar III	35	26	3490	365	74.3	16.2	25.0
Instar IV	10	6	1411	292	60.0	20.7	32.0
Instar V	5	4	222	108	80.0	48.6	52.2

The following parasites have been reared from the eggs, larvae, and pupae of *C. lacinia*.

*Egg*: *Trichogramma fasciatum* (Perkins) (Trichogrammatidae) is the only parasite thus far reared from the eggs of *C. lacinia*. Generally all the eggs in an infested egg mass were parasitized. Ten out of eighty-two egg masses (12.2%) were parasitized from collections made at three localities (Stockdale, Wilson Co.; Czestochowa and Kenedy, Karnes Co.) on June 18, 1968.

*Larvae*: One species of Braconidae, *Apanteles lunatus* (Packard) was found to parasitize all larval stages. A full grown *Apanteles* larva never emerged from a host before the host had reached the third instar. Upon emerging, the parasite spun a bright yellow cocoon on the leaf beside the moribund host. The frequency of larval parasitism was directly correlated with the stage of development as indicated in Table 3. Individuals in broods collected in the first instar and held in the laboratory were rarely parasitized (0.6%), while approximately 48.6% of all fifth instar larvae collected in the field were parasitized.

There was some indication that the three larval morphs were differentially parasitized. Orange larvae, for instance, were rarely parasitized, while black larvae appeared to be more heavily parasitized by *Apanteles* than the striped larvae. These situations of selective parasitism are now under study.

*Pupae*: Three parasite species were reared from a few *C. lacinia* pupae collected as larvae or pupae in the field. The most frequently encountered parasite was a small pteromalid, *Pteromalus archippi* Howards, which oviposits in fully formed *lacinia* pupae. Two other Hymenoptera, the ichneumonid *Cratichneumon vinnulus* (Cresson) and the chalcid *Spilochalcis phoenica* Burke, have also been reared from pupae of *lacinia*. Apparently *S. phoenica* oviposits only on the pupal stage of this host, while *C.*

*vinnulus* was reared from a pupa which was collected as a fifth instar larva. Two species of Tachinidae, *Eupharocera daripennis* (Marquart) and *Siphosturmia melitaeae* (Cog.), are common *lacinia* parasites, but apparently oviposit on fourth and fifth instar larvae. Of 177 larvae collected at random in the last two instars, 14.1% were parasitized by these flies.

### Predation

Predators take a heavy toll of larvae and adults, but no field observations have yet been made on the predation of the egg and pupal states. One of the most frequently encountered predators was a metallic-blue pentatomid bug, *Stiretrus anchorago* (Fabricius). Most often attacking third and fourth instar larvae, these bugs would sometimes almost completely destroy a brood.

Members of the wasp genus *Polistes* (Vespidae) are also common predators of primarily fourth and fifth instar *C. lacinia* larvae. Two species, *P. annularis* (L.) and *P. exclamans* Viereck have been identified, but others undoubtedly prey on the larvae. Individuals of both species have been observed to return repeatedly to the same plant and systematically strip a sunflower of almost all larvae.

Birds and lizards, both common in the study areas, have never been observed to feed on any stage of this butterfly. Unidentified species of jumping spiders (Salticidae), on the other hand, have been observed capturing and feeding on adults and the butterfly has also been found in the webs of the orb spiders *Argiope aurantia* Lucas and *A. trifasciata* (Forsk.) (Argiopidae).

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### Summary

1. The nymphalid butterfly, *Chlosyne lacinia* Geyer, ranges from South America to the southwestern United States and is polymorphic in the larval, pupal, and adult

stages. Features of this species' biology make it exceptionally suitable for studies in ecological genetics of natural populations.

2. Techniques for rearing *Chlosyne lacinia* in the laboratory are described. The most satisfactory method of rearing is on an artificial medium (the Special Vanderzant-Adkisson Modified Wheat Germ Diet, with antibiotics and powdered *Helianthus* sunflower leaf additives) under a 16 hour photoperiod at about 25° C. Adults are kept in one-pint ice cream cartons with netting tops, and are fed daily with a honey (or sugar) water solution. Mating will take place in cages under florescent lights as well as sunlight.

3. The primary natural food plant from March through July in Texas is the sunflower, *Helianthus annuus* L. When this species dies in August, female butterflies begin to oviposit on *Ximenesia encelioides* Cav. Several other representatives of the Heliantheae (Compositae) which serve as secondary hosts are discussed.

4. *Chlosyne lacinia* lays large clusters of eggs and the larvae are gregarious until the fourth instar when they disperse. A silken trail is laid down and followed by larvae moving from one leaf to another during the gregarious phase. Black, orange, and black-with-orange-stripe color morphs occur in the larvae (clearly recognizable at fourth and fifth instar). Diapause occurs in the third instar, and may occur under natural conditions of food deprivation (in August) or on the approach of winter (in late November). Laboratory experiments indicate the winter diapause may be triggered by short-day photoperiod.

5. Pupation occurs at some distance from the host plant on which the larva fed. The pupae vary in color from pure white to almost black.

6. Newly emerged males are ready to mate the same day of emergence, but females must mature for one or two days prior to mating.

7. Parasites and predators discovered to date are discussed.

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