Notes on the bionomics, life history, and preparatory stages of *Chlosyne lacinia* (Geyer) (Lepidoptera: Nymphalidae)

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Introduction

Prior to 1964, the year the author started research on the three polymorphic color larval forms of *Chlosyne lacinia* (Geyer), there were no studies of this polymorphism or studies of any genetic aspects of this species. Additionally, little information exists prior to this time about its biology that are the subject of this paper. What exists are in older papers (i.e., Edwards, 1893). Gorodenski (Gorodenski, 1969) published the first genetics study that correctly identified the genetic mechanism of the polymorphic color larval forms. The mechanism had actually been discovered two years earlier by the author but there was no pressing need to publish the results until shortly before receiving his M.S. degree in 1970. Neck (1975) successfully applied these results to demonstrate that the similar looking larval forms of *C. gorgone* are governed by the same genetic mechanism. The purpose of this paper, albeit over forty years late, is to document the other side of the author's research, the bionomic, life history, and the preparatory stages of *C. lacinia*.

The research was on *C. lacinia* in Arizona in the cities of Mesa, Tempe, and Chandler, citrus and crop fields (cotton, and alfalfa) in and adjacent to these cities, and Granite Reef Dam at the confluence of the Salt and Verde Rivers. They will collectively be referred to as the Valley, although the term "Valley", excluding Granite Reef Dam, colloquially covers a larger area of cities that includes Phoenix. The Valley is at about 1,100 ft. elevation and is in the Sonoran desert. Due to the localized geographical area of the research, some of the descriptions and information of this paper may not be applicable to other areas in its range.

C. lacinia, the Patched Butterfly (one of many other common names), in the U.S. occurs primarily in California, Arizona, New Mexico, parts of Nevada, and Texas. Beyond the U.S. its range extends through Mexico, Central America, and South America (Godman and Salvin, 1882), including Argentina (Ehrlich & Ehrlich, 1961).

This is a highly variable protean species not only in wing coloration but also in the larval and pupal stages. There are no secondary sexual differences in wing color and pattern. The only differences are those characteristic of Nymphalids, such as males possessing very hairy prothoracic legs, those of the females being bare or almost so.

The Adult

Originally, twelve species were described because of the highly variable adult wing color and pattern (Godman and Salvin, 1882). Godman and Salvin had the foresight to recognize these as "varieties" of the same species. Of the twelve, three were known to occur in the U.S.: *adjutrix* (Scudder), *mediatrix* (Felder & Felder), and *crocale* (Edwards). *C. mediatrix* was described as being between *lacinia* and *saundersi* but Higgins (1960) later synonymized it with *adjutrix*. Forbes (1928) constructed a key to fourteen "forms" of *C. lacinia* (iincorrectly including *C. californica* as a form of *C. lacinia*).

Higgins (1960) recognized two principle forms occurring in the U.S.: *adjutrix* and *crocale*. He does not consider these as subspecies although he used the trinomial nomenclature. Dos Passos (1964) recognized five subspecies occurring in the Nearctic, but upon a revision (dos Passos, 1969) three were removed because they do not occur in the Nearctic. This left *adjutrix* and *crocale* which has held through all subsequent check lists up to the most recent (Pellham, 2008).

Higgins speculates "...more than likely...we are dealing with a range of colour forms, elaborated as components of different mimetic or cryptic associations, but without true geographical significance except possibly as determined by their models." This is consistent with the author's view that *adjutrix* and *crocale* are not subspecies but instead are either mere recombinational genetic forms, or representative of a cline. There is a higher frequency of *adjutrix* in Texas and a higher frequency of *crocale* in Arizona, but both forms occur side by side in both these states (Higgins, 1960). In the course of research by the author to determine the genetic mechanism of the larval forms (Gorodenski, 1969), *adjutrix*, *crocale*, and *nigrescens* (Edwards) (nigrescens was synonymized with crocale by Higgins) were routinely present in the offspring of single paired matings with an almost continual range of variation in between. These forms were also seen flying together in the field. The genetic and field observations are consistent with the author's view that the range of variation seen in the U.S. does not have any adaptive significance, such as cryptic or mimetic associations, and may possibly represent a cline.

Preparatory Stages

Egg Stage: The eggs are approximately 0.54 mm in diameter and 0.63 mm from top to bottom, are soft bodied, and are obovoid at the end attached to the host plant leaf. The top of the egg is slightly depressed and covered with a small bubble of a syrup like liquid. The egg appears to be covered with a thin layer of this same sticky material that may bind the eggs together as an egg cluster, and may attach the eggs to the leaf surface. Edwards (1893) reports twenty four rounded encircling ridges, but only eighteen were counted on eggs from Granite Reef Dam. Eggs change in color from a yellow-green, just after oviposition, to a deeper yellow a few days later, and then to gray prior to larval emergence.

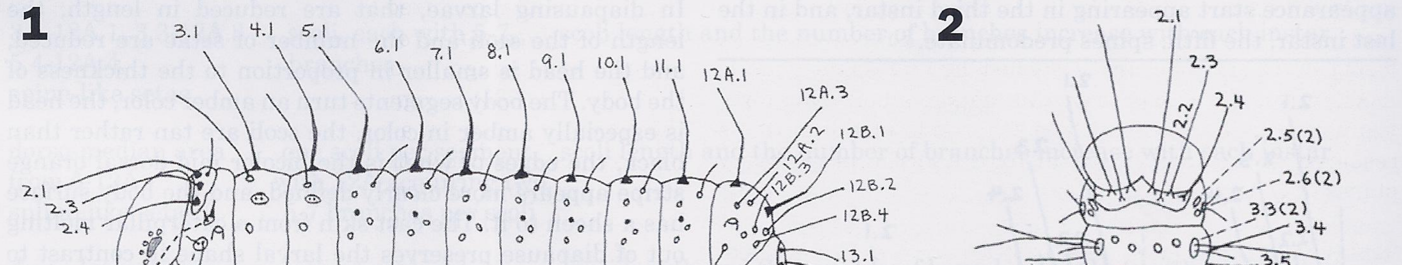
Larval Stage: Three polymorphic larval color forms exist: nigra, bicolor, and rufa (all described by Cockerell, 1893).

Nigra is the black form that is variable in the number of minute white spots dotted over the body surface. Another variable feature is a small yellow spot at the base of each mid-dorsal spine. In some individuals it is almost absent while in others it is very conspicuous.

Bicolor differs from nigra by having a mid-dorsal orange band extending the length of the body. This banded appearance is derived from large orange areas that are variable in size and pattern and occur one to each body segment, except for the prothoracic and anal segments.

The last form, rufa, is completely orange except for variable amounts of black pigment restricted to the segmented areas in the last instar. In some early instar larvae this pigment may be diffused between body segments to such an extent that a rufa can easily be mistaken for a bicolor. However, in the last instar the two forms are easily distinguishable.

There are five instars. Major setae and spine-like setae (setae somewhat more robust than a seta) are shown in figures 1-5 for the first instar. Setae not shown are those covering the head, between the thoracic leg segments (the three segmented legs), and the ventral region of the body. A few are shown on the prolegs. The setae and spine-like setae of only the first instar are illustrated because of their increasing number as the instars progress, the last possessing a confusing mass of setae on the whole ventral region and a sparser number over the rest of the body.



Figures 1 - 5. Setation on first instar larva of *Chlosyne lacinia*. (1) Lateral view. (2) Dorsal view. (3) Posterior view of last segments. (4) Dorsal view of first and second abdominal segments. (5) Dorsal view of last segments. See text, p. 144, for more explanation.

In figures 1-5, the number preceding the decimal point refers to the body segment, the head being segment 1. The number following the decimal point refers to the structural area containing setae or spine-like setae. Structural areas are imaginary divisions because there are no body demarcations that defines them. The structural areas are numbered from one to ten, consecutively, and starts from the mid-dorsal and proceeds to the mid-ventral. A number in parenthesis means there are that many setae or spine-like setae in that structure area. For example, 2.5(3) means the second segment (immediately behind the head) has three setae or spine-like setae in the fifth structure area. Segment twelve appears double segmented dorsally (see figure 2) but appears fused ventrally. As a result, instead of splitting this segment into segment twelve and thirteen, they are labeled 12A and 12B.

All the circles in figures 1 and 2 represent spine-like setae not shown. The black dots in figures 3-5 are setae not shown. In figures 1 and 2 the location of spiracles are indicated by blackened circles on segments 5-11.

The development of setae into spines on the dorsal plate is illustrated in figure 6. As can be seen, setae predominate in the first and second instar. Setae with a spine like appearance start appearing in the third instar, and in the last instar, the fifth, spines predominate.

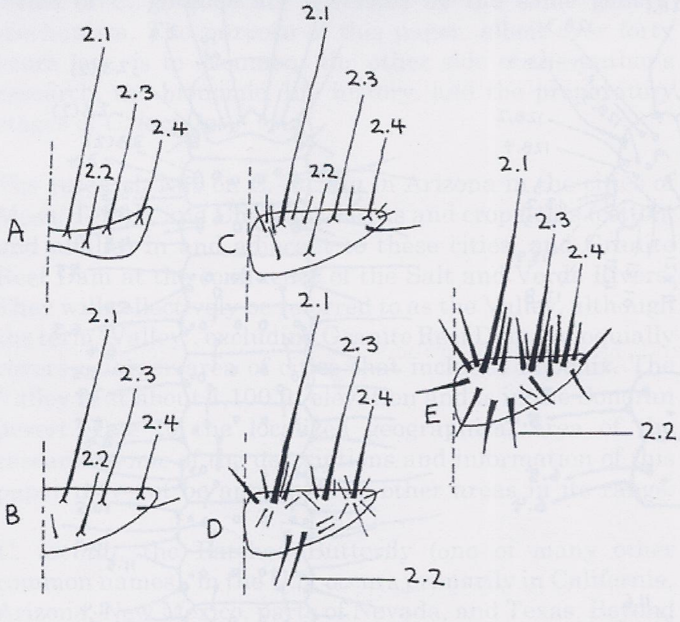


Figure 6. Setal development into spines on the dorsal plate of the pronotum. A through E represent the five successive instars.

Table 1 shows the development of setae, spines, and scoli for some of the body areas in figures 1-5. Unless indicated, the table gives modifications through instars of specific structures, or in one case a lack of a structure (the second row), as shown in column for the first instar. When a range is given, such as 7.5 to 10.5, it means only those structures in structural area 5, not 7.1, 7.2, 7.3...10.4, 10.5. As can be seen, branched spines, or scoli, make their appearance in some structural areas in the second instar. The major transition to scoli occurs in the molt to the third instar.

From larvae collected in 1964 the approximate larval lengths were found to be:

after emergence	1.5 mm
after first molt	2.3 mm
after second molt	4.5 mm
after third molt	6.0 mm
after fourth molt	13.0 mm
just prior to pupation	18-20 mm

Newly emerged larvae are pale yellow, but turn a yellow-green after feeding on the host plant. The second instar larvae are light brown but some are slightly darker if they are the nigra or bicolor forms. Being able to confidently, or with very little error, differentiate between the three larval forms occurs in the third instar.

The scoli are brown from the second to the fourth instar, the branches becoming blackened at the tips. At fifth instar the scoli turn a solid glossy black. The cordate head is black throughout all stages. Head size was a good way to estimate larval instar in the field.

In diapausing larvae, that are reduced in length, the length of the scoli and the number of setae are reduced, and the head is smaller in proportion to the thickness of the body. The body segments turn an amber color, the head is especially amber in color, the scoli are tan rather than black, the edges of what is the bicolor mid-dorsal orange stripe appears more clearly defined, and the body surface has a sheen to it. The cast skin from a caterpillar molting out of diapause preserves the larval shape in contrast to the shriveled skin of a normal molt.

<u>Pupal Stage</u>: The pupae vary from about 10-14 mm in length. The pupae of all larval forms are highly variable

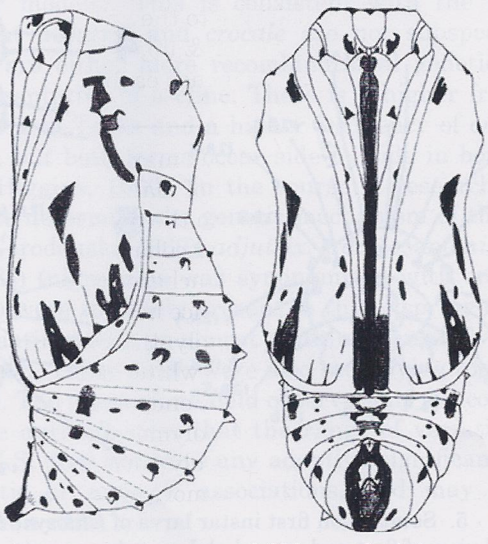


Figure 7. Representative pupa of *Chlosyne lacinia*.

Table 1. Setae and scoli development through instars.

1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
2.5(2), setae	2.5(2), setae	2.5(3) setae	2.5(3) setae	2.5(3) setae
between 2.5 & 2.6, anterior to spiracle, no setae	2 spine-like setae	no extra scoli	no extra scoli	no extra scoli
2.6(2) & 2.7(2), setae	2.6(3) & 2.7(3), setae	scoli with 10 branches per scoli	Length of scoli and number of branches increase with each succeeding insta	
3.2 & 4.2, setae	3.2(2) & 4.2(2), setae	no change	no change	no change
5.6 & 6.6, setae	no change	no change	spines slightly developed	scoli smaller than 5.5 & 6.5, 6 branches each
5.5, 6.5, and 11.5, setae	5.5(3), 6.5(3), & 11.5(3), setae	scoli less than half the size of dorso-median scoli	scoli remain half the size of dorso-median scoli but their length and the number of branches increase with each instar	
7.5 to 10.5, spine-like setae	each developed into two sets with two spine-like setae each	scoli less than half the size of dorso-median scoli	scoli remain half the size of dorso-median scoli but their length and the number of branches increase with each instar	
3.1-12A.1, 3.3-12A.3 5.4-12A.4, spine-like setae	scoli, each with 5-7 branches	scoli length and the number of branches increase with each instar		
dorso-median area from 5-12A, spline-like setae	one scoli per segment with 12A acquiring two, 5-7 branches per scoli	scoli length and the number of branches increase with each instar		
dorso-lateral area of 12B, spine-like setae	scoli on each side, pointing to the posterior, 5-7 branches each	scoli length and the number of branches increase with each instar		
area on side of anal plates, spine-like setae	scoli on each side, pointing to the posterior & up, 5-7 branches each	scoli length and the number of branches increase with each instar		

in the number and extent of black markings (see figure 7), ranging from one almost devoid of markings to one almost totally blackened by them, and in the presence of a creamy white or light yellow background coloration. Some type of correlation exists between the background coloration and the amount of black markings since heavily marked pupae are never yellow and, alternatively, those nearly devoid of markings are never white. The white and yellow phases are discrete (i.e., they are not the ends of a range), not sex-linked and, therefore, may be polymorphic or linked to a trait maintained by a balanced polymorphism. Rufa pupae were observed to generally have more black markings than bicolor and nigra.

Bionomics and Life History

C. lacinia is multibrooded and will go through as many generations as the season permits. It has a strong preference for sunflower (*Helianthus annuus* L.), but has been observed to transition to other plant species after a sunflower plant had been denuded. *Xanthium strumarium* (L.) (formerly *X. saccharatum* (Wallr.)), cocklebur, is in the sunflower family, but where the author has found *C. lacinia* larval colonies on sunflower along dry or running stream beds and washes, there were plentiful, often dominant stands of *X. strumarium*. Larval colonies were never found on cocklebur in the absence of sunflower, with one exception. In the fall of 1964 a thriving population was found on cocklebur at Granite Reef Dam. Granite Reef Dam is just below the joining of the Salt and Verde Rivers and is situated midway between Saguaro Lake and the city of Mesa, Arizona. The area immediately below the dam at one time was a favorite collecting spot for entomologists. This was the first and only time a thriving population on cocklebur has been observed. It was documented by Neck (1973) as a result of personal communication with the author. It was a quite unique occurrence, but, unfortunately, this area was completely destroyed by several major floods in the ensuing years and is now nothing more than the usual rocky and sandy river bed when water is not released.

The 1960's was a good time for agriculture and studying *C. lacinia* in the Valley. Citrus and agricultural crop fields (cotton and alfalfa) were very prevalent both within and adjacent to Valley cities. *C. lacinia* was very abundant, then, primarily because of agricultural practices that resulted in good sunflower stands along ditch banks and at irrigation water runoff ends of crop fields. Some of the sunflower stands occupied very large areas and supported large *C. lacinia* populations. However, because of changes in agricultural practices, urban development, and insecticidal spraying for west West Nile Virus, it is now extremely difficult to find *C. lacinia*. About 10 years ago the author found one alfalfa field inside Phoenix surrounded by homes. It had a good population of *C. lacinia* because the grower used the field for cattle grazing and so he did not apply herbicides or insecticides. The farm eventually was sold and is now the site of a housing subdivision. If the author had to do the research now that he did in the 1960's, he would have to rear larvae on artificial media, as researches have done since the author's work, and the field work to study the dynamics of the larval forms spatially and through time would not have been possible.

As initially mentioned, *C. lacinia* is multivoltine with the number of generations being a function of generation time. An estimate of generation time in the lab at 80^0 F can be obtained from the data of six matings given in Table 2. From this table, the average time from oviposition to emergence is 7.7 days, the average time spent in the larval stage is 15 days (the female column), and in the pupal stage 5.7 days. It is assumed it takes two days from time of female emergence to being able to fertilize eggs, and one day from copulation to ovipositing the first cluster. Adding all these numbers produces a laboratory generation time of 31.4 days – again, at 80^0 F. Assuming the same amount of time from adult emergence to the deposition of the first egg cluster, it has been observed a generation can be completed under field conditions in as few as 25 days. This is because the lab temperature was 80^0 F but the daytime temperatures in the field in the summer months are over 100^0 F. Later in the summer nighttime temperatures can be over 90^0 F. From this, it is estimated approximately 4-5 generations can be completed by the end of the season in the Valley.

A female oviposits eggs in clusters and oviposits a number of clusters during its lifetime. Some indication of fertility and egg cluster size from ten matings can be obtained from Table 3. In the laboratory, the average fertility per female was 432.6 eggs. One female, cross 23b, produced 1,169 eggs before dying. It oviposited seven clusters in its lifetime. Female 23a oviposited nine clusters but it did not have as high fertility. The average cluster size was 154.5 eggs with a high of 288 in a bicolor x rufa mating.

Adult females oviposit eggs near the base of the plant and, frequently, in such a manner that the first three instars are hidden from easy observation by a sampler walking through a field. When the larval population becomes larger, individuals can be found feeding along the entire length of the plant, except for the young leaves near the top.

Table 2. Life history data from laboratory reared progenies.

Mating Number	Duration of Larval Stage (in days)* M	F	Duration of Pupal Stage (in days)* M	F	Total number of egg clusters oviposited on successive days** V-29	V-30	V-31	VI-1	VI-2	VI-3	VI-4	VI-5***	Average Duration of egg stage (in days)	Average No. Offspring per Cluster
22b	15	15	5	6	-	1	1	2	1	1	-	-	7.7	180
22c	14	15	6	5	1	-	1	1	-	-	-	2	7.3	152
23a	14	15	6	6		1	2	2	2	1	-	1	7.8	95
23b	15	15	5	6	-	1	2	-	2	2	-	-	7.9	167
23c	14	15	6	5	-	1	1	-	2		-		8.0	264
23e	14	15	6	6	-	1	2	-	1	1	1	1	7.7	206

* M = male, F = female
** Copulation for each mating was performed on May 28, 1967.
*** Month and day.

Table 3. Fertility and cluster size by mating type.

	Egg	Larval Count			Grand
Mating Type	Cluster	Nigra	Bicolor	Rufa	Total
Nigra x Bicolor	1	124	117	-	241
	2	35	40	-	75
	3	95	74	-	169
Totals		254	231	-	485
Nigra x Bicolor	1	90	99	-	189
	2	66	77	-	143
Totals		156	176	-	332
Bicolor x Bicolor	1	7	32	-	39
	2	15	48	-	63
Totals		22	80	-	102
Bicolor x Bicolor	1	26	80	-	106
	2	10	27	-	37
Totals		36	107	-	143
Bicolor x Bicolor	1	26	93	-	119
	2	54	154	-	208
	3	38	90	-	128
Totals		118	337	-	455
Bicolor x Rufa	1	43	85	121	249
	2	23	57	84	164
	3	28	61	67	156
	4	37	101	150	288
Totals		131	304	422	857
Rufa x Rufa	1	-	22	45	67
	2	-	35	110	145
	3	-	54	138	192
	4	-	14	47	61
Totals		-	125	340	465
Rufa x Rufa	1	18	45	161	224
	2	8	25	81	114
Totals		26	70	242	338
Rufa x Rufa	1	17	36	156	209
	2	11	43	189	243
	3	18	40	146	204
	4	18	45	166	229
Totals		64	164	657	885
Rufa x Rufa	1	10	26	96	132
	2	12	19	101	132
Totals		22	45	197	264

Average fertility per mating: 4326/10 = 432.6 eggs
Average cluster size per mating: 4326/28 = 154.5 eggs

The eggs are oviposited underneath mature sunflower (or cocklebur) leaves in clusters from one to three egg layers deep. Eggs on the bottom layer (in contact with the leaf surface) are in contact with each other, stand upright, and exhibit an orderly arrangement. Those in the second layer deviate a little from being orderly, but in the third layer there is no order at all. The eggs are lopsided in every direction with spaces in between.

Larvae are gregarious from the first to the fourth instar, and molt together at the same time. The first to fourth instar larvae are leaf skeletonizers and feed in tightly packed colonies on a leaf. The first two instars are nearly always found underneath a leaf. Movement of the colony from a skeletonized leaf is usually to an adjacent leaf or those growing higher on the plant. The author has seen a colony migrate to a ragweed plant that happened to be in contact with the sunflower. During the migration to another leaf, the family unit may split into two or more groups. There may also be a merger of two or more colonies, which accounts for a mix of instars that is sometimes seen in field colonies, but a tightly packed feeding group is always reestablished. Thus, a colony in the field may be larvae from one egg cluster, a subset, or possibly a mixture of two or more colonies. When feeding they lay a network of silk wherever they go (except the last instar), possibly for better footing. In the last instar, the fifth, the larvae begin to disperse individually and eat through sections of a leaf rather than skeletonizing it.

Tachinid flies and Chalcid wasps have been observed to parasitize only last instar larvae. A caterpillar parasitized by a Tachinid can be easily recognized by the presence of minute (0.5 mm or less), white, elliptically shaped eggs attached to its dorsal scoli. When disturbed, larvae drop to the ground which is probably is also a strategy to counter ant predation. This was brought home when the author was doing his research on the genetics of the larval forms. When possible, the progeny of adult crosses were reared in the field by enclosing an entire sunflower plant in netting. Special measures had to be taken at the base of the plant to prevent red ants, about 1/16" long, from getting inside and devouring all larvae. The author has never seen predation by these red ants in the field under natural conditions. It may be larvae escape ant predation by dropping to the ground, but in a net when they drop they are still in it. Larvae also engage in a group response to a threat instead of dropping to the ground. In one instance, a small fly was observed inspecting a tightly packed second instar colony on the upper surface of a cocklebur leaf. All members of the colony were seen lashing about the anterior halves of their bodies in jerky movements until the fly left.

Molting begins by individuals of a colony congregating in a closely packed group usually under the leaf of the food plant. In the lab they may congregate in the corner of the petri dish, and, occasionally, those in the corner will spin a silk webbing around themselves, somewhat like tent caterpillars. On sunflower, a silk mat is spun into which are attached the anal prolegs. The larvae then remain in an inactive position for about 24 hours.

The molting process starts by the body becoming greatly distended, as though it was bloated from sickness. A series of pulsating movements begin, starting from the posterior to the anterior within one minute of the swelling. The head is the first to break through the skin, the body following with pulsations still occurring. The exuvia is left behind as a crumpled mass. In some instances the old head cuticle is still attached to the larva, but drops off immediately or within a minute of molting. Larvae having just molted resemble diapausing larvae in coloration, but normal color is restored within two hours, at which time they start feeding. Molting can sometimes be initiated prematurely in a larva close to molting by squeezing the body lightly with forceps.

Once pupation is started it is completed within 24 hours, the pupae hanging head downwards and attached to a silk pad by the cremaster.

Larvae start entering diapause in October even though there is still plenty of sunflower available. In *C. lacinia* this is not merely an inactive state with reduced metabolism. The larvae actually make a special molt into diapause, normally at the fourth instar but sometimes the third and rarely the fifth. Diapausing larvae move very slow and sluggishly, possibly because the thickened cuticle characteristic of diapause impedes movement. As mentioned previously, when a larva leaves diapause the cast skin holds its shape compared to the crumpled mass of a normal larva.

Entering diapause while food is still available indicates the trigger for diapause is not the scarcity of food. According to Patton (1963), photoperiod and temperature are the two principal factors stimulating diapause in insects, with photoperiod the one most likely triggering its onset. Lees (1955) states the termination of diapause in the majority of insects is controlled by temperature.

During the research to determine the genetic mechanism of the larval forms, the author made use of diapause to get larvae through the winter until sunflower was available the following year. Larvae from genetic crosses were forced into diapause by reducing the exposure to light (turning off an incandescent lamp) and lowering the temperature to 75° F. To take larvae out of diapause, they were first placed on a sunflower leaf in a 6" petri dish whose petiole was inserted in a slit in nine layers of thoroughly wet paper toweling. Then the temperature was raised to about 88° F with an incandescent bulb. During this period, while still in diapause, the larvae have the ability to feed and take in water and frequently do. However, only a small amount of feeding occurs in diapause.

C. lacinia exhibits what is termed a "long day" response (Lees, 1955), i.e., one to two weeks are required to leave diapause after the temperature and light level has been raised. After leaving diapause, feeding is sporadic, and a longer period of time is required to reach larval maturity than would occur in normal *lacinia* larvae at this stage of development.

Cockerell (Edwards, 1983) states larvae "hibernate" gregariously in dead curled up leaves of sunflower. This was observed, with the qualification that they are dried curled leaves on the plant, not on the ground. It was also observed that a silk mat is spun over the leaf surface and over any openings, thus creating an enclosed chamber, possibly serving for protection against spiders, Myrmeleontids, Tenebrionids, or other potential predators.

Larvae leave diapause by molting out of it, again head first. It was found in the lab that in the absence of fresh sunflower, larvae that leave diapause can sustain themselves on moistened dried sunflower or cocklebur leaves. Individuals in a natural population begin leaving diapause during the months of March and April. Based on the time it takes larvae to leave diapause (one to two weeks) after the initial stimulus to do so (raised temperature and constant light) and the long day response, it may be April or May before the females are laying eggs. It is usually not until June or July that large larval populations can be found.

It is not known how prevalent diapause is throughout the range of *C. lacinia*, whether it can be induced if not occurring in some of the non-desert Neotropical areas, and whether the extraordinarily thick cuticle is prevalent throughout its range or an adaptation to the xeric conditions in the SW U.S., Texas, and other similar xeric areas in its range from Mexico to Argentina. This would be an interesting study and may provide some insight into the environmental conditions under which *C. lacinia* initially evolved.

Acknowledgments

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