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WING POLYMORPHISM IN GRYLLODES SIGILLATUS

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STUDIES ON WING POLYMORPHISM IN GRYLLODES SIGILLATUS (Walk.)

by

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STATEMENT OF CONTRIBUTION TO KNOWLEDGE

In Gryllodes sigillatus (Walk.), the determination of wing polymorphism depends upon the temperature to which the nymphs are exposed in their early life. The phenomenon of wing polymorphism is also influenced by photoperiod, maternal inheritance and hormones. An extensive study of the influence of photoperiod showed an optimal photoperiod for wing development of 14 hr. This appears to be the first report in the literature of an "optimal" photoperiod as against a "critical" photoperiod for insects. The existence of maternal inheritance of the capacity for wing development due to the influence of photoperiod is demonstrated here for the first time in the Orthoptera. The ocelli are involved in the photoperiodic mechanism which controls wing polymorphism. This finding represents the first instance of its kind in any insect.

The neuroendocrine system is described here for the first time for any cricket. The histological evidence shows that macropterism in G. sigillatus is due to the level of the brain hormone(s). The juvenile hormone appears to play a key role in maternal inheritance.
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I. INTRODUCTION

According to Imms (1957), the phenomenon in which two or more forms of alary organs occur in the same species is referred to as wing polymorphism. Richards (1961) has made a brief review of the phenomenon in eight orders of insects among which the Orthoptera is one. In this order, wing polymorphism occurs only in the Tetrigidae and Acrididae (Imms, 1957; Richards, 1961). However, it is also known to be of common occurrence in Gryllidae. In an extensive study of the phenomenon in crickets, Sellier (1954) has shown that it occurs in Gryllus campestris Linnaeus, Pteronemobius heydeni (Fisher), Acheta xanthoneuroides (Chopard) [= Teleogryllus wernerianus (Karny)], Acheta [now Melanogryllus] desertus (Pallas), and Acheta [now Tartarogryllus] burdigalensis (Latreille). To this list must be added two more crickets, namely Nemobius yezoensis Shiraki and Gryllodes sigillatus (Walker). N. yezoensis is an univoltine ground cricket occurring in Japan (Masaki and Oyama, 1963), and is found only in the micropterous form in the field (Ochami, 1933, cited by Masaki and Oyama, 1963). Similarly G. sigillatus is also micropterous in nature (Ghouri and McFarlane, 1953b). Nevertheless these two species of crickets can be induced to develop wings under certain laboratory conditions.

Sellier's extensive study on diapause and wing polymorphism is confined to a single species i.e. G. campestris (Poisson and Sellier, 1947; Sellier, 1949, 1954, 1956).

* From Randell (1964).
Sellier (1954), with an aim to investigate the influence of external factors like temperature, light and humidity on wing development, carried out certain experiments from which he concluded that none of these factors were responsible for inducing wing development. In regard to internal factors, Sellier (1949) had already demonstrated that the implantation of brains of younger larvae into older ones eliminated diapause and induced wing development. Later, Sellier (1954) concluded that the sporadic occurrence of macropterism in this normally micropterous cricket was due to the level of the brain hormone.

*G. sigillatus* is a species with homodynamic development i.e., it has no diapause. Ghouri and McFarlane (1958b), while investigating the influence of temperature, noticed the occurrence of several macropterous forms. The authors predicted that higher temperatures in the optimal range favoured macropterism. In 1962, McFarlane presented quantitative evidence to confirm the prediction. In addition, the influence of diet, group effect, photoperiod and genetic constitution on wing development were also investigated (McFarlane, 1962, 1964). McFarlane (1962) showed that macropterism can be produced on diets containing glucose, casein, cellulose, cholesterol, inorganic salts and B-vitamins. The same author in 1964 demonstrated that the optimal level for both growth and wing development was an artificial diet containing 20% protein. As a result of an unfavourable photoperiod, crowding had little effect on growth and macropterism. Later McFarlane (personal communication), by using a 'selected' strain, demonstrated that group-reared larvae
(groups of 5) produced more macropterous forms than singly-reared ones. Then by conducting a preliminary experiment on photoperiod in summer he showed that growth retardation and wing development were due to a short photoperiod. Masaki and Oyama (1963) have also shown that the photoperiod has a definite influence on growth and wing development in N. yezoensis. But in this cricket, unlike C. sigillatus, it was the long (16 hr) and not the short (12 hr) photoperiod that was responsible for macropterism. Finally, the important role of genetic constitution was apparent as the offspring of macropterous females produced more macropterous forms. McFarlane also envisaged that the genetic effect may be mediated by the action of the brain hormone, as demonstrated by Sellier (1954) in C. campestris. Thus the determination of wing polymorphism in C. sigillatus probably depends on at least five factors: temperature, diet, group effect, photoperiod and genetic constitution.

The aim and scope of the author's research project have involved investigation of the following: the influence of initial rearing at 28°C and then at 35°C on wing development, the influence of different photoperiods (10 hr - 18 hr) on growth and wing development, maternal inheritance, photoperiodic mechanism and the role of neuroendocrine functions in determining wing polymorphism. The completion of the whole project has involved the examination of 3,720 insects - excluding the number of insects reared for maintaining the stock culture - and preparation and examination of 4,380 stained sections for histological work.
II. REVIEW OF LITERATURE

A. Wing Polymorphism in Insects

Wing polymorphism involves the occurrence of two or more forms of alary organs in the same species, which may or may not be correlated with sex (Imms, 1957). A brief review of the prevalence of the phenomenon in insects has been made recently by Richards (1961). According to Richards, it occurs only in eight orders of insects, namely Plecoptera, Orthoptera, Zoraptera, Psocoptera, Hemiptera, Diptera, Hymenoptera and Coleoptera.

In Plecoptera, various species of the genera Perla, Perlodes, Chloroperla and Nemura are dimorphic in the length of the male wings and the short-winged forms are more common at high altitudes (Schoenemund, 1924, cited by Richards, 1961). In Orthoptera, polymorphism as such is well known, frequent and of several kinds. But wing polymorphism occurs only in Tettigidae and Acrididae. In the former group, the fore wings are always vestigial, while the hind wings may be polymorphic (Imms, 1957).

In Zoraptera only a few species are known to be both winged and apterous, the latter form being more frequent. The Psocoptera includes several genera where one species is both winged and brachypterous or apterous.

The most complicated polymorphism occurs in Hemiptera (Apididae and Phylloxeridae) where eight or more different types of individuals, several of which occur simultaneously,
are involved. Simple brachyptery followed by long and short winged forms in one or both the sexes is known to be common in both Homoptera and Heteroptera.

In Diptera, *Leptocera fenestralis* (Fall.) and *L. nivalis* (Hal.), formerly known as two different species, are really long and short winged forms of only one species (Collin, 1956, cited by Richards, 1961). In *Copromyza pedestalis* (Mg.), normally a brachypterous species, Guibe (1939, cited by Richards, 1961) obtained macropterous forms in his laboratory culture.

In Hymenoptera, the wheat straw worm *Tetramesa* (= *Isosoma*) *grandis* (Riley) is known to have a wingless spring generation and a fully winged summer one (Webster and Reeves, 1909, cited by Richards, 1961). The Braconid parasite (*Sycosoter lavagnei* Pic. and Licht.) displays a more interesting feature. Its wing length is known to vary and alter progressively through the season (Lichtenstein and Picard, 1917, cited by Richards, 1961). An Ichneumonid, *Speophaga*, which parasitises social wasps, exhibits a peculiar dimorphism. Its two species in Britain have both short and long-winged forms, but differ in the structure of their cocoon. Several species of Pteromalids, Encyrtids and Bethylids have long and short-winged forms without any known relation to the environment. Salt (1952, cited by Richards, 1961), has shown that the food supply controls the wing size resulting in di-or-polymorphism in *Gelis*.

Finally in Coleoptera, variations in wing length are known to occur in Carabids, Dysticids and Curculionids as shown by some authors (Richards, 1961).
With reference to Hemiptera, in female Coccoidea and Aphidoidea (sexuales and some parthenogenetic forms), aterous forms are invariably found (Imms, 1957). Sometimes both aterous and alate males are present in both groups. Besides the two well-marked types, i.e. aterous and macropterous, sometimes intermediate or brachypterous forms occur in different families. The phenomenon is very clear in Gerroidea (Larsen, 1931, cited by Imms, 1957), Anthocoridae and Reduviidae among Heteroptera and certain Flumgoroidea (Delphacidae) and Jassidae among Homoptera. According to Southwood (1961) the phenomenon is widespread in Heteroptera, prevailing in all its major groups except Pentatomoidea and Hydrocorisae. Brinkhurst (1959a, 1960, 1963), who has made an extensive study of wing polymorphism in Heteroptera, has outlined the types of polymorphic cycles observed in British Heteroptera (1963).

Hille Ris Lambers (1966), has very recently made a comprehensive review of polymorphism in Aphididae. In many Phyllaphidine aphids fundatrices and all other viviparae are always alate, but their oviparae are aterous. On the other hand, in a few aphid species all viviparae are aterous, but oviparae are alate. By taking into account all the published literature, Hille Ris Lambers states that each holocyclic species has 5 - 6 morphs: males, and, as females, fundatrices, aterous and alate virginoparae or gynoparae and oviparae.

In regard to the wing polymorphism in Orthoptera, Imms (1957) and Richards (1961), mention its occurrence only in the
Tetrigidae and Acrididae. Nevertheless it is certainly known that the phenomenon is also of common occurrence in Gryllidae. Sellier (1954), in an extensive study of wing polymorphism in crickets, has proposed a tentative classification of the various species on the basis of their wing form. The species known from only one form are *Gryllus bimaculatus* De Geer and *Acheta domesticus* Linnaeus, both macropterous but latently polymorphic, whereas *Nemobius sylvestris* (Bosc) is micropterous. Species known in two forms where one form predominates and the other is occasional are *Gryllus campestris* and *Pteronemobius heydeni*. Belonging to this same group is *Teleogryllus wernerianus* which exhibits both the forms simultaneously. Finally there are two species namely *Melanogryllus desertus* and *Tartarozygryllus burdigalensis* which appear in three different forms. However, Sellier failed to include two more species in his classification, namely *Nemobius yezoensis* and *Gryllodes sigillatus*. *N. yezoensis* is an univoltine ground cricket which occurs in Japan (Masaki and Oyama, 1963). According to Ochami (1933, cited by Masaki and Oyama, 1963), this cricket is unique in that only the micropterous form is found in the field. *G. sigillatus*, the wing polymorphism of which forms the subject of the author's present study, is also micropterous in nature (Ghouri and McFarlane, 1958b).

**B. Determination of Wing Polymorphism**

In regard to the polymorphic organism, Wigglesworth (1954) is of the opinion that such an organism is generally endowed with the potentialities for all its diverse forms.
Although the form to be developed is sometimes determined by the genetic constitution, the latter is also sometimes over-ridden by the environmental conditions to which the growing stages are exposed. Wigglesworth (1954, 1960, cited by Southwood, 1961) has also suggested "that many forms of polymorphism may be due to hormones acting directly or via the cytoplasm on the gene system in the nucleus". Lees (1961), specifically dealing with the control of wing polymorphism in aphids, states that the agents responsible for the formation of alatae are temperature, quality and quantity of food, water deficiency in the plants, crowding and many other kinds of environmental stimuli, and that these important agents differ from species to species. Bonnemaison (1950, cited by Hille Ris Lambers, 1966), studying the development of wings in Aphididae, has also suggested more or less the same factors viz. nutrition, withering of the host, starvation, overpopulation, humidity, temperature and light.

1. External Factors:

We might recall here that Salt (1952, cited by Richards, 1961) has shown that the food supply controls the wing size in *Gallis*.

Poisson (1924) was the first person to notice the increase in the number of short-winged forms in summer in the British water bugs *Gerris* and *Valia*. Brinkhurst (1959a) has given a detailed account of the life cycles and wing polymorphism of these insects. Polymorphism occurs in most bivoltine *Gerris*
species in summer generation only but in *G. lacustris* and *G. lateralis* in both the generations. In other words, the polymorphic bivoltine species is entirely macropterous in the overwintering generation, but in most species the summer generation is mixed. Brinkhurst has observed the polymorphic forms being more common on mountains and in more rigorous climates. According to him, polymorphism is determined by an environmental switch mechanism—cold seemingly an important factor in all species, but with a small degree of genic segregation in *G. lacustris* and *G. lateralis*. In a detailed study of the phenomenon in Gerroidea, Brinkhurst (1958, 1959a) has suggested that although genetic constitution may be responsible in some cases, usually it seems that the environmental factor switches the phenotype to one or other type of morph. In 1959b, he postulated that such a factor is temperature and also described an experiment wherein by raising the temperature long-winged forms were produced but colder and normal conditions produced short-winged ones. Later, working on *G. odontogaster* (Zetterstedt), Brinkhurst (1963) found that the first batch of eggs, developed within their mothers in winter, most probably gave rise to short-winged forms, whereas the eggs which matured after spring emergence and those which developed in succeeding generation gave rise to macropterous forms.

Kisimoto (1959a, 1959b, cited by Danilyevsky, 1965) has observed that the larvae of the rice leaf-hopper *Nephotettix cineticeps* (Homoptera: Cycadellidae) enter diapause during short
days and give rise to small and relatively short-winged imagoes. But when they develop under continuous long day conditions, they produce larger long-winged summer forms.

The phenomenon of wing polymorphism has been studied in detail in aphids. According to Wilde (1962), "the seasonal polymorphism in insects is often day-length dependent". Marcovitch (1924, cited by Wilde, 1962) was the first to recognize the role of photoperiod in controlling the autumnal appearance of the sexual forms in aphids. Later Shull (1928, 1929 and 1930, cited by Wilde, 1962), working on Macrosiphum euphorbiae (Thomas) (= M. solani folii), detected the appearance of winged gynoparae due to the combinations of moderate temperatures and short day, and the formation of apterous viviparae as a result of high temperatures and long day.

As reviewed by Hille Ris Lambers (1966), the spontaneous appearance of great numbers of alatae in certain aphid species which have apterous virginoparae has been known for a long time. Bonnemaison (1949, 1950, cited by Hille Ris Lambers, 1966), working on the formation of wings in Brevicoryne brassicae Linné, came to the conclusion that crowding was the chief factor in the production of alatae. Since prolonged association between the mother and the larvae results in the production of alate forms in this species, Bonnemaison in fact has shown a maternal influence on wing formation acting on the larvae postnatally by crowding. Investigating the phenomenon further in Dysaphis plantagines, Bonnemaison (1951) noticed that crowding in apterous exules was unable to produce alate
virginoparae. But later (1964a, 1964b, cited by Hille Ris Lambers, 1966), he found that in this species the crowding combined with temperature and day-length is effective in the production of alate virginoparae. The alatae are produced when crowding is combined with short photoperiod and temperature below $22^\circ C$, but not when combined with long photoperiod and high temperature. In Myzus persicae Sulzer, Bonnemaison (1951) also discovered the influence of crowding but acting in a different interrelation i.e., usually only among the larvae. Lees (1961), working on Megoura vicina Guckea, discovered a somewhat different mechanism. When the apterous virginoparae were reared singly they produced only apterous progeny, whereas when crowded they gave rise to alate progeny. In this case, wing determination was complete before the birth of the larva and the control mechanism operated through the crowded mothers. Johnson and Birks (1960), while studying the phenomenon in Aphis craccivora Koch, concluded that the production of apterae is under the rigid maternal control of young alate mothers. In this species, at the beginning of the reproductive period, alatae usually produce apterae but later alatae. These facts led Johnson and Birks to conclude that all virginoparae commence their development as presumptive alatae but the apterae produced later are morphs deviated from this developmental path. Thus in several species of aphids, crowding has an important role to play in determining the wing form, though it operates differently in different species (Hille Ris Lambers, 1966).
Lees (1959) has made a detailed investigation of the photoperiodic regulatory mechanism of development in *M. viciae*. The seasonal cycle of this aphid is summarized by Danilyevsky (1965). During spring and summer, the species produces a series of generations of virginoparae developing with or without wings, and each generation usually includes some males. In autumn, they produce a bisexual generation with winged egg-laying females (oviparae), which after being fertilized are destined to produce wintering diapause eggs. Thus apterous virginoparae of this species are capable of producing three kinds of daughters i.e., parthenogenetic winged and wingless virginoparae and sexual oviparae, in addition to males. Lees (1959) has demonstrated that this kind of polymorphism is mainly controlled by the external factors which act through a series of maternal "switch mechanisms" and that these mechanisms in turn direct the oocytes and embryos into different pathways of development. He has also shown that high temperatures and long photoperiods influence the embryos to become virginoparae but the reverse conditions oviparae. However, the photoperiod as such has almost no effect on wing production.

Lees (1961) is of the opinion that the control of wing polymorphism and embryonic development in *Megoura* are under maternal control. Such a maternal switch mechanism is located in the head of the parent insect (Lees, 1959, 1960b), and the photoperiodic centre begins to operate well before the birth of the offspring (1959). The mechanism acts as an ovipara
determiner under short-day conditions and as a virginópara
determiner under long-day conditions. Lees (1963) also sets
a critical daily photoperiod approximately as 14 hr 55 min
at 15°C. He has demonstrated that photoperiods longer than
15 hr 23 min strongly favour virginópara production and those
shorter than the critical photoperiod promote ovipara production.
The prenatal light regime of long photoperiods which influences
the progeny sequence 'switches on' the controlling ('endocrine?')
system. In this aphid, the area most sensitive to photoperiod
is located near the midline of the head (1959).

As reviewed by Danilyevsky (1965), no data are available
in the literature regarding the inheritance of the photoperiodic
reaction and the investigations made so far are on the inheritance
of diapause. Such a concept of "maternal physiology" was first
introduced by Simmonds (1948) who studied its influence on the
incidence of diapause. The concept gained importance by the
investigations of numerous authors (see Lees, 1959; Morchoshi,
1959; Wilde, 1962; Roger, 1965; Hille Ris Lambers, 1966;
Saunders, 1966). The work of some of these authors pertaining
to hormones will be reviewed briefly under the internal factors.

Sellier (1954) has put forth certain ideas regarding the
various aspects of wing polymorphism in Gryllidae. In A. 
*domesticus* the phenomenon is latent, as it was not differentiated
in a definite way; in *M. desertus* and *T. wernerianus*, it is
current, constant and already foreseen in the larvae and seems
to be genetically determined; in *G. campestris* it is unexpected,
non-inheritable and appears only as an occasional fact and as an accident of growth; and finally in *P. heydenii* genetic factors seem to intervene in a complex way and the occasional onset of imaginal macropterism is generally accompanied by the arrest of the growth of the gonads. 

Sellier's experimental work on diapause and wing polymorphism is centered around only one species i.e. *G. campestris* (Poisson and Sellier, 1947; Sellier, 1949, 1954, 1956). This species passes through ten larval stadia, before reaching the imaginal stadium. The 9th stadium shows a larval duration much longer i.e. several months in nature and 35 - 40 days when reared at 30°C, than the 8th or the 10th stadium. Thus it obviously enters diapause (Sellier, 1949). Sellier (1954), in order to investigate the influence of external factors such as light, temperature and humidity on wing development in this species, carried out certain experiments. He reared these insects at more or less strong illumination, at 'intermittent' illumination and also in complete darkness. Besides, he also reared them at laboratory temperature, at a constant temperature (30°C) and also at low temperatures followed by warming. He did not give any consideration to nutritional factors. From these experiments, Sellier concluded that none of these factors considered singly or collectively had any effect on wing development and that the latter was due to the level of the brain hormone.

*G. sigillatus*, which has a wide spread tropical and subtropical distribution, has now transformed itself into a
cosmopolitan household and greenhouse pest (Blatchley, 1920; Chopard, 1938). Ghouri and McFarlane (1958b) reported that only the micropterous form of this species is known. In other words, so far no one has collected and/or described the macropterous form of this species from the field. This species is neither univoltine nor multivoltine but one with homodynamic development having no diapause. Ghouri and McFarlane (1958b), while investigating the effect of temperature, discovered the occurrence of several macropterous individuals among groups of nymphs reared at a variety of temperatures (23, 28, 33, 35, 38 and 41°C). The observation enabled the authors to predict that macropterism partly depends on the temperature of rearing and higher temperatures in the optimal range favoured the production of the winged form. The prediction was later confirmed by quantitative evidence (McFarlane, 1962). When the progeny of either micropterous or macropterous parents was reared at 28°C, it gave only micropterous forms, but rearing at 35°C produced some macropterous forms. Moreover, intermediates also appeared. The normal micropterous female has very small tegmina of the size of the larval lobes, but the male has short tegmina covering about half the abdomen. Both the sexes have wing pads lying completely beneath the tegmina. Macropterous females and males show fully developed tegmina and wings. On the other hand, the intermediates have slightly elongated tegmina but are devoid of wing growth. Since the intermediate males show macropterous tegmina and soon shed their wings, they should be considered as macropterous forms (McFarlane, 1964). Ghouri and McFarlane (1958b) also reported the following observations:
more frequent macropterism among females than among males, no difference between micropterous and macropterus forms either in the duration of the nymphal stage or in the weight gained by these adults, and the importance of genetic constitution on wing development. The last observation was evident as the winged condition could be selected for.

In addition to temperature, McFarlane (1962, 1964) also investigated the influence of diet, group effect, photoperiod and genetic constitution on wing development. The macropterus form could be produced on artificial diets containing glucose, casein, cellulose, cholesterol, inorganic salts and B-vitamins (McFarlane 1962). In 1964, the same author demonstrated that the optimal level for both growth and wing development was an artificial diet containing 20% protein. Even though this diet was not as good as baby rabbit pellets for growth, it was equally good for wing development. Crowding had little effect on growth and produced no macropterus forms, probably as a result of an unfavourable photoperiod. However, Ohmachi and Nakamura (1960, cited by Masaki and Oyama, 1963) have shown that a high density of rearing favoured the occurrence of macropterus forms in G. sigillatus. Later McFarlane (personal communication), by using a 'selected' strain, showed that the appearance of macropterus forms was much greater among group-reared larvae (groups of 5) than among singly-reared ones; but this is scarcely crowding. Regarding photoperiod, by conducting a preliminary experiment in summer he showed that a short photoperiod greatly retarded the growth but induced wing
development. Finally the importance of genetic constitution was obvious since the offspring of macropterous females gave more macropterous forms than the offspring from the stock culture.

McFarlane (1964), summing up the factors responsible for wing polymorphism in G. sigillatus, reported that the phenomenon probably depends on at least five factors: genetic constitution, temperature, larval diet, photoperiod, and group effect. He also envisaged that the genetic effect may be mediated by the brain hormone, as shown by Sellier (1954) in G. campestris.

Masaki and Oyama (1963) investigated the influence of photoperiod on growth and wing-form in a small ground cricket, N. yezoensis. They subjected this cricket to two different photoperiods i.e., 12 hr and 16 hr per day, and found that although the species is normally micropterous in the field, macropterous forms appeared in certain batches of long day (16 hr) treatment but none at all in short day (12 hr) treatment. Therefore in this cricket, unlike G. sigillatus, it was the long and not the short photoperiod that induced the formation of macropterous forms. Moreover the nymphal diapause was maintained for a long time in a short photoperiod but was readily terminated by a long one. The reason for the non-existence of macropterous forms in the field was due to the fact that the nymphs experienced short days in their late instars, whereas for the development of wings they must be exposed to long days beyond the middle of August, when the
day length is actually decreasing in the field. Therefore Masaki and Oyama concluded that the conditions required for wing development are non-existent in nature. However, the existence of the latent character for wing development has enabled the authors to trace the evolutionary history of the species.

It is possible that the species might have been derived from a multivoltine ancestor in which the micropterous and macropterous forms alternated seasonally. Later in the course of evolution, as the life cycle was channelled into the present univoltine and nymphal hibernation species, the insect was deprived of a chance to develop macropterous forms in the field. Nevertheless the retention of a concealed genetic potential for macropterism may be of some survival value in nature. The other findings by Masaki and Oyama were: an increase in the body size of the adults in the long photoperiod, females always outnumbering the males in short photoperiod, occurrence of both sexes in equal numbers in the long photoperiod, faster growth of macropterous individuals than the micropterous ones, and the tendency of the macropterous condition to be more frequent in females than in males. The last but one finding is in agreement with that of Sellier (1954) and the last two with those of Sellier (1954), and Ghouri and McFarlane (1958b).

The structures involved in the photoperiodic mechanism are thought to be variously trichiod sensilla, epidermis, ocelli, and cerebral neurosecretory cells (Danilyevsky, 1965). In addition, structures which have been definitely shown to respond to photoperiodic stimuli are the anterior portion of the hind gut of the European corn borer, Ostrinia nubilalis.
(Beck and Alexander, 1964b) and the terminal abdominal ganglion of *Periplaneta americana* L. (Ball, 1965). In the European corn borer the structure produces a developmental hormone called proctodone, which is also known to be present in *Galleria mellonella* (Beck and Alexander, 1964a). So far as the author knows, no attempt has been made experimentally to detect the photoperiodic mechanism or its receptors in crickets. This is also true of the influence of this mechanism on wing polymorphism in any of the insects.

2. Internal Factors

Polymorphism in insects is known to be genetically determined, but in many cases the genetic constitution allows a plasticity of form, whereby the final morph is determined by the changes in the environment through the action of hormones (Southwood, 1961; Wigglesworth, 1965a). A familiar concept in the study of insect metamorphosis is that environmental factors by exercising their influence on the sensitive stage of the insect operate a 'switch' mechanism which will decide which form is realized. According to Wigglesworth's (1954) conception, insect development is controlled by the change in the balance between growth and moulting hormone on one hand, and juvenile hormone on the other, and metamorphosis is due to a relative decrease in the activity of the latter.

The neuroendocrine system, its associated structures and their functions in insects have been described in general terms by Snodgrass (1935), Imms (1957) and Wigglesworth (1965b). The subject of neurosecretion and hormones as such has played an important role in three international symposia and has been
reviewed from time to time by some authors (see Wigglesworth 1954, 1957, 1964, 1965a; Van der Kloot, 1960; Gilbert, 1964; Highnam, 1964). Some important descriptions of the neuroendocrine system in insects are as follows: Hanström (1940) for Saltatoria, Highnam (1961) for Schistocerca gregaria, Willey (1961) for Periplaneta americana L. and other Blattaria, Khan and Fraser (1962) for P. americana, Scharrer (1962) for Leucophaea maderae, Johnson (1962, 1963) for aphids, Clark and Langley (1963) for Locusta migratoria migratoroides (R. & F.), Ganagarajah (1965) for a beetle, Nebria brevicollis (F.), Strong (1965 a, b) for Schistocerca sp. and Clark (1966) for Locusta migratoria L.

Cazal (1948, cited by Clark and Langley, 1963) has described the retrocerebral endocrine glands in a variety of insects.

According to Wigglesworth (1965a), the neuroendocrine system of insects, in general, consists of four principal components: neurosecretory cells, neurohaemal organs, endocrine glands and neurohormones. The neurosecretory cells are known to occur in all the ganglia and are recognized by their peculiar staining properties and also by their bluish appearance under dark-ground illumination. The neurohaemal organs are the paired corpora cardica of nervous origin. They are made up of three components, namely the bulbous endings of the axons of the cerebral neurosecretory cells, secretory cells of nervous origin and glial cells. The corpus cardiacum of each side lying just behind the brain is always in intimate relation with the corresponding corpus allatum. The endocrine glands are of ectodermal origin and include ventral, thoracic or prothoracic
glands - depending upon their position in the body - and corpora allata. The latter lie behind the brain and are in close association with the sympathetic nervous system. They are commonly paired but in Hemiptera and higher Diptera they unite to form a single median structure. Finally the neurohormones, whose exact origin is uncertain, can be extracted from the ganglia including the brain.

According to Beck (1964), in spite of the variations in the anatomical details of the neuroendocrine system, the sequence of humoral functions implicated in moulting and metamorphosis could be generalized as follows. The median and lateral cerebral neurosecretory cells produce secretions which are transported through the axons to the corpora cardiaca. The latter release these secretions into the blood stream from where they reach the prothoracic glands to stimulate the production of the growth and moulting hormone called ecdysone. Ecdysone in turn stimulates certain body tissues to initiate differentiation and moulting.

Weyer (1935, cited by Van der Kloot, 1960) was the first to discover the neurons showing cytological indications of secretory activity in the brain and other ganglia of the honey bee. Later Hanström (1938, cited by Wigglesworth, 1964) recognized them in the pars intercerebralis of the protocerebrum of Rhodnius. Following this observation, Wigglesworth (1939, 1940b, cited by Wigglesworth, 1964), by the implantation technique showed that they are the source of a hormone which triggers moulting. This was really the first experimental
proof of endocrine function for the neurosecretory cells in any animal. Since then several authors have employed aldehyde fuchsin and chrome-haematoxylin-phloxin staining methods as standard techniques for the demonstration of neurosecretory cells. These methods show the secretory granules in the perikarya and quite often stain the granules in the axons, so that the course of the axons can be traced easily (Van der Kloot, 1960). Since the stainable material is presumed to be a 'carrier protein' for the actual brain hormone(s), it is deduced that the hormone is present wherever the stainable material also occurs (Highnam, 1961).

Median neurosecretory cells of the brain synthesize a hormone in their perikarya and the hormone is known to be transported within the secretory granules moving down the axons of nervi corporis cardiaci I (NCC I) (Van der Kloot, 1960). Scharrer (1952) and Thomsen (1954), who performed an unmatched feat of ligaturing this nerve of Calliphora erythrocephala (Meigen), demonstrated the movement of the secretory granules down the axons. The material may then be stored at the axon endings in the corpora cardiaca and corpora allata from where it is released into the circulatory system (Van der Kloot, 1960). Several authors including Highnam (1961) have observed that the material in the median neurosecretory cells and NCC I is identical in its staining properties. Highnam (1961) postulates that the developmental events in insects may be controlled either by varying the rate of production of the material or by varying the amount of the material to be
released from the corpora cardiaca. In regard to the activity and inactivity of the neurosecretory system (i.e. median neurosecretory cells, NCC I and corpora cardiaca), Highnam (1962b) concluded that small amounts of material indicate active synthesis and release of developmental factors while large amounts signify some synthesis but no release of these factors. For example, when female desert locusts were reared without males the system exhibited large amount of material signifying inactivity and the development of oocytes was arrested (Highnam and Lusis, 1962). Similarly a greater amount of material accumulating within the system in matured females than in the immatured ones suggests a positive control over ovarian development (Highnam, 1962a). According to Fraser (1959), the best criterion of the activity of the median neurosecretory cells is furnished by the evidence of axonal transport of the material.

A bewildering variety of functions such as triggering the prothoracic glands, stimulating the proteinase synthesis in the gut, stimulating oviposition, promoting water retention, probably controlling the rhythmic activity etc., are attributed to the secretions from the protocerebrum (Van der Kloot, 1960). It is not certain whether a distinct hormone is essential to carry out each function or whether only one serves all purposes. Very recently Fraenkel and Hsiao (1965) have discovered a new hormone named bursicon secreted by the neurosecretory cells of the pars intercerebralis. They have shown that bursicon is necessary for the tanning of adult flies and other insects.

Wigglesworth (1965a) considers the corpora allata as among the most important endocrine glands in insects. They
are known to receive neurosecretory material from the brain (Gilbert, 1964). According to a review by Highnam (1964), the material is not only found in the glands but also in nervi corporis allati I and II (NCA I & II) in several insects. There is also evidence to show that the corpora allata store the brain hormone in this way and as a result they are stimulated or inhibited by the brain (Van der Kloot, 1960). Moreover Scharrer (1962) has emphasized that the morphological basis for the neurohormonal control mechanism of corpora allata is evident by the presence of neurosecretory axon terminals within the glands. Khan and Fraser (1962), working with *P. americana*, have observed an abundant accumulation of neurosecretory material in the corpora allata of freshly moulted last instar nymphs and adults, but very little in the sexually matured adults. The former observation is presumably related to the restraining action of the cerebral neurosecretory cells on the corpora allata (Scharrer, 1946, cited by Khan and Fraser, 1962; Scharrer 1952). On the contrary the decrease in the amount of stainable material, accompanied by an increase in the size of the glands, indicate relaxation of such a restraint (Khan and Fraser, 1962). The volume of the corpora allata is often used as criterion of their activity (Highnam, 1964). An active gland which is secreting a hormone shows an increase in volume accompanied by an increased amount of cytoplasm and scattered nuclei; the reverse is true for the inactive one (Strong, 1965b). For example, Highnam (1962a) found that in desert locusts as the terminal oöcytes develop,
the corpora allata also increase in volume, but when they are fully developed the glands decrease in volume. Moreover, when the females are reared without males, the corpora allata become small in size and the oocytes may cease to develop. Ganagarajah (1965), working on the neuroendocrine complex of the adult beetle *N. brevicollis* and its relation to reproduction, has also observed the accumulation of neurosecretory material around the corpora allata at the end of active breeding, suggesting its non-utilization by the glands. On the other hand, its absence suggests a rapid utilization. According to him, the material is presumably derived from the median neurosecretory cells as the staining properties of both are similar.

The corpora allata produce a hormone(s), the important functions of which have been reviewed by Wigglesworth (1964), Gilbert (1964) and Oberlander and Schneiderman (1966). Some of them are: the prevention of the maturation of the immature insects (Wigglesworth, 1955; Gilbert, 1962), gonadotrophic effect (Wigglesworth, 1964), general metabolic effect (Wilde and Stegwee, 1958; Thomsen, 1949, both cited by Oberlander and Schneiderman, 1966), activation of the accessory glands of male *Rhodnius prolixus* (Wigglesworth, 1936), involvement in the control of protein and RNA synthesis (L'Hélias, 1953; Vanderberg, 1963), causing marked increase in the rate of RNA synthesis by the prothoracic glands (Oberlander et al., 1965), etc.
Corpora allata control metamorphosis in an insect by retention of its larval or juvenile characters and by suppression of its adult characters (Wigglesworth, 1954). The abnormalities like metathetely (i.e., adult insects capable of reproduction still retain larval characters) and prothetely (i.e., the insect still in the larval condition develops adult characters like reproduction) are due to an upset in the hormonal balance or abnormal environmental conditions. Thinking in the light of hormonal balance, excessive influence of the juvenile hormone produces a metathetely and its lack—resulting in a failure of the prothoracic glands and consequently of ecdysone—produces a prothetely (Southwood, 1961). A short wingedness in insects is now known to be a juvenile character (Cousin, 1935; Sellier, 1949; Wigglesworth, 1954) which is likely to arise due to the excessive secretion of the juvenile hormone (Southwood, 1961). As shown by Wigglesworth (1948) and Lees (1961), by disturbing the normal hormone balance at the final moult, short-winged phenotypes can be produced. For example, when Lees (1961) gave an application of extra juvenile hormone to the fifth stage larvae of the aphid M. vicieae which were destined to produce winged forms, they produced wingless adults similar to the normal apterous forms. Von Dehn (1963, cited by Wigglesworth, 1964) also obtained similar results with the larvae of Aphis fabae when treated with farnesol. Lees has also shown that in aphids the alate structures developed in morphs which were destined to be apterous. Similarly small but adultiform wings develop in Rhodnius (Wigglesworth, 1936, 1948).
Brinkhurst (1963), who has made an extensive study of wing polymorphism in Heteroptera has written "..... the alary apparatus may similarly develop along two diverging lines, genetically determined, in which the tissues respond differently to a normal hormone balance". In Gerris the determination of wing polymorphism is two-fold: genetic segregation and temperature. As far as this is concerned, it is the wing forming tissue and not necessarily a change in the amount of juvenile hormone which seems to be a determining factor in nature (Southwood, 1961). In the light of Brinkhurst's (1963) observation that the general morphology - both in the wing and the abdomen of brachypterous forms of Gerroidea - is that of the adult, Wigglesworth (1964) has made an interesting suggestion. The short wings may develop as a result of the overactivity of moulting hormone, which brings about increased deposition of the cuticle over the wings. However, in those brachypterous Heteroptera inhabiting high altitudes, since a longer time is spent in the larval state due to low temperature, the condition results from being exposed to the influence of juvenile hormone for a longer period (Southwood, 1961). In certain Heteroptera, the males develop usually faster than the females (Giles, 1959, cited by Southwood, 1961). Since these females are mostly brachypterous and males generally macropterous, it is possible that the female larvae are under the influence of juvenile hormone for a longer time, leading to brachypterism and metathetely (Southwood, 1961).
Lees (1961), working on *M. vicinae*, concluded that the mechanism of wing development is under direct prenatal maternal control and is also humoral. When the apterous mothers are isolated, they elevate the activity of the corpora allata in older embryos and apterous progeny develop; crowded mothers exercise a reverse influence resulting in an alate progeny. In intermediate winged forms the corpora allata are partially "turned on". In view of these interpretations the mode of operation of the maternal endocrine system on the corpora allata of the late embryos determines the alate or apterous condition. As known already, the system itself is in turn under the influence of both the environment and various intrinsic timing mechanisms. Although young larvae may respond directly to the environment, the control of embryonic development is more frequently through maternal physiology. Johnson and Birks (1960), after performing a very interesting experiment in aphids, suggested that the maternal regulatory mechanism is situated in the brain, as the progeny of decapitated apterous mothers were apterous whereas those of non-decapitated ones were alate. They also suggest that the corpora allata have a key role to play in wing polymorphism.

Lees (1960a) thinks that the timing mechanism in aphids is located in the cytoplasm and that the elements may self-duplicate during transformations. The maternal control system enables the egg or the embryo to develop along alternate paths by responding to the suitable stimuli of the environment. Roger (1965) thinks that photoperiod acting on maternal
physiology stimulates the production of a hormone in the mother which now becomes incorporated in the unlaid egg and remains to act at a later stage. Working on the maternal influence on diapause in *Coeloides brunneri* Vier., Roger has shown that the parent female is responsive to photoperiodic stimuli and transmits the determination for diapause to the next generation through its eggs. Gilbert and Schneiderman (1961, cited by Wigglesworth, 1964) have detected the presence of juvenile hormone in the eggs of *Hyalophora cecropia*. The hormone is derived from the mother but from about the seventh day onwards it is produced by the developing embryo itself. It is found in all the larval stages but not prior to the final moult.

The maternal physiology of diapause has been studied in detail in *Bombyx mori* L. by Fukuda (1951, 1952), and Hasegawa (1951, 1952) (both cited by Morohoshi, 1959) and by Morohoshi (1959). Fukuda and Hasegawa found independently that the transplantation of the suboesophageal ganglion produced diapause eggs. Morohoshi believes that the suboesophageal ganglion hormone may be a growth-inhibitory hormone and the corpora allata hormone a growth-promoting one. In transplantation experiments, the former transformed a "non-diapause" individual into a "diapause" one and the latter vice-versa. The production of hormone in both is controlled by the brain through the nerve commissures.

White (1965) has obtained some interesting results by studying the changes in the volume of corpora allata in aphids of known ages. The alate parents of *Brevicoryne brassicae* (L.)
give birth almost exclusively to apterous progeny while the apterous parents, either apterous or alate progeny (Bonnemaison, 1951). Johnson and Birks (1960) have presented evidence that the young of alate parents are determined as apterae prenatally, while those of apterae, as apterae or alatae by about the second instar. White found that the corpora allata of alatae had doubled in size after 24 hours of imaginal moult, while in contrast those of adult apterae decreased considerably at about the same age. These results are interpreted as follows. By presuming that the form of the embryos is under the influence of the mother's hormones, an active corpus allatum of the alate mother would thus pass on more juvenile hormone into the embryos and direct them to become apterous. On the other hand, the inactive corpus allatum of apterous mothers would translocate less hormone and its effect could be modified postnatally by the external factors. Interestingly enough, the corpora allata of apteriform aphids during the 3rd and 4th nymphal instar were larger than in alatiform aphids. According to White, this observation on nymphal stages supports the theory that the apterous condition may result from a relatively high concentration of the juvenile hormone (Johnson, 1959, cited by White 1965; Lees, 1961).

Wigglesworth (1954) thinks that in *G. campastris* also there may be more juvenile hormone in the later stages. Brachypterism which is a juvenile character appears always to be associated with a diapause in the 9th stage and also with a
deficiency of moulting hormone. Sellier (1949) has demonstrated that by implanting the brains of younger larvae into the older ones, not only could diapause be eliminated but also wing development is stimulated and macropterous forms are obtained. Later Sellier (1954) concluded that the sporadic appearance of macroptery in this cricket must be attributed to an abnormal morphogenic functioning of the brain, which upsets the equilibrium in the phenomenon of wing growth. In 1956, Sellier also reported that the brain produces a certain factor which triggers moulting, inhibits diapause and exercises a morphogenic action on the wing leading to macroptery.
III. MATERIAL AND METHODS

*G. sigillatus* served as the laboratory animal. Eggs, newly hatched nymphs, ultimate forms and adults were obtained from a stock culture, which had been selected for macropterous forms for three generations, as and when required to carry out the experiments.

A. Maintenance of Stock Culture

The stock culture of *G. sigillatus* was maintained after the method described by Ghouri and McFarlane (1958a). The nymphs as well as adults were supplied with "Baby Rabbit Pellets" as food, obtained from Ogilvie Flour Mills Ltd., Montreal. Newly hatched nymphs were placed in large candy jars and were provided with a water source, crumpled strips of paper and food. The jars, after being covered with a muslin cloth and secured with rubber bands, were then placed in a large incubator maintained at 34 ± 2°C and at a relative humidity of 50 ± 5%. The required R.H. was maintained by a saturated salt solution of Ca(NO₃)₂·4H₂O. A fan and a thermostat fitted inside the incubator kept the temperature constant. The adults emerging from these nymphs were reared at 26 ± 2°C to lengthen their life span and period of active oviposition.

B. Collection and Incubation of Eggs

The method described by Ghouri and McFarlane (1958a) was employed for this purpose. Eggs were collected in jam cups (1½ oz., Lily Paper Cups Ltd., Toronto) half filled with
fine sterilized sand moistened with distilled water. The water vials were removed from the rearing jars and the cups so prepared were supplied to gravid females. The cups were left in the jars for about 24 hours, after which adequate number of eggs were observed in the sand. The cups were then emptied onto a piece of paper towelling and the latter was placed in a 16 oz. ointment jar containing a small pad of moist cotton to provide 100% humidity. The jars were then tightly covered with lids and the eggs were incubated at $34 \pm 2^\circ C$ for about nine days when they hatched. The newly emerged nymphs were transferred to a glass container from where they were picked up by an aspirator for further transference to large candy jars for rearing.

C. Description of Incubators

Large incubators used for incubating the eggs and also for maintaining the culture have been described previously. In order to carry out the experiments on photoperiod, three small incubators each measuring 2' x 2' x 1.66' were used. Each incubator was provided with a 14 watt fluorescent tube in the ceiling. According to Wilde (1962), since the photoperiodic induction in insects is independent of light intensity, the latter is of little importance once a certain threshold is exceeded. The required length of photoperiod was automatically controlled by a time switch to ± 6 minutes per day. The starting time in all the experiments was 8 a.m. as a matter of convenience. Different photoperiods were obtained according to the needs of the experiments by
manipulating the time switch. The temperature and relative humidity were maintained at 33.5 ± 1.5°C and at 50 ± 5% respectively. In view of the small size of the incubators, thermostat, time switch, and fan were all fitted outside the incubators. The incubators were examined regularly to ascertain the accuracy of the temperature and photoperiod.

D. Rearing the Nymphs for Experiments

The newly hatched nymphs picked up by an aspirator were transferred to 16 oz. ointment jars. They were reared in a group of ten in the small or large incubator depending upon the type of experiment carried out. As a source of water supply a 32 ml shell vial filled with distilled water and plugged with aseptic cotton was placed inverted in the jar. A shallow boat prepared from filter paper was filled with ground baby rabbit pellets and kept on the side opposite the water vial. With a view to increasing the available surface area and to provide a place for moulting a folded sheet of filter paper was placed between the water vial and the boat. The jars were then covered with a muslin cloth held fast by a rubber band to prevent the escape of the nymphs. The jars were randomized in each experiment for rearing the nymphs. Regular examination was undertaken to maintain an adequate supply of food and water. After about four weeks the jars were examined every day for emerging adults and the data regarding their date of emergence, sex, wing condition and weight were recorded.

Any alterations made in the above methods will be mentioned under experimental procedures.
E. **Anesthetization**

In order to facilitate removal and easy selection of the required nymphs, the insects were anesthetized with carbon dioxide. An adjustable metal hose fitted to a compressed gas cylinder was held against the muslin cloth of the rearing jar and the gas was gradually released. When the insects were immobilized, the gas flow was stopped and selection and removal were carried out. Insects treated in this way showed normal activities after the anesthetic effect disappeared.

F. **Cauterization**

Winged nymphs in penultimate stages were anesthetized with carbon dioxide. Cauterization was performed by means of a Hyfrecator under a binocular microscope. One group of insects were cauterized on their lateral and median ocelli and the other group at three different spots other than the ocelli on the head. The latter group served as a control. Extreme care had to be taken in cauterizing so that the effect was just enough for the experimental purpose but not enough to seriously injure the insects.

G. **Slide-Preparing Techniques**

In order to study the neuroendocrine system of *G. sigillatus*, it was essential to prepare a large number of slides. Ultimate and adult stages were used for this purpose. The details in regard to the exact type of material used will be described under experimental procedure.

It is a common experience that the insect cuticle interferes with proper sectioning. Therefore Highnam (1961)
and Willey (1961) recommended that the cuticle be removed before embedding and sectioning. Using this method, the author experienced not only loss of orientation but also great difficulty in recognizing the various structures. Sectioning of the whole head capsule without removing the cuticle was therefore attempted. The method proved to be extremely satisfactory. The sections were quite good and enabled the author to recognize the orientation and various structures contained in them.

Two different techniques, one for studying the neurosecretory cells and the other for corpora allata, were employed. In both cases, the insects were decapitated with a very fine pair of scissors and the heads were allowed to drop down immediately into the fixative. Prior to decapitation, the antennae were cut off. Fixation was done "in vacuo" to eliminate air from the large air-sacs in the head so that rapid and unrestricted fixation would take place (Highnam, 1961). Since certain modifications were incorporated into the techniques, it may be worthwhile to mention them below wherever necessary.

1. Neurosecretory Cells

A modified Bouin's suggested by Halmi (1952) was used as a fixative. Heads fixed for 24 hr were washed under tap water for 15 hr, then transferred to 70% alcohol containing lithium carbonate for 24 hr with several changes to remove the picric acid. Dehydration was continued with 90% and absolute alcohols for 12 hr each. In the case of absolute
alcohol three changes were necessary to ensure complete dehydration. The heads were then left in benzene for 3 hr for clearing. The procedures for impregnation, embedding, sectioning paraffin ribbons and transference of sections to slides were employed as described by Gray (1958). The heads were sectioned serially at different planes but at the same thickness i.e., 10 microns.

The simplified aldehyde fuchsin (PF) staining technique described by Cameron and Steele (1959) proved highly satisfactory for staining the neurosecretory cells. The technique includes some of the modifications of Gabe (1953) together with a counterstain of Halmi (1952). The stain was prepared according to Gabe's recipe with some modifications suggested by Cameron and Steele.

Sections were deparaffinized and hydrated in the usual way. Since picric acid was still present, lithium carbonate was again used in 70% alcohol. After several trials oxidation in Gomori's fluid for 1 min, staining with PF for 5 min and counterstaining with Halmi's mixture for 20 sec were found to be the most suitable timings for the sections. Canada balsam was used as a mounting medium.

It is very important to preserve the liquid stain in a thoroughly air-tight condition. Otherwise the alcohol vaporizes and makes the stain more concentrated than actually required. Such a stain would give unsatisfactory results. Surprisingly enough, sometimes even the well-preserved stain does not show up in sections immediately. However the stain
does appear distinctly within a few days after keeping the slides on a hot plate.

Recently Ewen (1962) has described an improved aldehyde fuchsin staining technique. In view of the numerous slides to be prepared and the fact that Cameron and Steele's procedure proved satisfactory, this more time-consuming technique was not employed.

2. Corpora Allata

Heidenhain's Susa, a fixative recommended by Mendes (1948), was prepared according to the directions given by Pantin (1946). Heads fixed for 12 hr were dehydrated in 96% and absolute alcohols for 12 hr each. The 96% alcohol was made pale brown with iodine. Paraffin blocks were prepared as described before. Here also the heads were sectioned serially at different planes but at a thickness of 7 microns.

Deparaffinization and hydration were done in a routine manner. In order to get rid of the HgCl₂ from the fixative, Lugol's solution, running water and 5% sodium thiosulfate were used as recommended by Humason (1962). Ehrlich's Haematoxylin (3 min) and eosin (2½ min) were used as stains as suggested by Mendes (1948). Sections were mounted in Canada balsam.

The foregoing technique was later abandoned as the technique for the neurosecretory cells also proved useful for studying the corpora allata.
IV. EXPERIMENTAL PROCEDURES, OBSERVATIONS AND RESULTS

All the experiments described under this heading were carried out with offspring of females of the stock culture. Each experiment was carried out with insects from a different generation. The newly hatched nymphs, ultimate forms and adults were selected at random and the rearing jars were also randomized in the incubator.

A. Initial Rearing at 28°C and then at 35°C

The experiment was designed to investigate the influence on wing development of initial rearing of the newly hatched nymphs at 28°C, followed by rearing at 35°C. For this purpose, 1,000 nymphs were selected and were divided into 100 groups of 10 individuals each. Initially all the nymphs were reared in the large incubator maintained at 28 ± 2°C. After every week 10 jars were transferred to another large incubator maintained at 35 ± 2°C. The data regarding sex and wing condition of the emerging adults were recorded every day after a lapse of about 4 weeks from the date of the experiment. The numbers of adult males obtained were 41, 29, 36, 36, 30, 42, 42, 47, 48 and 44 at the end of 1-10 weeks respectively. On the other hand, those obtained for adult females were 36, 50, 29, 50, 60, 53, 44, 39, 38 and 46 at the end of the same weeks. The experiment was started on August 17, 1964 and continued until November 10, 1964.

The results of the experiment are presented in Figures 1 and 2. It is obvious that as the number of weeks at 28°C
Fig. 1. Percentage of macropterous males from larvae reared first at 28°C, then at 35°C.
Fig. 2. Percentage of macropterous females from larvae reared first at 28°C, then at 35°C.
increases the percentages of macropterous males and females decreases. There is no significant difference in the percentages for macropterous males and females during the first two weeks. But from the end of the 2nd week onwards, the percentages for both sexes drop abruptly, suggesting that wing development is determined in early larval life, depending upon the kind of temperature to which the nymphs are exposed. Once the condition for micropterism is determined, even an exposure to an optimum temperature of 35°C does not reverse this condition. This fact is quite apparent during the last 4 weeks when very few or no macropterous forms were obtained.

B. Influence of 10 hr, 12 hr, and 14 hr Photoperiods

The object of the present experiment and the succeeding ones described under the sections C, D and E, was to investigate the photoperiodic influence on growth and wing development in G. sigillatus. Six hundred newly hatched nymphs were allotted to 60 groups of 10 individuals each. The nymphs were divided into 3 batches, each consisting of 200 nymphs, in 60 jars. One batch was exposed to a photoperiod of 10 hr (8 a.m. - 6 p.m.), the second to 12 hr (8 a.m. - 8 p.m.) and the third to 14 hr (8 a.m. - 10 p.m.). The experiment was commenced on November 12, 1964 and terminated January 17, 1965.

The results are listed in Tables I and II. The maximum percentage of macropterous males and females was obtained at 14 hr, and no macropterous forms were produced at the 10 hr photoperiod. Few were obtained at 12 hr. Survival
TABLE I. Incidence of wing development in adult males and females, and percentage survival, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micro-pterous</td>
<td>Macro-pterous</td>
<td>% Macro-pterous with intermediate</td>
<td>Macro-pterous</td>
</tr>
<tr>
<td>10</td>
<td>69 0 0 0</td>
<td>108 0 0 0</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td>12</td>
<td>56 0 1 2</td>
<td>93 3 0 3</td>
<td>3</td>
<td>77</td>
</tr>
<tr>
<td>14</td>
<td>49 24 2 35</td>
<td>76 35 2 31</td>
<td>33</td>
<td>94</td>
</tr>
</tbody>
</table>
TABLE II. Average weight of the adults (≤ 24 hr old), and average duration of the larval stage, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>Adult Males Obtained</th>
<th>Adult Females Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Average Wt. (mg)</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>206.3 ± 47.0</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>159.7 ± 38.8</td>
</tr>
<tr>
<td>14</td>
<td>75</td>
<td>177.0 ± 34.6</td>
</tr>
</tbody>
</table>
was better at 14 hr than at the 12 hr and 10 hr photoperiods (Table I). With reference to Table II, the results for average weights of males showed a significant difference at the 1% level between any two photoperiods. The same is true of females excepting that there is no significant difference between the average weights at the 10 hr and 14 hr photoperiods. Therefore the males and females gain the maximum and minimum weights at 10 hr and 12 hr photoperiods respectively. But at 14 hr they show more weight than those at 12 hr. All the results for average duration of larval stage for both males and females showed a significant difference at the 1% level. Therefore the insects require maximum time to become adults at 10 hr and minimum time at 14 hr photoperiod.

C. Influence of 12 hr, 14 hr and 16 hr Photoperiods

The same number of nymphs and groups were used as in the preceding experiment. One batch of nymphs was subjected to a photoperiod of 12 hr (8 a.m. - 8 p.m.), the second to 14 hr (8 a.m. - 10 p.m.), and the third to 16 hr (8 a.m. - 12 p.m.). The experiment was begun on January 17, 1965 and terminated March 28, 1965.

The results are presented in Tables III and IV. As in the preceding experiment, the maximum percentage of macropterous forms occurred at the 14 hr photoperiod. The percentage obtained at 16 hr was far more than at 12 hr. Survival was better at 16 hr than at the other photoperiods (Table III). In regard to the average weights for both males and females, there was a significant difference at the 5% level only in the
TABLE III. Incidence of wing development in adult males and females, and percentage survival, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>MALE</th>
<th>% Macropterous with intermediate</th>
<th>FEMALE</th>
<th>% Macropterous with intermediate</th>
<th>Total % Macropterous</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>55</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>42</td>
<td>29</td>
<td>4</td>
<td>44</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>16</td>
<td>55</td>
<td>20</td>
<td>3</td>
<td>30</td>
<td>72</td>
<td>35</td>
</tr>
</tbody>
</table>
TABLE IV. Average weight of the adults (≤ 24 hr old), and average duration of the larval stage, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>Adult Males Obtained</th>
<th>Adult Females Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Average Wt. (mg)</td>
</tr>
<tr>
<td>12</td>
<td>59</td>
<td>175.2 ± 36.4</td>
</tr>
<tr>
<td>14</td>
<td>75</td>
<td>190.6 ± 37.0</td>
</tr>
<tr>
<td>16</td>
<td>78</td>
<td>184.1 ± 38.8</td>
</tr>
</tbody>
</table>
case of males at the 12 hr and 14 hr photoperiods (Table IV). The same statement holds for average duration of larval stage. The males therefore gain more weight at 14 hr than at 12 hr and show a longer average duration at 12 hr than at 14 hr.

D. Influence of 14 hr, 16 hr, and 18 hr Photoperiods

The number of nymphs and groups used for this experiment was the same as in the previous two experiments. Three batches of nymphs were reared at photoperiods of 14 hr (8 a.m. - 10 p.m.), 16 hr (8 a.m. - 12 p.m.), and 18 hr (8 a.m. - 2 a.m.). The experiment was started on April 12, 1965 and terminated June 18, 1965.

The results are given in Tables V and VI. As usual the 14 hr photoperiod yielded the maximum percentage of macropterous forms. The 18 hr photoperiod yielded a smaller percentage of macropterous forms than the 16 hr photoperiod. The percentage survival was better at 18 hr (Table V). Due to an unexpected rise in temperature up to 39°C in the incubator maintained at the 16 hr photoperiod, 90 nymphs died on May 14, 1965. The dead nymphs were removed from the jars but the data regarding their sex and wing condition were recorded. Table VI shows that in males there was a significant difference at 1% level between average weights at 14 hr and 18 hr. On the other hand, in females, a significant difference at the same level appeared at 16 hr and 18 hr. So it is apparent that the males gain more weight at 18 hr than at 14 hr and the females gain more weight at 18 hr than at 16 hr. The average duration of the larval stage in males was the same in all treatments and the same is true for females at 16 hr and 18 hr. However in females there were
TABLE V. Incidence of wing development in adult males and females, and percentage survival, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>MALE</th>
<th>FEMALE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micro-pterous</td>
<td>Macro-pterous</td>
<td>Inter-pterous with intermediate</td>
</tr>
<tr>
<td>14</td>
<td>51</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>1) 27</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>11) + 44</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>67</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

* Indicates the figures obtained from the survived adults.

+ Indicates the figures obtained from the survived adults as well as from the dead nymphs.
TABLE VI. Average weight of the adults (≤ 24 hr old), and average duration of the larval stage, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>Adult Males Obtained</th>
<th>Adult Females Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Average Wt. (mg)</td>
</tr>
<tr>
<td>14</td>
<td>76</td>
<td>171.7 ± 32.0</td>
</tr>
<tr>
<td>16</td>
<td>29</td>
<td>175.3 ± 35.9</td>
</tr>
<tr>
<td>18</td>
<td>86</td>
<td>184.5 ± 28.1</td>
</tr>
</tbody>
</table>
significant differences at the 1% and 5% levels between the average durations at 14 hr and 16 hr, and at 14 hr and 18 hr respectively. Therefore, the females show greater average durations at 16 hr and at 18 hr than at 14 hr.

E. Influence of 10 hr, 14 hr, and 18 hr Photoperiods

In order to obtain more macropterous forms two candy jars containing nymphs were exposed to 14 hr photoperiod on June 4, 1965. When the adults emerging from these nymphs were examined, it was surprising to notice that only 5% macropterous forms were obtained. This finding was suspected to be due to the fact that the parents of this offspring had been exposed to natural long day light in the large incubators. Therefore, it was thought that maternal inheritance played some important role in producing macropterous forms. Consequently an experiment was designed to test this hypothesis. The plan of the experiment was to obtain the offspring from the parents treated with a 14 hr photoperiod and to expose them to 10 hr, 14 hr, and 18 hr photoperiods. In addition the offspring from the stock culture reared at natural long day light were to be exposed to the same photoperiods so that they would serve as a control. But for some unknown reason the females treated with the 14 hr photoperiod did not oviposit, in spite of several attempts to collect the eggs. After dissection of the females the spermathecae were found to be devoid of sperms. Therefore this part of the experiment could not be started. But the finding did help to carry out the succeeding experiment on maternal inheritance.
The remaining part of the experiment whose description follows was started on July 27, 1965 and continued until September 25, 1965. Unlike previous experiments, only 300 nymphs were selected and were divided into 30 groups of 10 individuals of each. Three batches of nymphs each consisting of 100 individuals were exposed to three different photoperiods, viz. 10 hr (8 a.m. - 6 p.m.), 14 hr (8 a.m. - 10 p.m.), and 18 hr (8 a.m. - 2 a.m.).

The results are given in Tables VII and VIII. As anticipated the maximum percentage of macropterous forms was obtained at 14 hr and none at all at 10 hr. As in the previous experiments, macropterous forms occurred at 18 hr but were far less numerous than at 14 hr. Survival proved to be better at 18 hr (Table VII).

Table VIII reveals that the males showed the same average weights in all treatments. In the case of females there were significant differences at the 1% level at 14 hr and 18 hr, and at 10 hr and 18 hr photoperiods. Consequently the females gain more weight at 18 hr than at the 10 hr and 14 hr photoperiods. Regarding the average duration of the larval stage, the results for both males and females showed significant differences at the 1% level between 10 hr and 18 hr, and between the 10 hr and 14 hr photoperiods. That is to say, the males show a longer duration at 10 hr than at the 14 hr and 18 hr photoperiods. On the other hand, the females show a longer duration at 18 hr than at 10 hr and also a longer duration at 14 hr than at 10 hr. However, both males and females showed the same duration at the 14 hr and 18 hr photoperiods.
TABLE VII. Incidence of wing development in adult males and females, and percentage survival, of larvae reared at various photoperiods.

| Photoperiod in hours per day | MALE |       |       | MALE |       |       | FEMALE |       |       |      |      |       | FEMALE |       |       |       | Total |       |       | Survival |
|-----------------------------|------|--|---|------|--|---|---|------|--|---|---|---|---|---|--|---|---|---|---|---|---|---|
| 10                          | 18   | 0  | 0  | 0    | 53 | 0  | 0  | 0    | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 76  |
| 14                          | 23   | 12 | 2  | 38   | 26 | 22 | 3  | 42   | 40   | 90 |
| 18                          | 29   | 7  | 3  | 26   | 42 | 11 | 0  | 21   | 23   | 92 |

53
TABLE VIII. Average weight of the adults (< 24 hr old), and average duration of the larval stage, of larvae reared at various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Adult Males Obtained</th>
<th>Adult Females Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Average Wt. (mg)</td>
<td>Average duration larval stage (days)</td>
</tr>
<tr>
<td>10</td>
<td>18 165.2 ± 24.8</td>
<td>43.9 ± 6.1</td>
</tr>
<tr>
<td>14</td>
<td>37 177.3 ± 36.1</td>
<td>38.4 ± 3.4</td>
</tr>
<tr>
<td>18</td>
<td>39 170.1 ± 36.4</td>
<td>37.9 ± 2.5</td>
</tr>
</tbody>
</table>
As far as the results for wing development are concerned, they are approximately the same as in the previous experiments. Since the offspring were from the stock culture reared at natural long day light, maternal inheritance was expected. The expectation did not materialize probably because the culture had been transferred to another incubator where they remained in darkness for 11 days.

F. Maternal Inheritance

The experiment was designed to investigate the role of maternal inheritance in determining the macropterous condition. In order to obtain the eggs for this kind of experiment, parents were exposed to 10 hr, 14 hr, and 18 hr photoperiods. The eggs could not be obtained from parents exposed to the 10 hr photoperiod. Dissections showed that the males were devoid of spermatophores and the spermathecae of females were without any sperms. As it seemed possible that the 10 hr photoperiod might prevent mating and the formation of spermatophores, 14 hr males were transferred to the 10 hr culture from which the 10 hr males were completely removed. When this modified culture was then treated with a 14 hr photoperiod, the females laid eggs and the latter hatched. However, the number of newly hatched nymphs was not quite adequate to start the experiment. Nevertheless when the experiment was carried out and the results were analysed the survival was found to be very poor. Therefore, it was thought that the results of this part of the experiment are not worth including here.
It was also interesting to observe that the 14 hr photoperiod gave many eggs, 18 hr far less and 10 hr none at all. These observations seem to be in conformity with the percentages obtained for macropterous forms at the same photoperiods. It is possible that 14 hr photoperiod may be the most suitable for mating, production of spermatophores, development of eggs and for oviposition.

The eggs collected from the parents exposed to 14 hr and 18 hr photoperiods were incubated as usual. The newly hatched nymphs from the 14 hr culture were divided into 24 groups of 10 individuals each. The groups were then divided into 3 batches of which one was exposed to 10 hr (8 a.m. - 6 p.m.), the second to 14 hr (8 a.m. - 10 p.m.), and the third to 18 hr (8 a.m. - 2 a.m.) photoperiods. A similar procedure was followed for the offspring of parents treated with the 18 hr photoperiod. The experiment was started on October 3, 1965 and continued until December 5, 1965.

The results are presented in Table IX. The total number and percentage of macropterous forms obtained at different treatments are arranged in the decreasing order from above downwards.
TABLE IX. Incidence of wing development in adult males and females, and percentage survival, of larvae reared at various photoperiods, from parents exposed to various photoperiods.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>MALE</th>
<th>FEMALE</th>
<th>Total</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>To Parents</td>
<td>To Progeny</td>
<td>Micro-pterous</td>
<td>Macro-pterous</td>
<td>Intermediate</td>
</tr>
<tr>
<td>14 14</td>
<td>14 9 0</td>
<td>39</td>
<td>12 17 0</td>
<td>59</td>
</tr>
<tr>
<td>14 18</td>
<td>29 5 0</td>
<td>15</td>
<td>17 11 0</td>
<td>39</td>
</tr>
<tr>
<td>18 14</td>
<td>22 4 0</td>
<td>15</td>
<td>29 5 0</td>
<td>15</td>
</tr>
<tr>
<td>18 18</td>
<td>14 1 0</td>
<td>7</td>
<td>36 1 0</td>
<td>3</td>
</tr>
<tr>
<td>18 10</td>
<td>5 0 0</td>
<td>0</td>
<td>23 0 0</td>
<td>0</td>
</tr>
<tr>
<td>14 10</td>
<td>6 0 0</td>
<td>0</td>
<td>23 0 0</td>
<td>0</td>
</tr>
</tbody>
</table>
G. Investigation of Photoperiodic Mechanism by Cauterization Method

During the course of the histology work (see next section), it was observed that the dorsal and median ocelli appeared to have nervous connections with the median neurosecretory cells of the pars intercerebralis (Plate I, A, B; Plate II). Therefore, it was suspected that the ocelli might be involved in the photoperiodic mechanism. In order to test this hypothesis the following experiment was carried out. There were 140 penultimate winged nymphs and were divided into two groups of 70 individuals each. After anesthetization one group was cauterized on their lateral and median ocelli to destroy the receptor cells and the other group was cauterized at three different spots other than the ocelli on the head to serve as a control. The nymphs were reared in the small incubator maintained at the 14 hr photoperiod. The experiment was begun on January 5, 1966 and continued until January 31, 1966. The data regarding sex and wing condition of the emerging adults were recorded.

The results are listed in Table X. The insects which served as a control gave a total of 12% macropterous forms and those cauterized on the ocelli none at all. Survival was better in the former group than in the latter.
TABLE X. Incidence of wing development in adult males and females, and percentage survival, of winged larvae with ocelli cauterized in penultimate stadium.

<table>
<thead>
<tr>
<th>Ocelli</th>
<th>MALE</th>
<th>FEMALE</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micro-pterous</td>
<td>Macro-pterous</td>
<td>Intermediate</td>
<td>Macro-pterous</td>
</tr>
<tr>
<td>Cauterized</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Not Cauterized</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
</tbody>
</table>

Survival
H. Role of the Neuroendocrine System in Wing Polymorphism

Since the object of this research was to study wing polymorphism, the neuroendocrine system in *G. sigillatus* will be dealt with only briefly so as to enable us to understand its role in determining the phenomenon. For this purpose the heads of micropterous and macropterous crickets in the ultimate stage were used. However, the neuroendocrine system described here also includes observations made from sections of the heads of adult crickets. The slides were prepared according to the techniques described in Section G of "Material and Methods". As already mentioned, the whole head capsule was sectioned and the sections proved to be quite satisfactory (See Plate III as a model example).

The neuroendocrine system of *G. sigillatus* conforms to the basic plan common to pterygote insects. The dorsal part of the pars intercerebralis region of the protocerebrum consists of two closely apposed groups of median neurosecretory cells (Plate IV). These cells stain dark purple suggesting the presence of PF-positive material i.e. the neurosecretory material itself (Plate V). The material can be located easily throughout the system wherever it occurs by its dark purple colour. In addition there are other groups of neurosecretory
cells in the brain. Since these cells and their axons do not show a distinct PF-positive reaction, their location and course cannot be studied satisfactorily. However, they do not appear to play an important role in determining wing polymorphism as will be evident a little later. The combined axons of each group of median neurosecretory cells constitute a nerve called nervus corporis cardiace I (NCC I). The course of these two nerves through the brain can be easily traced due to the presence of PF-positive material along their length (Plate IV). Both the nerves traverse vertically downwards through the brain for some distance and then cross. The axons of each nerve after crossing emerge from the brain and innervate the corpus cardiacum of the opposite side.

The corpora cardiaca are elongated paired structures lying behind the brain, and on the dorsal side of the gut. They are in close association with the hypocerebral ganglion and are closely applied to the walls of the aorta (Plates VI and VII). Since the cricket head is hypognathous, in its normal posture, the long axes of the glands are directed upwards. Therefore their 'posterior' parts are, in fact, dorsal to their 'anterior' parts. In order to have a better comparison with the corpora cardiaca of other insects it is preferable to use here the terms anterior and posterior instead of ventral and dorsal. The posterior parts of the corpora cardiaca are bounded by a transverse strip of aortal tissue. The glands actually form the lateral walls and to some extent the ventral wall of the aorta. Posteriorly, the corpora cardiaca consist
of two large lobes, which appear to project into the lumen of the aorta. But ventrally they fuse to form an unpaired lobe, which lies directly on the hypocerebral ganglion. A pair of short nerves which also show PF-positive material in them, connect this lobe to the hypocerebral ganglion (Plate VIII). The paired as well as unpaired lobes consist of nervous tissue derived from NCC I as suggested by the presence of PF-positive material in them (Plate VI). In addition the paired lobes, along their borders, usually show some pale-looking cells with clear boundaries. These cells are suspected to be intrinsic cells which are known to produce their own secretion. The unpaired lobe gives off a pair of nerves called nervi corporis allati I (NCA I) which innervate the corpus allatum of either side (Plates IX and X).

The corpora allata are paired, compact, glandular structures containing closely packed cells whose cell boundaries are not well demarcated. Each of them lies on the dorsolateral side of the gut in close association with the corpus cardiacum (Plate IX). As mentioned before each corpus allatum receives a nerve (NCA I) from the unpaired lobe of the corpus cardiacum. Another nerve called nervus corporis allati II (NCA II) is also seen at the other end of the gland. The NCA II is known to emerge from the suboesophageal ganglion and to innervate the corpus allatum. Such a connection may exist in this cricket too. The corpus allatum, NCA I and NCA II all show the presence of PF-positive material in them (Plates IX, X and XI). It is interesting to observe that the corpus allatum is attached
to the lateral side of the gut, probably for support (Plate XII).

Although they are not strictly relevant, two more observations will be mentioned here. Situated a little in front of the brain is an approximately pear-shaped body, the frontal ganglion, with its apex directed posteriorly (Plate XIII). From this apex the recurrent nerve is given out. Along the border of the frontal ganglion there are small and also very large neurosecretory cells fully packed with PF-positive material. Similarly the suboesophageal ganglion also shows neurosecretory cells on its lateral and ventro-median sides (Plate XIV). The neurosecretory cells in the ventro-median region are much larger and contain a large amount of neurosecretory material.

With a view to investigating the role of hormones in determining wing polymorphism, the heads of micropterous and macropterous crickets in the ultimate stage were sectioned. The observations made by examining these sections are seen in Plates IV, V, XV, and XVI. In the macropterous form, the median neurosecretory cells show a small amount of PF-positive material which is seen being released into their axons where it is clearly visible (Plate IV). On the contrary, in the micropterous form, the median neurosecretory cells are densely packed with the material but it is not released into their axons (Plate V). In regard to the corpora allata of the micropterous and macropterous forms, their size appeared to be the same in both cases (Plates XV and XVI). However, in order to prove this, the length and
width of the corpora allata were measured by using a micrometer and the volume of the glands was calculated presuming them to be approximately ellipsoidal. Serial sections of corpora allata of heads—five macropterous and five micropterous forms—were selected for this purpose. The length and width of ten serial sections from each head were measured. In all 50 measurements of length and the same number for width were made for each form and their average, standard deviations and volumes were calculated. The results are presented in Table XI, and they are not at all significant.

I. Role of the Neuroendocrine System in Maternal Inheritance

The discovery of maternal inheritance due to the influence of photoperiod led the author to carry out the experiment described here. Newly emerged micropterous adult females were reared singly in the small incubators. One group of insects was exposed to a 14 hr photoperiod and the other to an 18 hr photoperiod. The insects were decapitated at the end of 1, 2, 4, 8 and 16 days and the slides were prepared as per the techniques for neurosecretory cells described in section G of "Material and Methods".

In the 1-day-old insects treated with the 14 hr photoperiod for 1 day, PP-positive material was abundant in few of the median neurosecretory cells and very little material was seen in their axons (Plate XVII). On the other hand, in insects of the same age but treated with the 18 hr photoperiod, the median neurosecretory cells contain comparatively less material but their axons contain more material (Plate XVIII).
TABLE XI. Average length and width, and volume of corpora allata of macropterous and micropterous females in ultimate stages.

<table>
<thead>
<tr>
<th></th>
<th>Average length (microns)</th>
<th>Average width (microns)</th>
<th>Volume (microns)$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macropterous</td>
<td>$116.5 \pm 10.2$</td>
<td>$87.4 \pm 8.6$</td>
<td>407,959</td>
</tr>
<tr>
<td>Micropterous</td>
<td>$121.2 \pm 13.2$</td>
<td>$86.0 \pm 12.6$</td>
<td>401,080</td>
</tr>
</tbody>
</table>
The two observations mentioned here were also noted in insects of different ages exposed to 14 hr and 18 hr photoperiods for the remaining days. The corpora cardiaca of 1 and 2-day-old insects exposed to the 14 hr photoperiod for 1 and 2 days showed abundant PF-positive material in them (Plates VIII and XIX). But at the end of 16 days, the corpora cardiaca showed less PF-positive material (Plate XX). In 1-day-old insects exposed to the 14 hr photoperiod for 1 day, PF-positive material was observed in the corpora allata, NCA I and also in NCA II (Plates IX, X, XI and XXI).

In regard to the corpora allata, analysis of small samplings showed that the average length and width and hence the volume of the glands appeared to increase in insects of different ages exposed to the 14 hr and the 18 hr photoperiods; maximum figures were noticed in insects at the end of 4 days. In order to know the exact size difference in the corpora allata of 4-day-old insects exposed to 14 hr and 18 hr photoperiods, their average length and width, standard deviations and volumes were calculated. As in the preceding section, length and width were measured by using a micrometer and the volume was calculated presuming the glands to be approximately ellipsoid. Serial sections of corpora allata of 8 heads - 4 for 14 hr and 4 for 18 hr photoperiods - were selected for this purpose. In all 35 and 37 measurements of length and width were made from the insects exposed to 14 hr and 18 hr photoperiods respectively.

The results are presented in Table XII. There was a significant difference at the 1% level between the average
TABLE XII. Average length and width, and volume of corpora allata of micropterus adult female exposed to 14 hr and 18 hr photoperiods for 4 days.

<table>
<thead>
<tr>
<th>Photoperiod in hours per day</th>
<th>Average length (microns)</th>
<th>Average width (microns)</th>
<th>Volume (microns)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>157.2 ± 26.4</td>
<td>84.4 ± 10.9</td>
<td>450,739</td>
</tr>
<tr>
<td>18</td>
<td>184.6 ± 19.9</td>
<td>102.8 ± 7.5</td>
<td>795,366</td>
</tr>
</tbody>
</table>
lengths as well as between the average widths. The histological evidence regarding the difference in sizes of corpora allata is seen in Plates XXII and XXIII.
V. DISCUSSION

A. Initial Rearing at 28°C and then at 35°C

The results presented in Figures 1 and 2 apparently suggest that wing development is determined in early larval life, depending upon the kind of temperature the nymphs experience. Once the condition for micropterism is determined, it becomes irreversible despite an exposure of the nymphs to an optimal temperature of 35°C. This fact is supported by the results obtained during the last 4 weeks when very few or no macropterous forms appeared. These findings support the conception of 'canalisation', put forth by Waddington (1940). The occurrence of more frequent macropterism among females than among males was consistent with a similar observation made by Ghouri and McFarlane (1953b).

B. Influence of Various Photoperiods on Wing Polymorphism

In every experiment carried out on photoperiod, the maximum percentage of macropterous forms was always obtained at the 14 hr photoperiod (Tables I, III, V, and VII) and none were obtained at the 10 hr photoperiod (Tables I and VII). The 12 hr photoperiod did induce wing development but very few macropterous forms appeared (Tables I and III). A 16 hr photoperiod also induced wing development and macropterous forms occurred in greater numbers than at the 12 hr photoperiod (Tables III and V). A similar observation was made for the 18 hr photoperiod but this photoperiod yielded a smaller percentage.
of macropterous forms than the 16 hr photoperiod (Tables V and VII). Therefore, it is beyond any doubt that the 14 hr photoperiod is an optimal photoperiod for macropterism in G. sigillatus. This appears to be the first finding of an "optimal" photoperiod for insects, in contradistinction to a "critical" photoperiod at which an "all or none" type of response is determined. Any explanation of this phenomenon at the present time would be highly speculative.

However, in spite of provisions made for optimal temperature, optimal photoperiod and optimal diet for the 'selected' strain of the stock culture, the maximum percentage of macropterous forms obtained was only 44% (Table III). Why was it that the remaining 56% never became macropterous? The answer may be that these micropterous forms are not endowed with the genetic potentiality for macropterism. It is also worth observing that the short photoperiods (10 hr and 12 hr), shorter than the 14 hr photoperiod, inhibited macropterism more strongly than the long photoperiods (16 hr and 18 hr). Therefore it is obvious that the macropterism is more susceptible to short photoperiods less than 14 hr photoperiods than to long photoperiods. McFarlane (1964), by performing a preliminary experiment, suggested the importance of photoperiod in macropterism, and the inducement of wing development by a short photoperiod. Although the experiment was of a preliminary type, his observation that a short photoperiod induced wing development is in conformity with the author's observation of the same at the "short day" 14 hr photoperiod.
Masaki and Oyama (1963), working with a small ground cricket, *N. yezoensis*, have shown that macropterous forms appeared at a 16 hr photoperiod but none at all occurred at a 12 hr photoperiod. Therefore, in this small ground cricket, unlike *G. sigillatus*, it was the long and not the short photoperiod that induced wing development. However, Masaki and Oyama investigated the influence of only two photoperiods. They also found that females always outnumbered males in the short photoperiod and that both sexes occurred in equal numbers in the long photoperiod. In *G. sigillatus* females always outnumbered males irrespective of short or long photoperiods (Tables II, IV, VI and VIII). They also observed that the macropterous condition is more frequent in females than in males. But in *G. sigillatus* macropterous males and females occur more or less in equal numbers (Tables I, III, V, and VII). (Note that these results differ from those in the temperature experiment). Their observation regarding the faster growth of macropterous forms than micropterous ones is also similar in *G. sigillatus*. Sellier (1954), and Ghouri and McFarlane (1958b) have also made similar observations in *G. campestris* and *G. sigillatus* respectively.

The question arises as to why the macropterous form of *G. sigillatus* has not been collected and/or described from the field up till now, in spite of its distribution in tropical and subtropical parts of the world, where the photoperiod is around 14 hr i.e., optimal for this species. Lees (1961), while dealing with wing polymorphism in aphids, has stated that
external factors like temperature, quality and quantity of food, crowding etc., control the phenomenon. These factors differ from species to species. Bonnemaison (1950, cited by Hille Ris Lambers, 1966) also believes in the control of the phenomenon by external factors. In this context we have to recall that the determination of wing polymorphism in *G. sigillatus* is dependent on at least five factors: genetic constitution, temperature, photoperiod, larval diet and the group effect (McFarlane, 1964). *G. sigillatus* has a suitable genetic constitution for wing development and also the necessary photoperiod in nature. Therefore, it would be logical to suppose that the proper temperature, larval diet and group effect provided in the laboratory to induce wing development may be non-existent in nature. Masaki and Oyama suggest that the retention of a concealed genetic potential for macropterism may be of some survival value. They have also postulated that the non-existence of macropterism in the small ground cricket in the field is due to the fact that the nymphs experience short days in their late instars, which actually require long days for wing development. However, the same is not possible for *G. sigillatus* as it has a homodynamic development.

Sellier (1954), working on *G. campestris*, investigated the influence of external factors like temperature, light and humidity on wing development and concluded that none of these factors considered singly or collectively had any influence on
wing development. As pointed out by McFarlane (1964), the experiments carried out by Sellier were not critical enough to demonstrate the influence of these factors, and G. campestris, like G. sigillatus, is likely to respond to them to produce macropterous forms.

In all the experiments, survival proved to be better at long photoperiods than at short photoperiods (Tables I, III, V and VII), suggesting that the insects thrive better at long than at short photoperiods.

In regard to the average duration of the larval stage, it is obvious that the males require more time at the 10 hr and 12 hr photoperiods than at the 14 hr, 16 hr and 18 hr photoperiods to reach the adult stage (Tables II, IV, VI and VIII). This is also true for females in certain experiments (Tables II and VI). However, females did show the same time at 12 hr, 14 hr and 16 hr photoperiods (Table IV). In another case, females at the 14 hr and 18 hr photoperiods showed a longer developmental time than at the 10 hr photoperiod (Table VIII). In the latter case, the unexpected results were probably due to the fact that the culture had been transferred to another incubator where they accidentally remained in darkness for 11 days.

The results for average weights of both males and females are not consistent and so do not enable us to draw any definite conclusions as to an effect of photoperiod.
C. Maternal Inheritance

The results presented in Table IX prove beyond any doubt that maternal inheritance exists in *G. sigillatus* and plays a very important role in determining wing polymorphism. The existence of such a phenomenon is revealed here for the first time in crickets and for that matter in the whole order of Orthoptera. In discussing the results here, the photoperiod first mentioned refers to parents and the second to their progeny. It has been conclusively shown in the preceding section that 14 hr acts as an optimal photoperiod for wing development. Therefore it is not surprising to find the maximum percentage of winged forms i.e., 50% at the 14 hr and 14 hr photoperiods. Surprisingly enough at the 18 hr and 18 hr photoperiods only 4% macropterous forms were obtained. This finding supports the observation made by McFarlane (1964) that macropterous individuals do not occur in the laboratory culture during summer months. The 10 hr photoperiod completely suppressed the wing development in spite of the parents being exposed to 18 hr and 14 hr photoperiods. In this context, we recall here what has been mentioned under section F of Chapter IV. The 10 hr photoperiod appeared to prevent the insects from mating, and to prevent production of spermatophores and oviposition. Although the culture was later modified, only some eggs hatched and the survival of nymphs was very poor. On the contrary, the 14 hr photoperiod appeared to be the most favourable for
mating, production of spermatophores and for oviposition as it gave many eggs. The eggs collected from the culture at 18 hr were far less in number and none were available from 10 hr culture. These observations appear to be in conformity with the percentages of macropterous forms obtained at the various photoperiods.

In G. sigillatus, we have seen that photoperiod (10 hr-18 hr) plays a very important role in wing development and also in maternal inheritance. According to Wilde (1962), "seasonal polymorphism in insects is often day-length dependent". The phenomenon of wing polymorphism has been investigated in detail in British water bugs and aphids. The appearance of short-winged forms in summer in water bugs was first noticed by Poisson (1924). Later Brinkhurst (1958, 1959a, 1963) made an extensive study of the phenomenon and the seasonal occurrence of winged and wingless forms. He has shown that although the genetic constitution is important in some cases, usually an environmental factor like temperature switched the phenotype to one or the other type of morph (Brinkhurst, 1958, 1959a). In 1963, he also observed that in Gerris odontogaster, the first batch of eggs, developed within their mothers in winter, usually gave rise to short-winged forms, and those maturing after spring emergence produced macropterous forms. On the other hand, in several species of aphids, crowding plays an important role in determining wing form and also in maternal inheritance, though it operates differently in different species (Hille Ris Lambers, 1966).

Lees (1961) has stated that wing polymorphism and
embryonic development in *M. viciae* are under maternal control. The mechanism responsible is located in the head of the parent insect (Lees, 1959, 1960b), and the photoperiodic centre begins to operate well before the birth of offspring (Lees, 1959). Kisimoto (1959a, 1959b, cited by Danilyewsky, 1965) working on the larvae of the rice leaf-hopper has observed that short-days produce short-winged imagos and long-days, long-winged forms.

D. Investigation of Photoperiodic Mechanism by Cauterization Method

The results in Table X suggest that the ocelli - median as well as lateral- do participate in receiving the photoperiodic stimuli. So far as the author is aware, no attempt has been made experimentally to detect the photoperiodic mechanism or its receptors in Orthoptera. This is also true for the influence of this mechanism on wing polymorphism in any insect.

When the insects were cauterized on their ocelli, the photoperiodic mechanism responsible for macropterism was impaired and consequently no macropterous forms were produced. The insects which served as a control should have produced more macropterous forms as they were incubated at optimum conditions. Instead they produced only 12% macropterous forms, the reason for which may be the injury that they received. The painting of the ocelli with an opaque substance seems in the event to be a better means of blocking ocellar function.

In 1940, Hanström stated that in Saltatoria, the ocellar nerves terminate in the neighbourhood of the protocerebral bridge. But in *G. sigillatus* these nerves appeared to have
nervous connections with the median neurosecretory cells of the pars intercerebralis (Plates I, A and B, and Plate II).

E. Role of the Neuroendocrine System in Wing Polymorphism

There is no description in the literature of the neuroendocrine system of any cricket. The system has been described here for the first time. Since the object of the research project was not to study the neuroendocrine system in detail but to investigate its role in wing polymorphism, this discussion will be brief.

In view of the description in section H of Chapter IV, it is obvious that the neuroendocrine system of G. sigillatus is in conformity with the basic plan common to pterygote insects including Orthoptera. The innervation of corpora cardiaca by NCC I, crossing of these two nerves, innervation of the corpora allata by NCA I arising from the corpora cardiaca, the position of the corpora allata lateral to the corpora cardiaca, and connection of the corpora cardiaca to the hypocerebral ganglion by two stout nerves, all described and reviewed in Saltatoria by Hanström (1940), are also observed in G. sigillatus. The nervi corporis cardiaci II (NCC II), which connect the corpora cardiaca to the brain, mentioned by Hanström, were not seen in this insect. This is probably because the nerve is too slender to be observed in dissection (Hanström, 1960), and does not show the PF-positive material in its axons. The neuroendocrine system of G. sigillatus is, in general, similar to that described for Schistocerca gregaria.
Highnam, 1961), *Locusta migratoria migratoroides* (Clark and Langley, 1963), *Schistocera* sp. (Strong, 1965a and b), and *Locusta migratoria* (Clark, 1966). However, the posterior paired lobes of the corpora cardiaca and the intrinsic cells within them, and the NCC II described by Highnam (1961), were not observed in *G. sigillatus*. Instead in *G. sigillatus* some pale looking cells with clear boundaries along the borders of the paired lobes of corpora cardiaca were observed (Plate VI) and suspected to be intrinsic cells. The attachment of the corpus allatum to the gut (Plate XII), probably for support, appears to be the first observation of its kind in insects. Neurosecretory cells also occur in the suboesophageal ganglion (Plate XIV) and in the frontal ganglion (Plate XIII). The only other record of the occurrence of these cells in the frontal ganglion is that of Kobayashi (1957, cited by Van der Kloot, 1960).

The role of the brain hormone in determining wing polymorphism in *G. sigillatus* is evident from the observations in Plates IV and V. In the ultimate macropterous form, the median neurosecretory cells display a comparatively small amount of PP-positive material within them but it is seen being released into their axons where it is distinctly visible (Plate IV). In contrast to this, in the ultimate micropterous form, the median neurosecretory cells are densely packed with the material but their axons are devoid of it, suggesting that the material is not released into them (Plate V). In regard
to the activity and inactivity of the neurosecretory system, Highnam (1962b) has stated that small amounts of material indicate active synthesis and release of developmental factors, while large amounts signify some synthesis but no release of these factors. According to Fraser (1959), the best criterion for the activity of the median neurosecretory cells is the axonal transport of the material. Moreover the stainable material is presumed to be a 'carrier protein' for the actual brain hormone(s) (Highnam, 1961). In view of these authors' observations, it is certainly possible to conclude that the median neurosecretory cells in the macropterous cricket are active and release the brain hormone(s), whereas those in the micropterous cricket are inactive and do not release the brain hormone(s).

Sellier (1949), working on *G. campestris*, demonstrated by implantation of the brains of younger larvae into older ones that diapause was terminated and macropterous forms were produced. He did not study the activity of the corpora allata. In *G. sigillatus* the histological evidence, confirms that macropterism is due to the level of the brain hormone. Wigglesworth (1954) thinks that in *G. campestris* also there may be more juvenile hormone in later stages leading to brachypterism and diapause in the 9th stage i.e., penultimate. However, in regard to the corpora allata of macropterous and micropterous ultimate forms in *G. sigillatus* their average lengths and widths, and their volumes are the same (Table XI),
and so presumably is the amount of juvenile hormone produced.
The corpora allata are, however, known to be inactive in
ultimate stages as shown in *Rhodnius* (Wigglesworth, 1964) and
in *Hyalophora cecropia* (Gilbert and Schneiderman, 1961, cited
by Wigglesworth, 1964), but these insects do not exhibit wing
polymorphism.

**F. Role of the Neuroendocrine System in Maternal Inheritance**

Among the results and observations mentioned under Section
I of Chapter IV, the most important for the phenomenon of
maternal inheritance are those pertaining to the corpora allata.
In the 4-day-old micropterous adult female exposed to an 18 hr
photoperiod for 4 days, the glands are larger than those of
insects of the same age exposed to a 14 hr photoperiod for the
same number of days (Plates XXII and XXIII). The volume of the
corpora allata is often used as a criterion of their activity
(Highnam, 1964). An active gland which is secreting a hormone
shows an increase in an volume and vice-versa (Strong, 1965b).
Therefore the corpora allata at an 18 hr photoperiod are more
active than at the 14 hr photoperiod.

The corpora allata are known to control metamorphism
in insects by retention of its larval characters and by
suppression of its adult characters (Wigglesworth, 1954). In
insects, short-wingedness is now known to be a larval or
juvenile character (Cousin, 1935; Sellier, 1949; Wigglesworth,
1954), which is likely to arise as a result of the excessive
secretion of the juvenile hormone (Southwood, 1961). Moreover
the abnormalities like metathetely and prothetely are due to some
upset in the hormonal balance or abnormal environmental conditions (Wigglesworth, 1954). For example, Lees (1961) has shown that the application of extra juvenile hormone produced wingless forms in *M. vicina* which are destined to produce winged forms. In this aphid, the mechanism of wing development is under direct prenatal maternal control and is also hormonal. The isolated apterous mothers elevate the activity of the corpora allata in older embryos and apterous progeny develop; crowded mothers exercise a reverse influence resulting in an alate progeny. Johnson and Birks (1960) suggest that the corpora allata have a key role to play in wing polymorphism. Roger (1965) is of the opinion that photoperiod acting on maternal physiology stimulates the production of a hormone in the mother which becomes incorporated in the unlaid egg to act at a later stage. Gilbert and Schneiderman (1961, cited by Wigglesworth, 1964) have detected the presence of juvenile hormone derived from the mother in the eggs of *Hyalophora cecropia*. The most interesting results—already reviewed—in respect of maternal inheritance in the aphid, were obtained by White (1965). In view of this work, it may be possible to explain the role of hormone(s) in maternal inheritance, as follows. The small and inactive corpora allata of micropterous females treated with the 14 hr photoperiod produce juvenile hormone which may be incorporated in the eggs to act at a later stage. However, the large and active corpora allata of micropterous females exposed to the 18 hr photoperiod produce comparatively more hormone which may also be incorporated in the
eggs to act at a later stage. However, more rigorous investigations are necessary to understand and to explain the phenomenon of maternal inheritance in determining wing polymorphism in G. sigillatus.
VI. SUMMARY

1. The initial rearing of larvae at 28°C followed by 35°C reveals that wing development is determined in early larval life, depending upon the temperature to which they are exposed. Once the condition for microptery is determined, it becomes irreversible despite an exposure to an optimal temperature of 35°C.

2. A larval photoperiod of 14 hr is optimal for wing development.

3. Larval photoperiod of 10 hr suppresses the development of wings completely.

4. The 10 hr and 12 hr short photoperiods inhibit wing development more strongly than the 16 hr and 18 hr long photoperiods.

5. Female insects always outnumbered males irrespective of the photoperiod.

6. Macropterous males and macropterous females occur more or less in equal numbers at 10 hr - 18 hr photoperiods.

7. The favourable conditions required to induce wing development in the laboratory are presumably non-existent in nature.

8. Survival is better at long photoperiods than at short ones.

9. The average duration of the larval stage is greater at 10 hr and 12 hr photoperiods than at 14 hr, 16 hr and 18 hr photoperiods.

10. Maternal inheritance due to the influence of photoperiod plays a very important role in wing polymorphism.
11. The 14 hr photoperiod appeared to be the most favourable for mating, production of spermatophores and for oviposition. On the other hand the 10 hr photoperiod appears to inhibit these processes.

12. The ocelli respond to photoperiodic stimuli and are involved in the photoperiodic mechanism that controls wing polymorphism.

13. The ocellar nerves appear to have nervous connections with the median neurosecretory cells of the brain.

14. The neuroendocrine system of G. sigillatus conforms to the basic plan common to the pterygote insects including Orthoptera.

15. The corpora allata are attached to the gut in the ultimate stage of the insect.

16. Neurosecretory cells are also present in the suboesophageal and frontal ganglia.

17. In the ultimate stage macropterous forms, the median neurosecretory cells display a comparatively smaller amount of PF-positive material but the material is released into their axons, where it is distinctly visible. In contrast to this, in the ultimate stage micropterous forms, the cells are densely packed with the material but their axons are devoid of the material. It is concluded that the median neurosecretory cells in the ultimate macropterous forms are active and release the brain hormone(s), whereas those in the micropterous forms are inactive and do not release the brain hormone(s).
18. In *G. sigillatus*, macropteryism is due to the level of the brain hormone(s).

19. The corpora allata in both micropterous and macropterus ultimate forms are of the same size.

20. The corpora allata of 4-day-old micropterus adult females exposed to 18 hr photoperiod for 4 days are larger and presumably more active, than those of their counterparts exposed to the 14 hr photoperiod. The juvenile hormone appears to play an important role in maternal inheritance.
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PLATE I (A, B).
PLATE I (A, B).

Cross section through the head of a cricket in the ultimate stage, showing a direct connection of the dorsal ocellar nerve with the pars intercerebralis.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X130.
PLATE II.

Frontal section through the head of a cricket in the ultimate stage, showing a direct connection of the median ocellar nerve with the pars intercerebralis.

Fixed in modified Bouin's, stained with FF, counterstained with Halmi's mixture, and sectioned at 10 microns. X130.
PLATE III.

Cross section through the whole head of a cricket in the ultimate stage, showing compound eyes, optic nerve on one side, brain and frontal ganglion.

Fixed in Heidenhain's Susa, stained with Ehrlich's Haematoxylin and eosin, and sectioned at 7 microns. X35.
PLATE IV.
Cross section of the median neurosecretory cells of the pars intercerebralis and NCC I of the macropterous form in the ultimate stage. Note the small amount of PF-positive material in the median neurosecretory cells and its release in large amount into the axons of NCC I which cross each other.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X360.
PLATE V.

Cross section of the median neurosecretory cells of the pars intercerebralis and NCC I of the micropterous form in the ultimate stage. Note the abundant PF-positive material in the median neurosecretory cells and its absence in the axons of NCC I which cross each other.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X360.
PLATE VI.
PLATE VI.

Cross section showing hypocerebral ganglion, corpora cardiaca with PF-positive material, and roof and lumen of aorta, of macroperous female in ultimate stage. A few pale intrinsic cells may be seen along the border of the corpora cardiaca.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X210.
PLATE VII.

Cross section showing hypocerebral ganglion, corpora cardiaca and lumen and roof of aorta, of macropterous male in ultimate stage.

Fixed in Heidenhain's Susa, stained with Ehrlich's Haematoxylin and eosin, and sectioned at 7 microns. X360.
PLATE VIII.

Cross section showing two stout nerves connecting the hypocerebral ganglion to the corpora cardiaca in 1-day-old micropterus adult female exposed to a 14 hr photoperiod for 1 day. Note the abundance of PF-positive material in corpora cardiaca.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture and sectioned at 10 microns. X360.
PLATE IX.
PLATE IX.

Cross section showing corpora cardiaca, NCA I, corpus allatum, NCA II and part of gut, of 1-day-old micropterous adult female exposed to a 12 hr photoperiod for 1 day. Note the PF-positive material in the corpora cardiaca, corpus allatum and NCA I and II. Also note the connection of NCA I with the median lobe of corpus cardiacum.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X230.
PLATE X.

Legend as in Plate IX.
Only part of corpus allatum is seen. X360.
PLATE XI.

Cross section showing the presence of PF-positive material in corpus allatum and NCA I and II of 1-day-old micropterous adult female exposed to 14 hr photoperiod for 1 day.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X575.
PLATE XII.
PLATE XII.

Sagittal section of corpus allatum and gut of macropterus male in ultimate stage. Note the attachment of the gland to the gut.

Fixed in Heidenhain's Susa, stained with Ehrlich's Haematoxylin and eosin, and sectioned at 7 microns. X360.
PLATE XIII.

Cross section showing abundant PF-positive material in the neurosecretory cells of frontal ganglion of macropterous female in ultimate stage.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X210.
PLATE XIV.

Frontal section of suboesophageal ganglion of micropterous female in ultimate stage. Note the neurosecretory cells filled with PF-positive material.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X210.
PLATE XV.

Cross section showing corpus allatum and part of gut, of macropterous female in ultimate stage.

Fixed in Heidenhain's Susa, stained with Ehrlich's Haematoxylin and eosin, and sectioned at 7 microns. X210.
PLATE XVI.

Cross section showing corpus allatum and part of gut, of micropterous female in ultimate stage.

Fixed in Heidenhain's Susa, stained with Ehrlich's Haematoxylin and eosin, and sectioned at 7 microns. X210.
PLATE XVII.

Cross section showing median neurosecretory cells of the pars intercerebralis of 1-day-old micropterous adult female exposed to 14 hr photoperiod for 1 day. The PP-positive material is abundant only in some cells and very little material is seen in the axons.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X360.
PLATE XVIII.

Legend as in Plate XVII, excepting that the insect was exposed to 18 hr photoperiod. X360.
PLATE XIX.

Cross section showing hypocerebral ganglion, lumen and roof of aorta, corpora cardiaca and part of the gut of 2-day-old micropterous adult female exposed to 14 hr photoperiod for 2 days. Note abundant accumulation of FF-positive material in the corpora cardiaca.

Fixed in modified Bouin's, stained with FF, counterstained with Halmi's mixture, and sectioned at 10 microns. X230.
Cross section showing hypocerebral ganglion, corpora cardiaeca, and part of gut of 16-day-old micropterous adult female exposed to 14 hr photoperiod for 16 days. Note the PF-positive material in the corpora cardiaeca.

Fixed in modified Bouin's, stained with PF, counterstained with Haliim's mixture, and sectioned at 10 microns. X230.
Cross section of corpus allatum of 1-day-old micropterous adult female exposed to 14 hr photoperiod for 1 day. Note the PF-positive material in the gland and also in NCA II.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X360.
PLATE XXII.
PLATE XXII.

Cross section of corpus allatum of 4-day-old micropterus adult female exposed to 14 hr photoperiod for 4 days.

Fixed in modified Bouin's, stained with PF, counterstained with Halmi's mixture, and sectioned at 10 microns. X230.
PLATE XXIII.
PLATE XXIII.

Legend as in Plate XXII, excepting that the insect was exposed to 18 hr photoperiod, X230.