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Title: SEASONAL AND ALTITUDINAL REGULATION OF EMBRYONIC AND NYMPHAL DEVELOPMENT IN ALLONEMOBIUS FASCIATUS (ORTHOPTERA: GRYLLIDAE)

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Abstract approved: Victor J. Brookes

The physiological adaptations involved in the seasonal and altitudinal regulation of the life cycle in Allonemobius fasciatus were studied.

This species maintains a univoltine life cycle with an embryonic diapause over an altitudinal gradient of as large as 1,000 m. A more than 1.5-month difference exists in hatching time between the highest and lowest altitudes studied. Little or no difference was detected among populations from different altitudes when various developmental traits such as diapause intensity, postdiapause development, and nymphal development, were compared in the laboratory. The photoperiodic regulation of nymphal development seems to play an important role in compensating for the shorter growing season at higher elevations.

The embryo of A. fasciatus showed seasonal variation in diapause characteristics. Diapause intensity measured as the duration of the egg stage at 20°C was greater in eggs laid early in the season than in those laid later. At 20°C, all embryos entered "winter diapause" at the end of the appendage-formation stage. However, when incubated at higher temperatures, another type of diapause was manifested before that stage. The higher the temperature the earlier was the stage at which this developmental suppression (called "summer diapause") was imposed. The induction of summer diapause depended not only upon the temperature of incubation but also upon the time of ovi-

position. The capability of entering summer diapause at a high temperature was decreased as the time of oviposition was delayed. The effects of temperature and moisture on embryonic development were examined using eggs with or without summer diapause and it is suggested that the role of summer diapause is not to ensure the survival of eggs under dry, warm conditions in summer but to avoid untimely hatching before winter, and to stabilize the univoltine life cycle in this species.

Non-diapause development was induced when eggs were transferred from 20 to 27°C in early embryonic stages, although they entered diapause when constantly held at either of these temperatures. Based on this and other results, a model for the possible mechanism underlying the control of induction and intensity of diapause was proposed.

SEASONAL AND ALTITUDINAL REGULATION OF EMBRYONIC AND NYMPHAL DEVELOPMENT IN *ALLONEMOBIUS FASCIATUS* (ORTHOPTERA: GRYLLIDAE)

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SEASONAL AND ALTITUDINAL REGULATION OF EMBRYONIC AND NYMPHAL DEVELOPMENT IN ALLONEMOBIUS FASCIATUS.

I. INTRODUCTION

The striped ground cricket, Allonemobius fasciatus DeGeer is distributed over a wide range of North America (55^{o}-25^{o}N), although the information is scanty from the western states (Fulton 1931,1951; Vickery,personal communication). This species is known either as a pest of fruits, seeds, and vegetables (Phipps 1930; Neunzig and Gyrisco 1955; Evans et al. 1965; Hoveland et al. 1966; Nielsson and Bass 1967) or as a beneficial insect preying on aphids and mites and making a major contribution to the reduction of apple maggot pupae (Monteith 1971). It is univoltine in the north and multivoltine in the south (Fulton 1951; Nielsson and Bass 1967; Van Hook 1971; Vickery and Johnstone 1973). In Oregon, this cricket produces one generation each year and overwinters as an egg. Among several crickets belonging to the same genus found in Benton county, Oregon (44.3^{o}N), it is the only species that also occurs at a high elevation of about 1,100 m. At a low altitude (76 m), adults of this species can be heard chirping in midsummer when the temperature is the highest and precipitation the lowest in the year. In midsummer, most grasses and forbs were wilted and the surface of the soil becomes firmly caked. Under such conditions, if eggs are laid in the soil, they would be exposed to high temperatures under dry conditions. The main purposes of the present study were to elucidate the physiological mechanisms which enable A. fasciatus to maintain its univoltine life cycle over an altitudinal gradient of as large as 1,000 m and to prevent the egg from hatching under warm conditions encountered in summer at low altitudes.

A. fasciatus shows wing dimorphism as commonly found in other species of the same genus (Alexander 1968; Masaki 1972,1978; Harrison 1980). In those crickets, the macropterous form has long tegmina, well developed hind wings, and flight muscles as compared with the micropterous form. The former can fly and probably migrate, but the

micropterous form with the atrophied wings is unable to do so (Masaki and Oyama 1963; Alexander 1968; Tanaka 1976,1978a; Tanaka et al. 1976). A preliminary sampling of A. fasciatus conducted in 1979 showed that most adults collected at an elevation of 1,100 m were macropterous while less than 5 % were macropterous at 76 m of altitude. This, together with the fact that the density of this species at higher altitudes appears to be quite low compared with that at lower altitudes, might indicate that macropterous adults migrate to higher elevations each summer. Although migration of adults can not be excluded completely, some development must occur at the higher elevations because nymphs as well as adults were caught in the autumn of 1980. The univoltine life cycle of this cricket at both high and low elevations requires that its growth must be adjusted to the length of the growing season. While many workers have been interested in latitudinal adaptation of insects (Masaki 1961, Danilevskii 1965; Saunders 1976; Beck 1980), much less effort has been made to study adaptations to altitudinal gradients.

Among various phenological events, the timing of oviposition would be of pivotal importance for the survival of crickets which overwinter as eggs (Masaki 1972). If adults appear and lay eggs too early, the risk of untimely hatch of their eggs would be large because the diapause may be terminated before winter. If they appear too late, their breeding would be prevented by cold weather or their eggs would be killed before reaching the diapause stage (Masaki 1967). There appear to be several possible ways in which a population of A. fasciatus at a high elevation can compensate for the smaller amount of heat available for development in order to maintain the univoltine life cycle. In the egg or nymphal stage, populations at high elevations may have a lower temperature threshold for development and/or a smaller heat requirement (day degree) for hatching or completion of nymphal development than do those at low elevations. Such adaptations would depend upon genetic modification. If no genetic difference exists among populations from different altitudes, it is likely that the duration of nymphal development is environmentally controlled so

that adult emergence is ensured to occur in autumn. Photoperiodic regulation of nymphal development is widespread among crickets (Alexander 1968; Masaki 1972,1978; Ismail and Fuzeau-Braesch 1976; Harrison 1978; Tanaka 1983). In the spring ground cricket, Pteronemobius nitidus, photoperiod has been demonstrated to influence the duration of nymphal development by changing not only the duration of each instar but also the number of instars (Tanaka 1978b,1979). Comparison of several physiological traits among populations from different altitudes has been made in the laboratory to test the above possibilities and the results will be discussed in Chapter II.

In grasshoppers, several adaptations to a dry and warm environment have been reported. Oedipoda miniata, for example, passes a summer in reproductive diapause so that oviposition does not take place until the summer is over (Pener and Broza 1971; Broza and Pener 1972; Orshan and Pener 1979). This ensures that eggs are not exposed to environmental adversity in summer. The embryo of Chortoicetes terminifera can survive warm, dry conditions in a state of quiescence (Wardhaugh 1980). Diapause at early embryonic stages provides a means for Austroicetes cruciata to overcome summer adversity (Andrewartha 1943,1952; Birch and Andrewartha 1942,1944).

Preliminary observations showed that adult females of A. fasciatus collected in summer in Corvallis started laying eggs within a few days in the laboratory (at room temperature). This may rule out the possibility of reproductive diapause in this species. Therefore, in this study, experiments were designed and carried out mainly to examine the effect of temperature and moisture on the development of embryos. Although none of the above phenomena observed in grasshoppers have been reported in crickets, some crickets apparently start laying eggs when the temperature is still high and two different physiological mechanisms may have evolved under such conditions. The female of Gryllus pennsylvanicus lays eggs with different intensities of diapause in different seasons (Rakshpal 1962): the diapause in eggs laid in summer is more intense than in those laid in autumn. On the other hand, the embryonic diapause of Teleogryllus emma is intensified if the egg is exposed to a high temperature during the early embryonic life (Masaki

1962). Eggs of these species laid in summer are able to avoid untimely hatching before winter because of the high intensity of diapause. In Chapters III and IV, the effects of temperature and environmental moisture on the embryonic development will be examined and the possible involvement of summer diapause and its ecological significance will be discussed.

The hormonal control of embryonic diapause has been extensively studied in Bombyx mori. In this species, eggs are induced to diapause by a neurohormone, the diapause hormone, secreted from the suboesophageal ganglion during pupal-adult development of the female parent (Fukuda 1951; Hasegawa 1951,1957). A similar mechanism for the determination of diapause has been suggested in Origia antiqua (Kind 1965), although the precise mechanisms remain unknown. The diapause hormone, which consists of two principles characterized as peptides (Isobe et al. 1973), is known to stimulate the accumulation of lipids (Ichimasa 1976) and glycogen (Yamashita and Hasegawa 1970: Yamashita et al. 1972) in oocytes. However, little is known about the physiological mechanism controlling the actual onset and intensity of embryonic diapause, although various physiological changes occurring before, during, and after diapause have been observed in B. mori (Chino 1957a,b,1958; Kai and Nishi 1976; Yamashita et al. 1981), A. cruciata (Andrewartha 1943) and Teleogryllus commodus (McFarlane and Hogan 1966). The diapause hormone in B. mori has been found both in adult ovaries and laid eggs and the possible involvement of this hormone in the control of onset and maintenance of diapause has been suggested (Kai and Kawai 1981). However, the definite action of this hormone in the control of these phenemena remains unknown.

Lees (1955) has suggested the possibility that the immediate causes of embryonic diapause are not necessarily humoral in nature. In Melanoplus differentialis, Bucklin (1953) found that when embryos were explanted to hanging drops of Ringer's solution, diapause could be averted or terminated. That is, the diapausing embryo seems to be capable of responding at any time if the conditions favorable for growth, notably the free access to water, are restored (Lees 1955).

Okada (1971) obtained a similar result in B. mori and suggested that diapause would be due to the deficiency of oxygen created by the decreased permeability of the chorion to air. Ando (1978) reached a similar conclusion with Atrachya menetriesi. Whatever the immediate cause(s) may be, some mechanism regulating the onset and intensity of diapause must exist.

Based on her studies of the effect of organic solvents on diapause, Silfer (1946) concluded that the embryonic diapause in M. differentialis is caused by the water deficiency due to the presence of a wax layer in the hydropyle. According to her hypothesis, the onset of diapause is occasioned by the secretion of a substance with wax-like properties at the hydropyle which prevents water from entering the egg. Diapause can be prevented or terminated by exposure of the eggs to low temperature because the wax is altered physically in some way so that it is no longer able to prevent the entry of water. As pointed out by Lees (1955), the regularities of termination of diapause suggest that some less casual process must be involved. Furthermore, this hypothesis can not be applied in those grasshoppers which absorb water before or during the period of diapause (Andrewartha 1952; Lees 1955). Preliminary observations suggest that the egg of A. fasciatus also absorbs water before entering diapause.

Another approach to this problem has been made by several workers, e.g. Watanabe 1924; Bodine 1932, Muroga 1951; Ando 1978 etc., who assumed growth inhibitory substances or diapause factors to explain the diapause phenomenon in the eggs of different species. According to Muroga (1951), for example, diapause in the egg of B. mori results from the presence of an inhibitory substance transmitted from adult females and ends when this substance has been consumed in the egg. Based on this assumption, he calculated the consumption coefficient of the inhibitory substance at different temperatures. However, this hypothesis seems to encounter difficulty if the intensification of diapause noticed by Lees (1955) occurs in the eggs incubated at a high temperature. Ando (1978) has explained the onset of diapause and the reversible changes in diapause intensity in the egg of A. menetriesi by assuming a dia-

pause factor produced from another substance, the precursor, transmitted from the adult females. At present, evidence for the presence of these substances is lacking in any species. However, this may reflect the difficulty of the subject rather than the absence of such mechanisms.

During the course of this study, I found that eggs of A. fasciatus did not enter diapause when transferred from 20 to 27°C during the pre-diapause stage, although they did so when constantly kept at either of these temperatures. The proportion of eggs which apparently hatched without diapause depended not only upon the time of shift in temperature but also upon the level of the initial incubation temperature. In most species studied, diapause can be prevented only after a treatment of eggs during the pre-diapause stage with hydrochloric acid (Watanabe 1935), organic solvents (Slifer 1946) or a low temperature below 10°C (Slifer 1932; Muroga 1951; Dean and Hartley 1977; Ando 1978). In A. fasciatus, however, such a drastic treatment is not required for the prevention of diapause. Furthermore, the intensity of diapause was also found to be influenced by the temperature at which eggs were incubated. These observations suggest that some physiological processes responsible for the determination of diapause characteristics proceed in the laid egg and they are considerably sensitive to temperature. Therefore, this species might provide excellent material with which the physiological mechanisms controlling the onset and intensity of diapause are studied. In Chapter V, based on the responses of eggs to various temperature regimes, possible mechanisms underlying the control of onset and intensity of diapause will be discussed and a model to account for the observed phenomena including the seasonal variation in diapause intensity will be presented.

II. ALTITUDINAL ADAPTATION

Introduction

Allonemobius fasciatus occurs at a high elevation (1,100 m) as well as at lower levels (76 m) in Benton county (44.3°N), Oregon. A 1,000 m difference in altitude is approximately comparable to a 6°-difference in latitude in terms of the effect on the body size of a field cricket in Japan (Masaki 1967). A 6°-difference in latitude is apparently large enough to result in some genetic difference for climatic adaptation between two populations in some species. Such a difference is often related to the difference in the critical photoperiod for diapause induction, diapause intensity, or developmental rate (Lees 1955; Masaki 1961; Danilevskii 1965; Saunders 1976; Beck 1980). While many workers have been interested in latitudinal adaptation of insects, much less effort has been made to study adaptations to altitudinal gradients.

The physiological mechanisms that enable A. fasciatus to inhabit an altitudinal gradient is dealt with in this chapter. A large difference was expected to exist in heat available for development of this species between the lowest and highest altitudes of its habitats. Because the cricket is univoltine throughout this altitudinal gradient, there is the possibility that populations at higher altitudes have some physiological mechanisms by which they can compensate for the smaller amounts of available heat. Developmental traits such as diapause intensity, postdiapause development, and nymphal development which might be responsible for such mechanisms were compared among populations from different altitudes.

Materials and Methods

Specimens collected at 1,100 and 76 m of altitudes on Marys Peak were sent to Dr. V. R. Vickery and identified as A. fasciatus. This species overwinters as an egg in a state of diapause both in Corvallis and near the top of Marys Peak, Oregon (Tanaka, unpublished

observations).

Because no meteorological data are available from higher altitudes of Marys Peak, the difference in available heat for development of A. fasciatus between different altitudes is not known. Eggs obtained in the laboratory were placed in autumn at two locations, i.e. Corvallis (76 m) and near the top of Marys Peak (1,100 m), and returned to the laboratory in the following spring to estimate the time of hatching in the field. All eggs used in this experiment were laid by females collected at 76 m of latitude. The eggs were kept on moist paper in Petri dishes for 1.5 months before they were transferred to the field on November 3, 1980. Three bags made of nylon gauze (2 x 3 cm) each containing about 50 eggs were placed at about 1 cm depth in soil at each of the locations. The sites where eggs were deposited were habitats of this species which were open and exposed to the sun during the day. The ground was covered with short grass. The nylon bags were taken back to the laboratory on different dates in the following spring and the time required for the eggs to hatch was determined at 20 $\pm$ 1°C (mean $\pm$ range).

The duration of so-called postdiapause development was compared among eggs from different altitudes. To obtain eggs for the experiment, more than 10 adult females were collected at 4 different sites which were approximately 1,100, 600, 100, and 76 m in altitude and will be called sites A, B, C, and D, respectively. The adults from each site were allowed to lay eggs into wet glass wool in a 2-liter glass jar at room temperature. Eggs laid within 24 hours were put on moist paper in Petri dishes (9 cm in diameter) and kept at 20°C for 30 days which ensured that they reached the diapause stage. They were then chilled at 3°C for 8 months and transferred to 20 $\pm$ 1°C to measure the time required for hatching.

Photoperiodic effects on nymphal development of this species were examined at 24 $\pm$ 1°C and compared between two populations from sites A and D. Nymphs were obtained at 27°C from eggs which had been laid by females collected from each site and chilled at 3°C for 3 months. Newly hatched nymphs were reared in a 2-liter glass jar with clean sand covering the bottom to a depth of about 1 cm. They were fed on hamster

pellets and slices of carrot with a continuous supply of water in a vial plugged with absorbent cotton. The photoperiods used were LD 12: 12, 14:10, 15:9, and 16:8 hr. There was no detectable thermoperiod created by the light source (a 7 W incandescent lamp) in each incubator. Because of the limited space available for the experiment, only one jar containing 70 newly hatched nymphs for each altitude was exposed to each photoperiod. All individuals which emerged as adults were removed from their jars every day and the time spent during nymphal development was recorded.

Three other jars each containing 50 nymphs from site A were kept in LD 12:12, 14:10, and 16:8 hr, respectively, at 24 $\pm$ 1°C to determine the number of instars by marking nymphs with white paint according to the method of Tanaka (1979).

Diapause intensity was compared among eggs from different altitudes by measuring the duration of the egg stage at 20 $\pm$ 1°C. To obtain eggs for the experiment, more than 10 adult females were collected at sites, A, B, C, and D on September 5, 1980, and kept by a window at room temperature under uncontrolled light conditions. From adults collected at each site, seven groups consisting of varying numbers of eggs laid during the period from September 9 to 27 were placed on moist paper in Petri dishes within 24 hours after being laid and incubated at 20 $\pm$ 1°C in darkness. Hatches were recorded daily for 35 weeks. When the hatching record was discontinued, a few eggs still remained unhatched in most dishes and they were not considered in the results.

Results

Available heat for development: As expected, embryonic development after overwintering was much more advanced at a low altitude than at a high altitude. The first transfer of eggs to the laboratory from sites A and D was made on May 25, 1981 (Table 1). The eggs from site D took 18.9 days on the average to hatch at 20°C and those from site A, 32.4 days. Eggs transferred from site D 5 days later hatched more quickly. Some eggs started hatching 4 days after being incubated at

20°C and the mean time taken by all eggs to hatch was 9.1 days, being shortened by about 10 days. This means that the prevailing temperature around the eggs had been higher than 20°C and suggests that hatching would have occurred in early June at site D. In fact, all nymphs had hatched before the last transfer was made on June 15. Two groups of eggs were transferred from site A on June 29 and July 20. Even the eggs of the second group were not yet ready to hatch at the time of transfer. They took about 16 days on the average ranging from 13 to 19 days. This suggests that hatching would probably have taken place in late July and early August at this elevation.

These results show a substantial difference in the available heat for the embryonic development in spring between 1,100 and 76 m of altitude.

Postdiapause Development: The above results may not necessarily indicate exactly when hatching occurs in the population at site A because all eggs used in the experiment were derived from site D. In other words, there is the possibility that, by having a smaller heat requirement for hatching, the populations of higher altitudes may compensate for the smaller amount of available heat, making it possible for them to hatch earlier than expected from the above results. In order to test this possibility, the heat requirement for hatching was compared among different populations. After an 8-month exposure to 3 °C, eggs from different altitudes were incubated at 20°C to measure the time required for hatching at 20°C. Although there is no evidence to indicate that diapause terminated during the chilling period, the results seem to suggest no significant difference in the heat requirement for the postdiapause development among these populations (Table 2). This, together with the results in Table 1, means that hatching would occur in the population at site A in midsummer about 1.5 to 2 months later than the hatching time at site D.

Nymphal Development: Differences in hatching time among populations at different altitudes will expose them to different photoperiods during the nymphal stage in the field. In order to compare the photoperiodic effects on the duration of the nymphal stage between popu-

lations from different altitudes, an experiment was carried out with nymphs derived from the populations at sites A and D. Figure 1 shows the similar effects of photoperiod on the duration of the nymphal stage in the two populations. Nymphal development was the most rapid at LD 14:10 hr and tended to be prolonged at either shorter or a longer photoperiod. There was no significant difference in the duration of the nymphal stage between the two populations at all photoperiods except at LD 15:9 hr. Although the difference at LD 15:9 hr was statistically significant in the male ($t = 2.76$; $P<0.05$), a more precise determination of the nymphal development with a larger sample size would be required to draw any meaningful conclusion.

Table 3 shows the effects of photoperiod on the number of instars at 24°C. The shorter the photoperiod the smaller was the number of instars.

Diapause Intensity: In some insects, the duration of the egg stage or diapause intensity varies with latitude and altitude (Masaki 1967; Bradshaw and Lounibos 1977).

To test the possibility of this in A. fasciatus, seven groups containing varying numbers of eggs (104-654) laid by females from different altitudes were incubated at 20°C. The percent hatch from these groups was determined on a weekly basis and plotted against the time from deposition in Fig. 2. All 4 populations showed essentially the same pattern of hatching with the 50 % hatching time occurring around the 17th week. The mean time for 50 % hatching in the seven groups of eggs from each population was calculated (the data are not presented here) and tested by an analysis of variance, which showed no significant difference in the mean between the 4 populations ($F = 0.73$; $P>0.05$).

Discussion

Little is known about the biology of crickets in the western states of North America (Alexander 1968). In eastern states, A. fasciatus is univoltine in the northern areas and multivoltine in the

southern areas (Fulton 1931,1951). In Oregon (44.3°N), this species produces one generation a year and maintains a univoltine life cycle over the altitudinal gradient studied (Tanaka, unpublished observations).

The present results suggest that a 1,000-m difference in altitude brings about a more than 1.5 month difference in hatching time between two populations of this species. After overwintering, hatching took place about the beginning of June in Corvallis (76 m)(Table 1). No significant difference in heat requirement for hatching was apparent among eggs overwintering at different altitudes (Table 2). At 1,100 m of altitude, the eggs were not yet ready to hatch in mid-July. The seasonal life cycles of A. fasciatus at the two altitudes are summarized in Fig. 3. The nymphs of this species responded to photoperiod in such a way that nymphal development was rapid at LD 14:10 hr and more prolonged at either a shorter or a longer photoperiod (Fig. 1). Because nymphs of this species do not encounter a photoperiod as short as LD 12:12 hr at 44.3°N, their photoperiodic response would be similar in effect to that of a short-day type in the field. This seems important for this cricket in compensating for the delayed hatching time at higher altitudes at least to some extent. At site A, for example, nymphs hatch in midsummer when the photoperiod decreases rapidly as shown in Fig. 3. Their development would, therefore, be accelerated under the influence of intermediate photoperiods and the temperature which is highest at this time of the year. At site D, on the other hand, nymphs hatch from eggs in early June (Table 1). The mean temperature on the ground at this time of the year must be higher than 20°C because, as mentioned before (p. 10), eggs which remained in the field developed faster than those transferred to 20°C in late May. The increasing temperature towards the midsummer would be favorable for rapid growth. However, they are exposed to photoperiods longer than 15 hours for the first two months. Because such photoperiods tend to increase the duration of the nymphal stage with greater numbers of instars (Fig. 1 and Table 3), precocious emergence of adults would be avoided. In fact, this species was not heard to chirp at site D

until the end of July, 1981 (Tanaka, unpublished data). Maturation of those nymphs which are still young in early August would be accelerated because of the short-day type of response which is known to serve as a mechanism synchronizing adult emergence within a population in other univoltine crickets overwintering as eggs (Masaki 1972, 1978). As demonstrated in species such as Gryllus campestris (Ismail and Fuzeau-Braesch 1976) and Pteronemobius nitidus (Tanaka 1978,1979, 1983), which overwinter as nymphs, photoperiodic control of nymphal development involves variation in the number of instars. This is also true in A. fasciatus which tended to have additional instars (Table 3) and increased average body size (Tanaka, unpublished observations). In general, rapid maturation is associated with small body size when the development involves no diapause (Masaki 1967,1972). It is interesting that this does not hold in A. fasciatus when reared at LD 12:12 hr, a photoperiod not experienced by the nymphs in the field (Fig. 3). At this photoperiod, the duration of nymphal development is long (Fig. 1) and the number of instars small (Table 3), resulting in small adult body size.

The difference in the duration of the nymphal stage at LD 15:9 hr between populations from 1,100 and 76 m of altitude is statistically significant (Fig. 1). Although based on a rather small sample size, this difference is of particular interest because, at site A, nymphs started hatching in midsummer when the photoperiod was about 15 hours (Table 1 and Fig. 3). Selection for rapid development at a 15-hour photoperiod may occur in the high altitude population.

Diapause intensity is another physiological trait which is known to vary with latitude or altitude in various insects (Masaki 1961,1967; Danilevskii 1965; Bradshaw and Lounibos 1977). In some species, it tends to be deeper in the populations inhabiting the northern or cooler parts of the range than in those occupying the southern or warmer parts. The reverse is true in others. Such a variation has been proposed to be a factor in maintaining diapause before winter (Masaki 1961) or in controlling the timing of resumption of morphogenesis after overwintering (Bradshaw and Lounibos 1977). Among populations from

different altitudes of A. fasciatus, however, no significant difference in diapause intensity for overwintering was found (Fig. 2).

The present study failed to provide any conclusive evidence for a genetic difference in traits studied among populations from different altitudes. This, together with the fact that this species occurs in open, grassy areas at low density at higher altitudes, might suggest that populations may have become established at the higher altitudes relatively recently. The phenotypic plasticity as found in the photoperiodic regulation of nymphal development seems to enable this species to enter new habitats of as high as 1,100 m in altitude without genetic modification in these traits.

Figure 1. The effect of photoperiods on the duration of the nymphal stage of A. fasciatus from sites A (1,100 m) and D (76 m) at 24°C. Vertical lines indicate the standard deviation. An asterisk indicates a siginificant difference between the two populations (P 0.05).

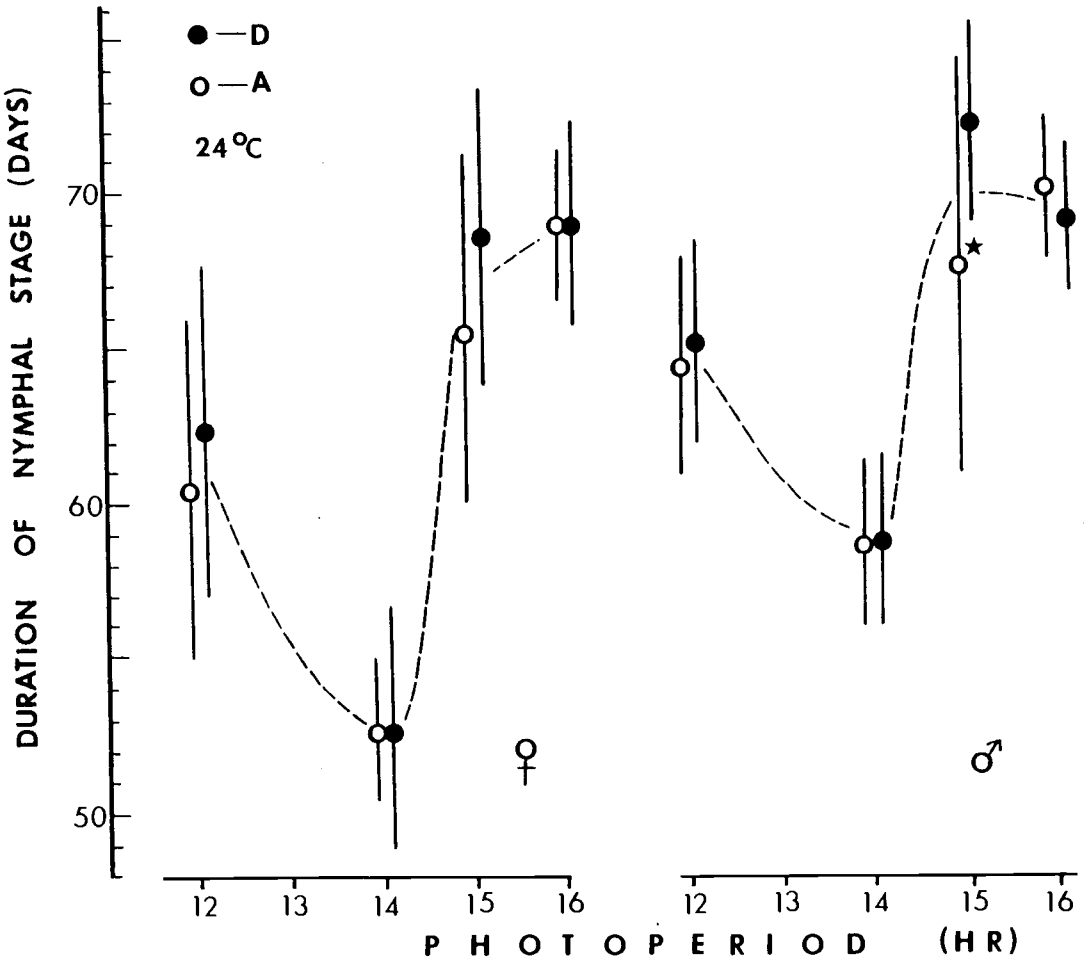
●—D
○—A
24°C
DURATION OF NYMPHAL STAGE (DAYS)
70
60
50
12
13
14
15
16
♀
♂
PHOTOPERIOD (HR)

Figure 1

Figure 2. A comparison of the duration of the egg stage among populations of A. fasciatus from different altitudes at 20°C.

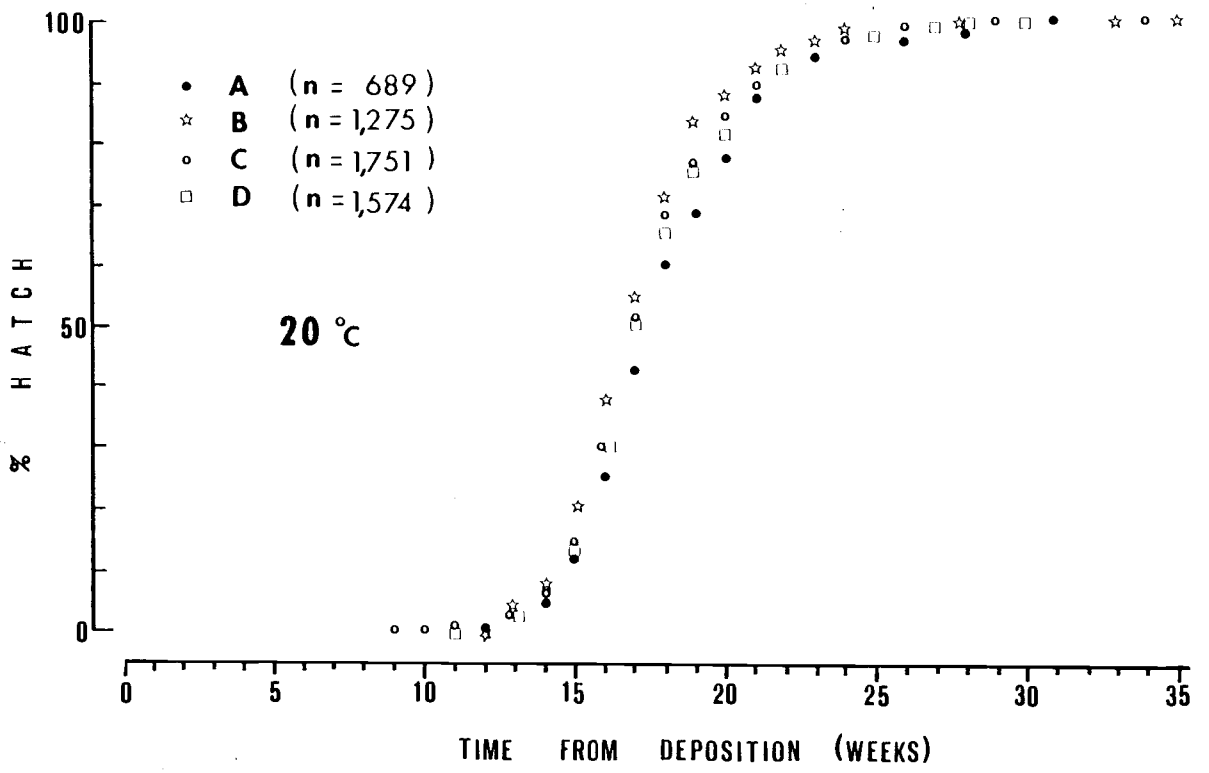
100
50
0
% HATCH
A (n = 689)
B (n = 1,275)
C (n = 1,751)
D (n = 1,574)
20 °C
0
5
10
15
20
25
30
35
TIME FROM DEPOSITION (WEEKS)

Figure 2

Figure 3. The seasonal life cycles of A. fasciatus at sites A (1,100 m) and D (76 m). The time of adult emergence is inferred based on unpublished data. The seasonal fluctuations in photoperiod (based on Beck 1980) and temperature at site D (Climatological data from OSU) are also given.

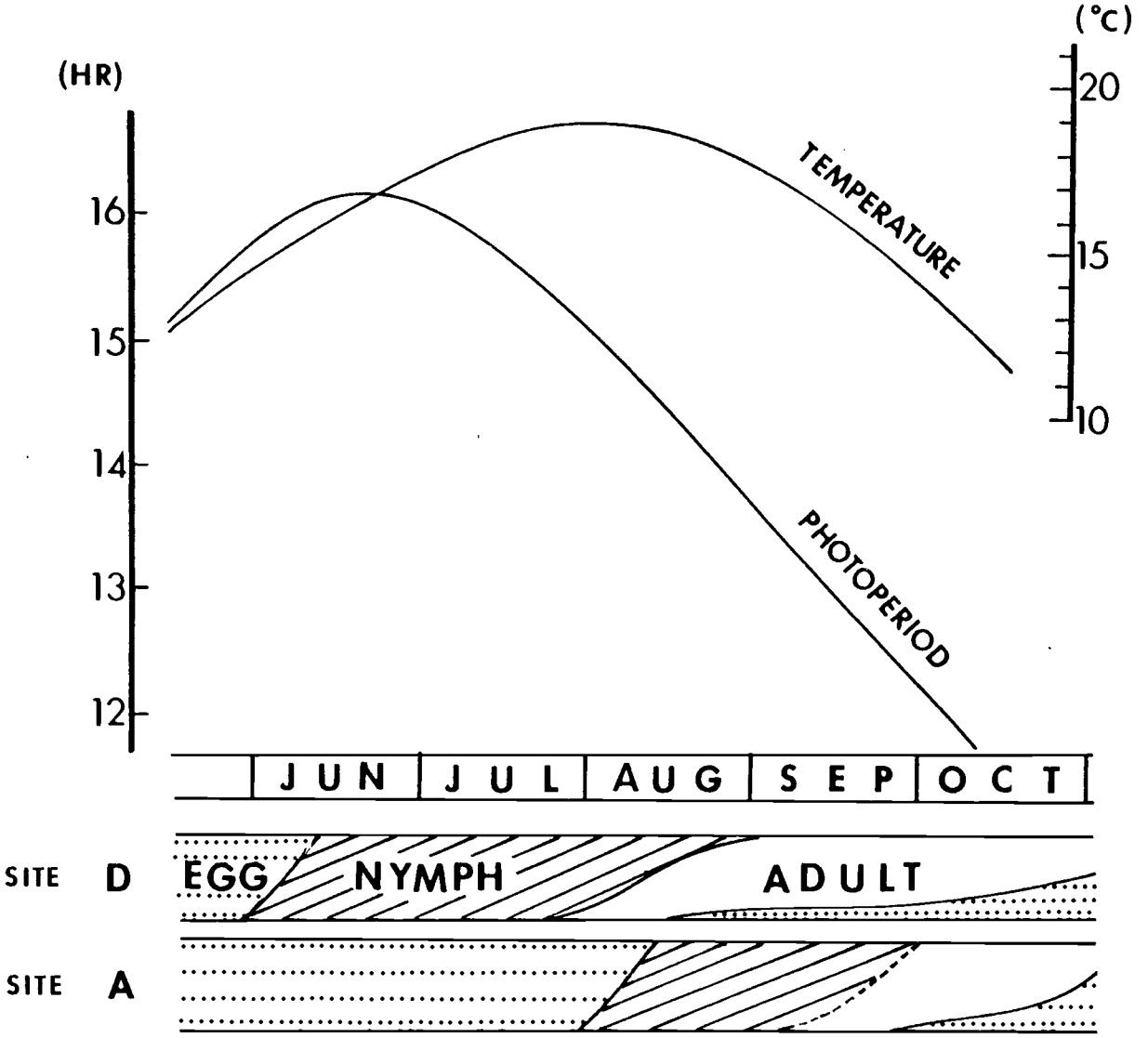
(°C)
(HR)
20
16
15
15
14
10
13
12
TEMPERATURE
PHOTOPERIOD
JUN
JUL
AUG
SEP
OCT
SITE D
EGG
NYMPH
ADULT
SITE A

Figure 3

Table 1. Time required for hatching of A. fasciatus eggs transferred from the field to the laboratory (20°C). The eggs used were laid by female adults collected at 76 m.

Date of transfer	n	Time required in days for hatching mean ± S.D.	range
from site D (76m)			
May 25	43	18.9 ± 1.7	16 - 23
May 30	42	9.1 ± 2.6	4 - 14
June 15[a]	45	———	———
from site A (1,100m)			
May 25	46	32.4 ± 3.5	28 - 42
June 29	43	26.4 ± 1.3	24 - 31
July 15	43	15.5 ± 1.2	13 - 19

[a]All eggs hatched before transfer.

Table 2. A comparison of *A. fasciatus* eggs from different altitudes in the time required for hatching at 20°C after an 8-month period of chilling at 3°C.

Original site	n	Time required in days for hatching mean ± S.D.	range
A (1,100 m)	30	33.5 ± 2.2	30 - 39
B (600 m)	29	33.0 ± 2.2	30 - 39
C (100 m)	33	33.5 ± 3.3	30 - 48
D (76 m)	31	33.0 ± 2.0	30 - 39

ANOVA TABLE

Source	df	SS	MS	F	
Altitude	3	7.683	2.561	0.41	(P>0.05)
Error	119	744.561	6.257		
Total	122				

Table 3. The effect of photoperiod on the number of nymphal instars of A. fasciatus from site A (1,100 m) at 24°C.

Photoperiod (hr)	% of animals with 6 instars	7 instars	8 instars	n
12	79	21	0	29
14	44	56	0	27
16	0	96	4	24

III. SEASONAL VARIATION IN DIAPAUSE CHARACTERISTICS

Introduction

In the striped ground cricket, A. fasciatus, adults start appearing and ovipositing in midsummer when the temperature is the highest and precipitation the lowest in Corvallis (Chapter II; climatological data from OSU). According to my laboratory observations, eggs are deposited at about 1 cm depth in soil. Eggs laid in summer are very likely exposed to high temperatures under dry conditions. Several species of grasshoppers are known to pass a dry summer in a state of quiescence (Wardhaugh 1980) or diapause (Andrewartha 1943, 1952; Birch and Andrewartha 1942,1944) at early embryonic stages before they reach the diapause stage for overwintering. No comparable phenomena are known in crickets belonging to Gryllidae.

I examined the influence of temperature on the duration of the egg stage in A. fasciatus and found that embryos exhibited seasonal variation in diapause characteristics which might be involved in the adaptation of this species to warm and dry conditions. The results of experiments to show this seasonal variation in diapause characteristics are reported here. I also compared the rate of embryonic development by determining the stages at different temperatures. The results of this observation and a model to explain the temperature responses observed in this species are also described.

Materials and Methods

To examine if the time of oviposition influences the duration of the egg stage, I collected more than 10 adult females on 8 different occasions between August 5 and November 4, 1981, in Corvallis. Adult females were maintained in a 2-liter glass jar at 27°C and LD 15:9 hr and allowed to oviposit into moist glass wool. Eggs laid during the first few days after collection of females were placed on moist paper in Petri dishes within 24 hours after being laid and incubated at 20, 27, and 30 $\pm$ 1°C. For each treatment, 3 batches, each consisting of 25 eggs, were used and hatches were recorded daily.

An index of embryonic stages was made to compare the rate of development at different temperatures (Fig. 4). Eggs obtained from females reared at 27°C and LD 16:8 hr were incubated at 20°C for different periods and dissected with two insect pins under a binocular microscope. To facilitate separation of embryos from the yolk, eggs were immersed in water at 75°C for 8 minutes according to the method described by Ando (1978). Dissected embryos were stained with acetic carmine (Ando 1978) and drawings were made of distinct morphological stages using a squared-ocular (Fig. 4). These drawings illustrate changes in morphology only and do not correspond to the mean lengths of embryos at each stage. Embryos younger than stage I were so fragile that isolation from the yolk was not possible. Therefore, these young embryos were not included in Fig. 4. The last embryonic stage, VIII, includes all those embryos beyond stage VII. The distinction between these stages was made by measuring the length of antennae: embryos with their antennae longer than 0.57 mm were categorized as stage VIII. After stage VII, the embryo rotates inside the shell and develops without interruption until it hatches.

The embryonic development was compared at 20, 24, 27, and 30 $\pm$ 1°C using the index in Fig. 4. I incubated about 500 eggs obtained in the same way as described above at each temperature and randomly selected 5 - 20 eggs from them for each dissection to determine the embryonic stage.

Results

Hatching at different temperatures: To examine the influence of the time of oviposition on the duration of the egg stage, I incubated eggs laid by adult females collected on different dates at 20, 27, and 30°C. When eggs were incubated at 20°C, the time required for the first hatch decreased as they were laid later in the season (r^2= -0.62; $P < 0.05$)(Fig 5a). The regression lines in this figure indicate that the number of days to hatch was reduced by 0.31 and 0.32 day for every day delay in collection of adult females for the first and 50 % hatch,

respectively. This variation in the duration of the egg stage (or the intensity of diapause) was attributed to the mothers because all eggs used were laid under the same conditions in the laboratory. Similar results were obtained at 27°C (Fig. 5b). As eggs were laid later, they required a shorter period to start hatching (slope= -0.97; r^2= -0.82; P<0.05) and to attain the 50 % hatch (slope= -0.67; r^2= -0.51; P<0.05). In spite of the 7°-difference in temperature, however, no significant difference was observed in the average time required for 50 % hatch when the data were pooled for each temperature and compared. At 30°C, hatching started within 20 days after deposition in all batches of eggs except in some batches laid in August (Fig. 5c). The time required for the first hatch decreased as the time of oviposition was delayed, although the correlation between the two variables was not statistically significant (slope= -0.13; r^2= -0.30; P>0.05). The 50 % hatch was also recorded within 20 days of incubation in those laid after September. However, it was not observed even after 200 days of incubation in those laid in August.

In Fig. 6, the average percentage of eggs that hatched within 200 days of incubation is given for the eggs from each group of adult females collected. The percent hatch increased as the time of oviposition was delayed, although the correlation between the two variables was statistically significant only at 20°C (r^2= 0.64; P<0.05) and 30°C (r^2= 0.93; P<0.05). Hatching became very sporadic after 200 days of incubation at all temperature and very few eggs looked "healthy" after 250 days of incubation. Thus, the percent hatch given in this figure probably reflects the survival rate of eggs at these temperatures.

Eggs incubated at 30°C showed two different modes of hatching (Fig. 7). One group hatched within 20 days of incubation and the other started hatching about 30 days after oviposition and continued to do so in a sporadic fashion. The latter group showed a hatching pattern characteristic of diapause eggs from some species which do not require a cold period for diapause termination (Hogan 1960; Masaki 1960; Rakshpal 1962). The earlier hatching eggs, on the other hand, hatched in somewhat synchronized fashion. They probably experienced little or no diapause and development resembled that of non-diapause eggs of

other crickets, e.g. Acheta domesticus (McFarlane and Kennard 1960), Teleogryllus sp. (Masaki 1960), Pteronemobius nitidus (Masaki and Oyama 1963), and T. oceanicus (Masaki et al. 1979). Thus, they were termed "fast-developing" eggs in a sense similar to that used by Walker (1980). The eggs that hatched within 20 and 22 days after incubation at 30 and 27 C, respectively, were regarded as fast-developing eggs.

Figure 8 illustrates the proportion of fast-developing eggs incubated at 30 and 27°C. When laid in early August, few eggs were fast-developing at 30°C. The proportion of fast-developing eggs increased as the time of collection of females from the field was delayed and most eggs laid in November were fast-developing. A similar tendency was observed for eggs incubated at 27°C. At this temperature, however, no fast-developing individuals were obtained in the egg batches laid in August and early September and the proportion of fast-developing eggs laid later was much smaller than those at 30°C. At 20 °C, the hatching pattern was continuous and no distinction could be made between the fast-developing eggs and diapause eggs. However, the time required for the first hatch was considerably reduced as the time of oviposition was delayed (Fig. 5a), raising the possibility that some eggs laid in late autumn develop without diapause.

These results indicate that eggs laid later in the season are more likely to develop without diapause (fast-developing) when exposed to a high temperature. This may be due to a maternal influence on eggs. The proportion of fast-developing eggs depends upon the temperature of incubation. This suggests that the response of the eggs to temperature is facultative.

Embryonic Development at Different Temperature: To examine the effect of temperature on embryogenesis, eggs laid by laboratory-reared females were kept at 20, 24, 27, and 30°C, and dissected at different intervals to determine the embryonic stage using the index in Fig. 4. The results are given in Tables 4, 5, 6, and 7.

All embryos incubated at 20°C were at stage I after 6 days of incubation (Table 4). Development was continuous until day 20 when most embryos reached stage VII. They remained at this stage for as

long as two months. After this stage, embryos rotate inside the shell and develop without interruption until hatching. The suppressed development at stage VII is probably due to the induction of diapause. Similar results were obtained at 24°C except that embryos took longer to reach stage VII (Table 5). Eggs incubated at 27°C reached stage II within 6 days but subsequent development was suppressed at stages II, III and IV and only a small proportion of eggs reached stage VII (Table 6). An even greater suppression of development was seen in the embryos at 30°C and after 60 days most embryos were still at stage II (Table 7). One hundred days after incubation at 30°C most embryos examined had developed beyond stage III. Out of 380 eggs that remained undissected by day 100, about 20 % hatched. There was no indication that embryos were suppressed to develop at stage VII at this high temperature (30°C). These results indicate that developmental suppression was imposed at an earlier stage as the temperature of incubation was increased.

In this experiment, no fast-developing eggs were obtained even at 30°C. The eggs laid by laboratory-reared females seemed similar in the response to temperature to those laid by females collected early in the season (Fig. 5 and Table 7).

To examine the nature of suppressed development at higher temperatures, eggs were first incubated at 27°C for 14 days and then transferred to different temperatures. The embryonic development was examined for 50 days after the transfer in the same way as in the previous observations. When eggs were dissected 10 days after the transfer, there was little developmental progress at 30°C (Table 8). It appears that development proceeded more rapidly as the temperature decreased, except when the temperature was below 20°C. A similar relationship between developmental rate and temperature was observed in later dissections. At 16°C, embryonic development proceeded at a lower rate probably because less heat was available for morphogenesis. Therefore, the embryo developed to stage VII most rapidly at an intermediate temperature of 20°C. The embryos transferred to 20°C attained stage VII in 20 days. They remained at this stage at least for 30 days. This indicates that the embryos whose development has been suppressed at a high temperature are induced to diapause at stage VII if transferred to

a lower temperature.

Discussion

Diapause occurs in various embryonic stages of insects. Umeya (1946,1950) described the embryonic diapause of 21 species and recognized 5 different stages. They are the pyriform, dumb-bell shaped, elongated, appendage-formation, and prelarval stages. Many insects studied enter diapause at one of these stages (Rakshpal 1962; Uvalov 1966) but some species such as Didymuria violescens (Bedford 1970) and Tipula simplex (Hartman and Hynes 1980) are known to do so before the formation of the blastoderm. In A. fasciatus, the embryonic development was suppressed at the elongated (stage II in Fig. 4) and the appendage-formation stage (stages III, IV, V, VI, and VII in Fig. 4) depending upon the temperature to which the embryo was exposed. At 20 ^{o}C, the embryo developed without interruption until it reached stage VII at which diapause was induced (Table 4). At 30^{o}C, development was strongly suppressed at stages II and III (Table 7). When an embryo suppressed at an early stage was transferred from a high temperature to lower temperatures, the development was resumed and then suppressed at stage VII due to the induction of diapause (Table 8). In this case, the developmental rate before reaching stage VII was inversely related to temperature within the range between 27 and 20^{o}C. One may argue that a high temperature has an inhibitory effect on the embryonic development of this species so that the embryo becomes quiescent under such conditions. However, this is not the case. As shown in Figs. 5 and 8, many of those eggs laid in late autumn developed rapidly and hatched in 3 weeks at 27^{o}C and 30^{o}C. The results clearly suggest that a high temperature ifself is not inhibitory to embryogenesis. Therefore, the suppressed development at early stages may be regarded as a diapause rather than a state of quiescence defined as a direct response to adverse environmental conditions (Andrewartha 1952; Lees 1955; Beck 1980). It may be called a summer diapause to distinguish it from the diapause induced at stage VII. The latter may be called a winter diapause.

The summer diapause seems different from the winter diapause in the range of the optimum temperatures for the induction and/or termination. As mentioned, the embryonic development suppressed at a high temperature was markedly accelerated after a transfer to 20°C at which winter diapause was induced and maintained (Table 8). The developmental suppression caused by the summer diapause became less obvious as the incubation temperature decreased (Tables 4, 5, 6, and 7). At 24°C, development was slow but appeared to be continuous before reaching stage VII. The time required to reach stage VII was further reduced at 20°C. Although winter diapause invariably occurred at this stage at this moderately low temperature, the summer diapause was neither induced nor maintained. This phenomenon is typical of thermal responses involved in summer diapause (Masaki 1980) and presents a striking contrast to the initial diapause in the egg of Leptophyes punctatissima which provides a means for overwintering (Deura and Hartley 1982). The present results indicate that embryogenesis can proceed even in a state of diapause.

A. cruciata which endures a dry summer in Australia (Birch and Andrewartha 1942; Andrewartha 1943) shows somewhat similar developmental behavior. This species enters diapause in early embryonic stages with morphogenesis strongly suppressed at high temperatures. Upon transfer to a lower temperature, morphogenesis resumes or accelerates but, by the end of appendage-formation stage (or the end of anatrepsis), a second diapause is induced (Uvalov 1966). Andrewartha (1943,1952) explained these phenemena in terms of the difference in the range of optimum temperatures between diapause development and morphogenesis. He did not separate the diapause of A. cruciata into two different types. In A. cruciata, diapause is obligatory and the range of optimum temperatures for the diapause development is considered to be constant throughout embryonic life (Andrewartha 1952).

In nature, as inferred from Table 5, eggs of A. fasciatus laid early in the season would enter summer diapause and remain in early stages as long as high temperatures prevail. In autumn when it becomes cooler, they would enter winter diapause. In those laid in autumn, on the other hand, only winter diapause would be induced because of the

relatively low temperatures prevailing. It is interesting that the ability of eggs to enter summer diapause in response to high temperatures gradually decreases as the time of oviposition is delayed (Fig. 8). Most eggs laid in late autumn underwent rapid development to hatch at high temperatures without any diapause.

Another seasonal variation in the response of eggs was observed in the intensity of diapause at 20°C (Fig. 5a). The later the time of oviposition, the shorter was the duration of the egg stage or the lower the intensity of winter diapause. A similar phenomenon was observed in Melanoplus differentialis (Burdick 1937), Locustana pardalina (Matthee 1951), and G. pennsylvanicus (Rakshpal 1962), and some maternal influence must be involved in the seasonal changes in the intensity of diapause in all these species including A. fasciatus. In G. pennsylvanicus, this phenomenon has been suggested to serve as a mechanism by which eggs laid in summer can remain unhatched before overwintering because of their high intensity of diapause (Rakshpal 1962). In A. fasciatus, however, winter diapause would probably not be induced until the autumn and precocious hatching of eggs laid in summer would be prevented by the induction of summer diapause. However, eggs may encounter warm weather even in autumn. Under such circumstances, it seems important to maintain the capacity of entering winter diapause with a decreased but still high intensity in order to avoid precocious hatching.

The seasonal variations in the induction and intensity of the summer and/or winter diapause of this species perhaps result from differences in the environment of the parents during their active life as suggested for the diapause in T. commodus (Browning 1952) and G. firmus (Walker 1980). Precise determination of the cause(s) for these phenomena in A. fasciatus is yet to be done.

Figure 9 illustrates a simple model in which these phenomena may be accounted for by assuming a humoral factor A. More of this factor is assumed to be produced and transmitted by female adults to their eggs in summer conditions than in autumn conditions. When it is large in amount, the embryo enters summer diapause in response to high temperature, e.g. 30°C. When kept at low temperature, e.g. 20°C, it would continue to develop to stage VII and enter winter diapause with a high

intensity. In autumn, adults produce eggs with less amount of factor A. Such eggs enter winter diapause with a low intensity at low temperature. However, at high temperature, no diapause is manifested and they hatch. Some eggs which contain little or no factor A might be produced in late autumn. They would show little or no diapause at either high or low temperature but, in the field, the prevailing low temperature would prevent their precocious hatching before winter. My preliminary observations suggest that eggs laid in late autumn encounter winter before they reach the winter diapause stage, but can overwinter and hatch in the next spring.

Differences in the proportion of fast-developing eggs between two temperatures (e.g. 27 and 30°C in Fig. 8) suggest that there is a threshold level of factor A below which an egg becomes fast-developing in response to a temperature. In other words, an egg with a certain level of factor A may enter diapause at a certain temperature but override it at a higher temperature because this level of factor A is below the threshold for diapause induction at the higher temperature.

A. fasciatus is widely distributed in North America and is bivoltine in the southern states (Fulton 1951). The occurrence of a bivoltine life cycle is difficult to reconcile with the existence of a summer diapause and with the model proposed in Fig. 9. However, if the production of factor A were controlled by an environmental cue such as photoperiod in the bivoltine populations, the adults in the first generation would be able to limit the production of factor A and lay non-diapause eggs even under summer conditions. Geographic variation in the photoperiodic control of embryonic diapause is quite common in many insects (Beck 1980) including crickets (Masaki 1972,1978) but very little is known about the nature of the factor or hormone controlling embryonic diapause except in the silkworm, Bombyx mori. In B. mori, diapause is induced by a neurohormone, the diapause hormone, secreted from the suboesophageal ganglion during pupal-adult development of the female parent (Fukuda 1951; Hasegawa 1951,1957). The hormone consists of two active principles characterized as peptides (Isobe et al. 1973). Studies of the diapause hormone have mainly been concerned with the determination of diapause and the quantitative relationship between the

hormone and the intensity of diapause is not known. However, Kai and kawai (1981) recently obtained active extracts of the diapause hormone from both adult ovaries and laid eggs. They suggested that the hormone might act not only on the ovaries during the pupal stage to initiate diapause but also on the embryo to regulate the onset and maintenance of diapause.

As shown in Fig. 6, the survival rate decreased as the incubation temperature was increased in eggs laid in August and September. On the other hand, it was highest at 30°C among eggs laid in October and November. Apparently, most of these eggs did not enter diapause at this temperature (Figs 5c and 8). The high mortality in the eggs laid early in the season may be related to a higher intensity of diapause. Diapause with a higher intensity would take longer for termination. If the temperature is favorable for morphogenesis but not for termination of diapause, a prolonged exposure to it may be harmful to diapausing insects (McLeod and Beck 1963; Ingram 1975; Tanaka 1978b; Hartman and Hynes 1980).

As described above, A. fasciatus shows several characteristics of embryonic development shared by some of those species which survive a dry summer in the egg stage. Summer diapause as found in this species is not known in any other species of crickets (Alexander 1968; Masaki 1972,1978,1980). This might be related to the fact that crickets in general are adapted to a wet micro-environment. Data of experiments to examine the ecological significance of summer diapause in A. fasicatus will be given in the next chapter.

Figure 4. The embryonic stages of A. fasciatus. Stage I: Embryo is dumb-bell shaped, 0.92 mm or larger in length. Stage II-b: Segmentation begins. Stage II-c: Antennae appear as evaginations lateral to stomodeum; labrum is visible above the stomodeal invagination. Stage III-a: Abdominal segmentation begins. Stage III-b: Gnathal segments are distinct; limb buds tiny. Stage IV-a: Each thoracic segment is distinctly separated across the sternum. Stage IV-b: Antennae, gnathal, and thoracic appendages elongate. Stage V: Appendages become directed into the mid-line; abdomen is completely segmented. Stage VI: Metathoracic legs are long enough to touch each other. Stage VII: Thoracic appendages have distinct tarsal, tibial, and femoral divisions; antennae are shorter than 0.58 mm. Stage VIII: All embryos with antennae equal or longer than 0.58 mm.

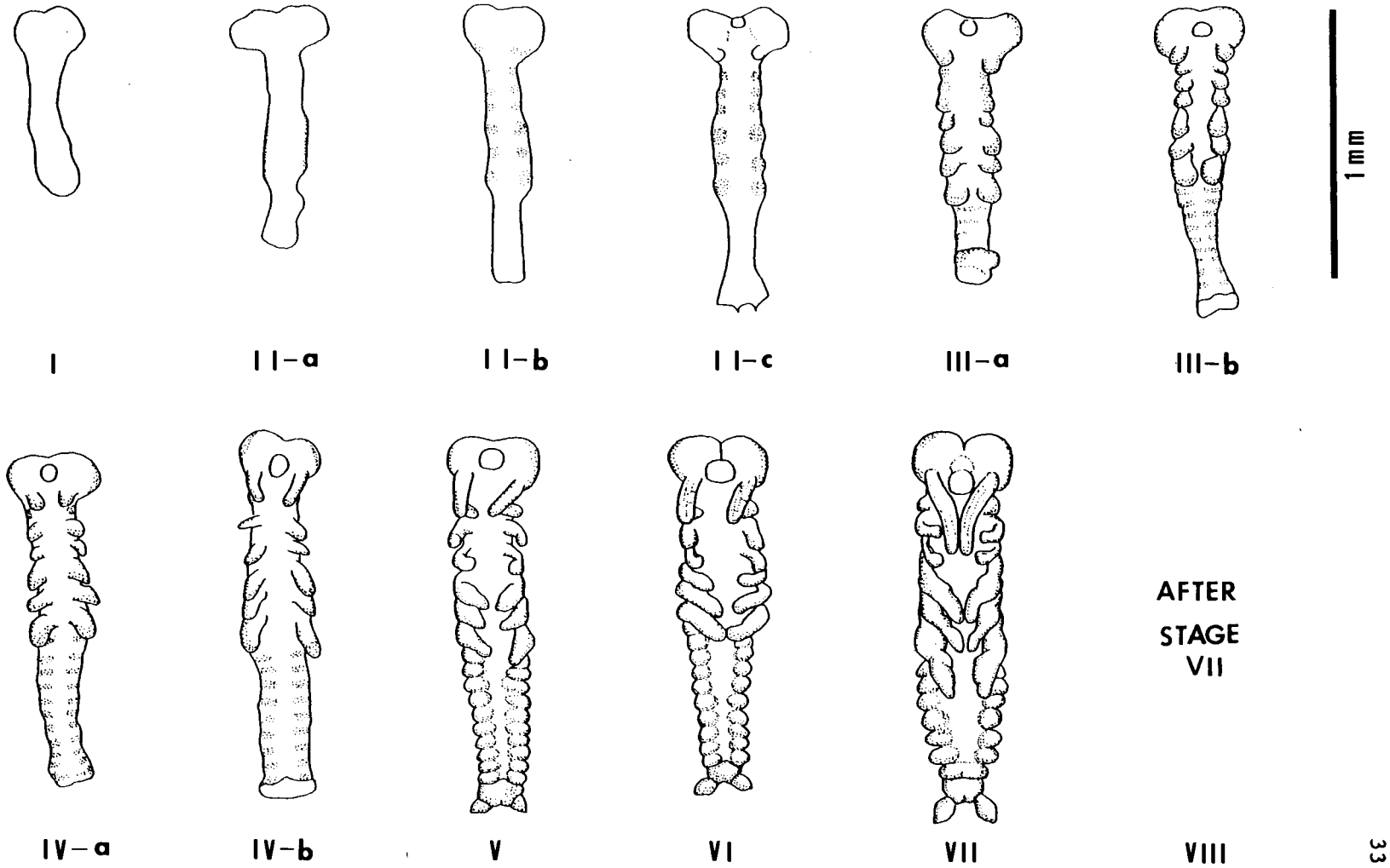
I
II-a
II-b
II-c
III-a
III-b
1 mm
IV-a
IV-b
V
VI
VII
AFTER STAGE VII
VIII

Figure 4.

Figure 5. Seasonal variation in the time required for the first egg to hatch (small dots) and for 50 % hatch (large dots) at different temperatures in A. fasciatus. The data are based on 3 groups of 25 eggs each.

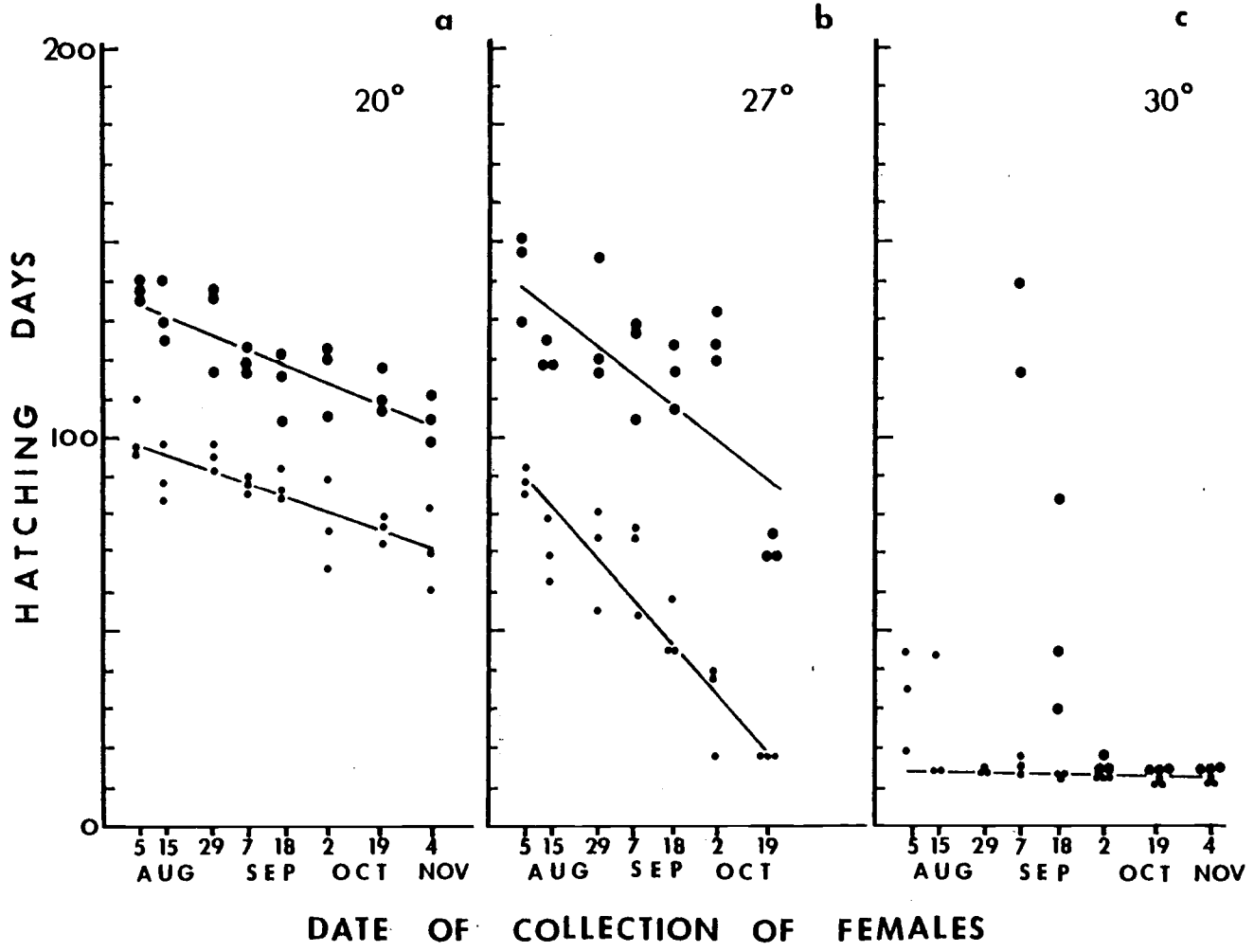
a
b
c
20°
27°
30°
200
100
0
HATCHING DAYS
5 15 29 7 18 2 19 4
AUG SEP OCT NOV
5 15 29 7 18 2 19
AUG SEP OCT
5 15 29 7 18 2 19 4
AUG SEP OCT NOV
DATE OF COLLECTION OF FEMALES

Figure 5

Figure 6. Seasonal variation in the mean proportion of eggs hatching within 200 days of incubation at different temperatures in *A. fasciatus*.

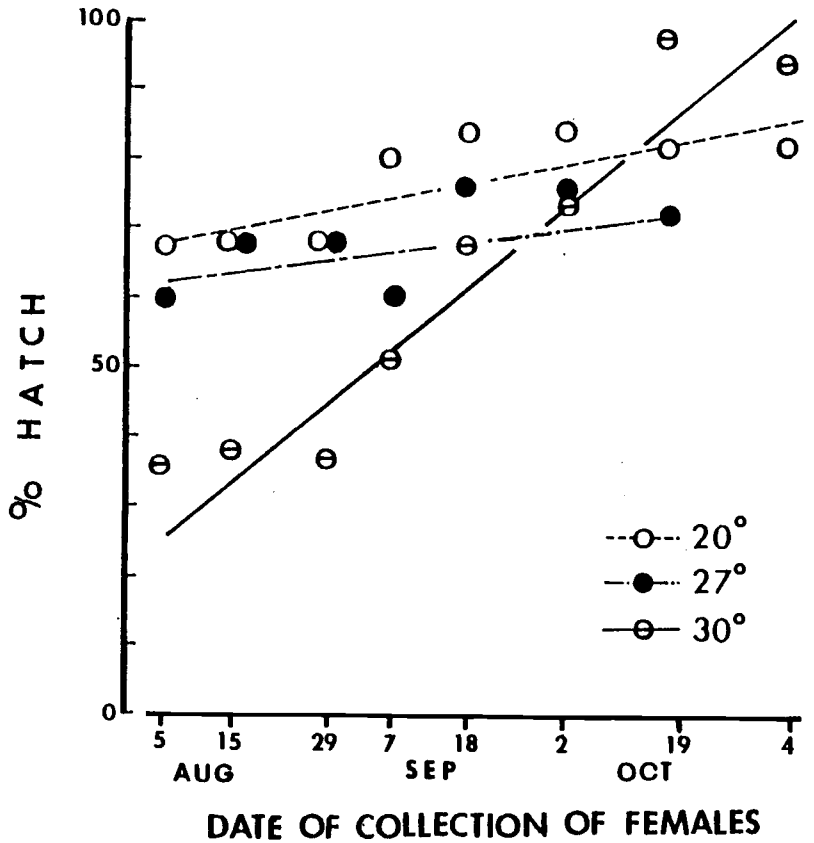
100
50
0
% HATCH
20°
27°
30°
5
15
29
7
18
2
19
4
AUG
SEP
OCT
DATE OF COLLECTION OF FEMALES

Figure 6

Figure 7. Hatching patterns of A. fasciatus eggs laid by females collected on October 19, 1981, and incubated at 30 and 27°C. The percentages in the figure indicate the proportions of eggs which hatched in 50 days of incubation.

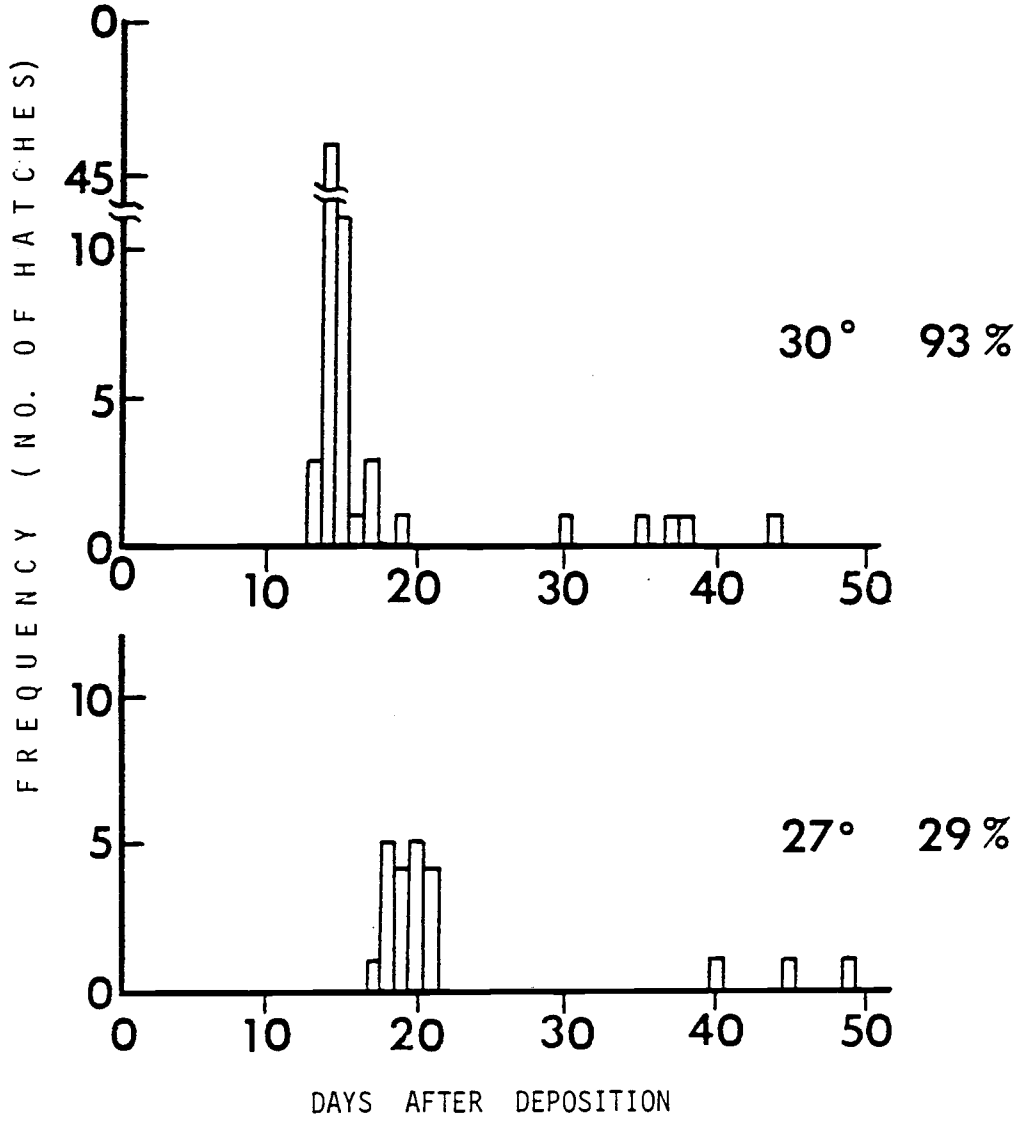
FREQUENCY (NO. OF HATCHES)
0
45
10
5
0
0
10
20
30
40
50
30° 93%
10
5
0
0
10
20
30
40
50
27° 29%
DAYS AFTER DEPOSITION

Figure 7

Figure 8. Seasonal variation in the proportion of fast-developing eggs of A. fasciatus.

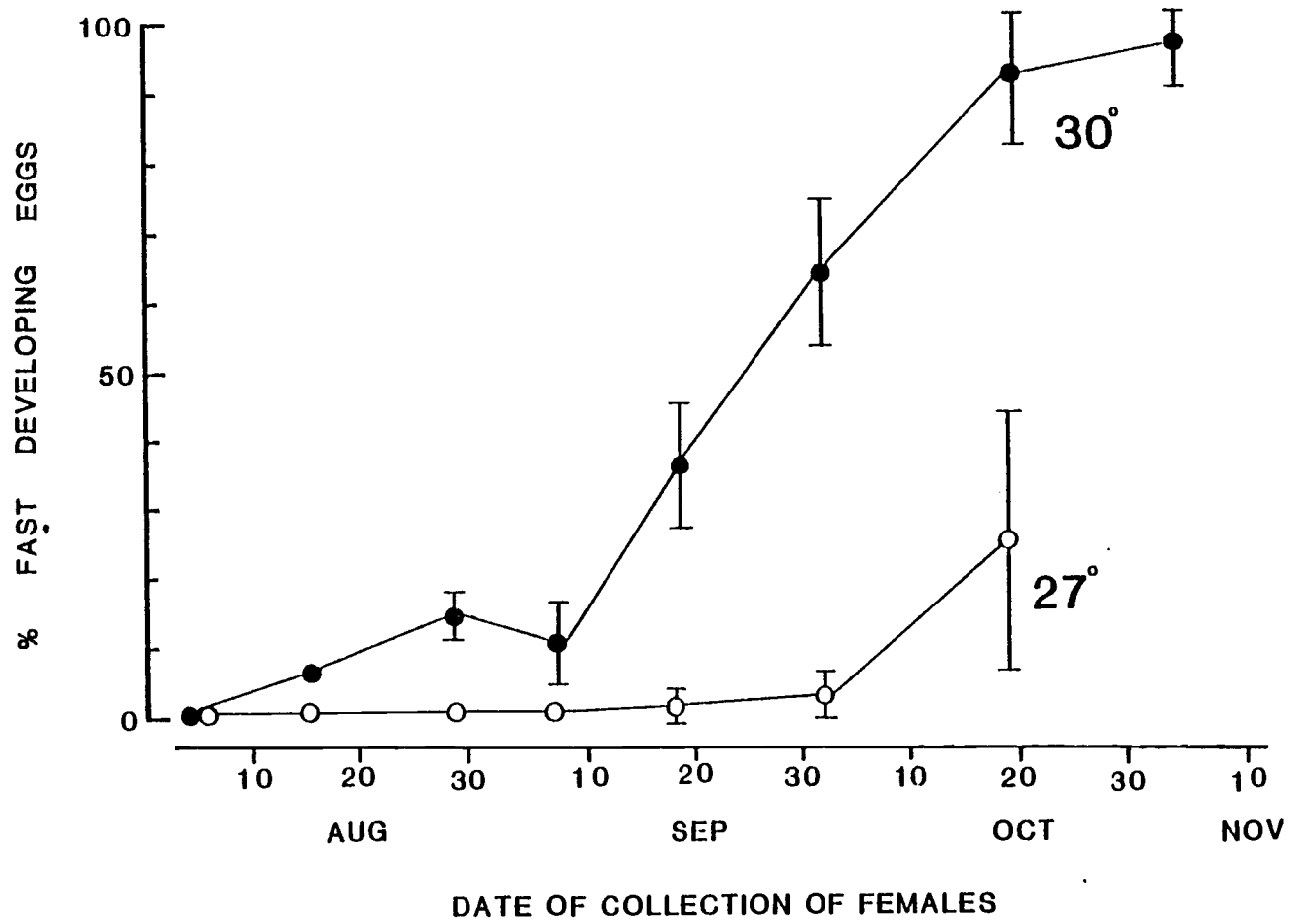
100
50
0
% FAST DEVELOPING EGGS
30°
27°
10
20
30
10
20
30
10
20
30
10
AUG
SEP
OCT
NOV
DATE OF COLLECTION OF FEMALES

Figure 8

Figure 9. A model for the seasonal variation in the response to temperature in eggs of A. fasciatus. The number of A's indicates relative amounts of the hypothetical factor which induces summer diapause (SD) and winter diapause (WD) with different intensities. More of this factor is produced and transmitted by adult females to their eggs in summer conditions than in autumn conditions.

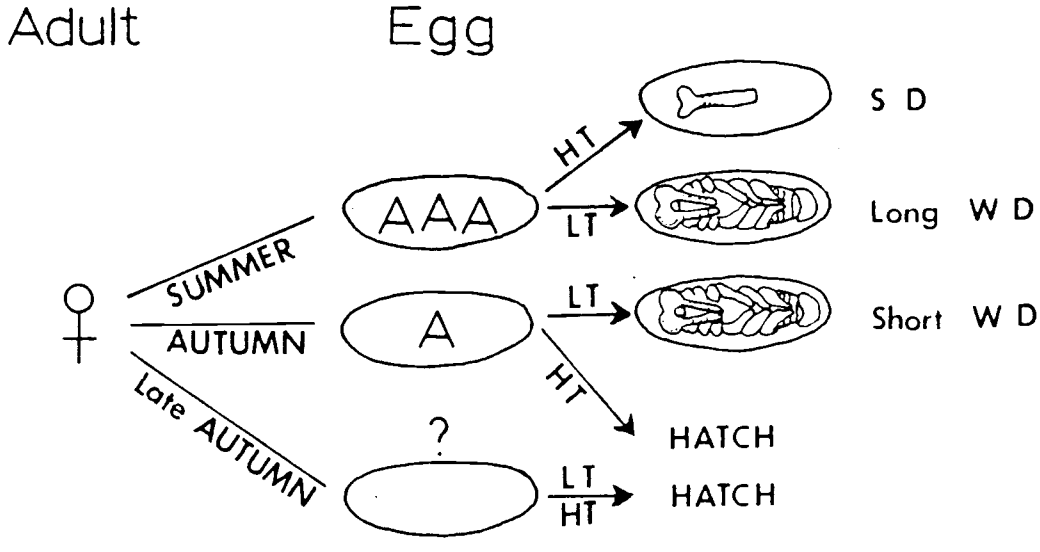
Adult
Egg
SUMMER
AUTUMN
Late AUTUMN
AAA
A
?
HT
LT
LT
HT
LT
HT
S D
Long W D
Short W D
HATCH
HATCH

Figure 9

Table 4. The embryonic development of A. fasciatus at 20°C.

Days of incubation	Embryonic stage								No. eggs dissected
	I	II	III	IV	V	VI	VII	VIII	
6	10								10
8	6	4							10
12		6	3	1					10
14		1	4	4					9
16				8	2				10
18					12	3			15
20					1	1	8		10
22.5						1	10		11
25					1	1	13		15
30							11		11
35		1					10		11
40							9		9
50							10		10
60							6		6
70							9		9
80							9	1	10
90							13	2	15

Table 5. The embryonic development of A. fasciatus at 24°C.

Days of incubation	Embryonic stage								No. eggs dissected
	I	II	III	IV	V	VI	VII	VIII	
8	2	5	3						10
12	1	5	3	1					10
16		4	4	2					10
20		3	4	2					9
25			4	2	4				10
30			4	3		4			10
40				4		3	3		10
50				1	1	4	3		9
60		1			2	4	3		10

Table 6. The embryonic development of A. fasciatus at 27°C.

Days of incubation	Embryonic stage								No. eggs dissected
	I	II	III	IV	V	VI	VII	VIII	
4	2	5							7
6		5							5
8		8							8
10		10							10
12		10	1						11
14		9	2						11
16		12	5	3					20
20		10		1					11
25		7	3						10
30		5	2	5					12
35		2	4	4					10
40		2	6	2					10
60		1	3	5	1				10
100				3	2	1	3	1	10

Table 7. The embryonic development of A. fasciatus at 30°C.

Days of incubation	Embryonic stage								No. eggs dissected
	I	II	III	IV	V	VI	VII	VIII	
4	3	7							10
6		9							9
8		10							10
10		9	1						10
12		8	2						10
20		9	1						10
30		10	1						11
60		9	1						10
100[a]			1		3	1	1	1	10

[a]Stages of 3 embryos could not be identified.

Table 8. The effects of shift in temperature from 27°C to different temperatures on the embryonic development of A. fasicatus. All eggs were kept for 14 days at 27°C before the transfer and dissected 0, 10, 20, 30, and 50 days

Days after transfer	(°C)	Embryonic stage I	II a	II b	III c	III a	III b	IV a	IV b	V	VI	VII	VIII
0	27		1	4	3	2							
10	30			3	3	4							
10	27			1	1	6	2						
10	24				2	4	3		1				
10	20						1		4	2	3		
10	16						4	5			1		
20	30				2	3	3	2					
20	27				1	3	3	3					
20	24				1	2	1	3	3				
20	20											10	
20	16							6	4				
30	30				2	3	5						
30	27			1		3	5						1
30	24			1			2	2	2	1		2	
30	20											10	
30	16							4	6				
50	30					4	1	3	1	1			
50	27							2	7		1		
50	24[i]										3	7	
50	20											10	
50	16								7	1	2		

[i] Temperature dropped to 22°C for the last 10 days.

IV. SUMMER DIAPAUSE AND ITS ECOLOGICAL SIGNIFICANCE

Introduction

A. fasciatus is the first species of crickets known to have a summer diapause in addition to a winter diapause in the egg stage (Chapter III). As an adaptation to warm conditions, the embryos of G. pennsylvanicus (Rakshpal 1962) and T. emma (Masaki 1962) have evolved a variable intensity of diapause. In these species, eggs enter diapause with a greater intensity in summer than in autumn. The intensity of diapause is influenced by the environmental conditions experienced by female adults of G. pennsylvanicus or by eggs of T. emma. As described in previous chapters, A. fasciatus also varies the intensity of winter diapause depending upon the environmental conditions to which both female adults and eggs are exposed. One may ask, then, why this species requires both a summer and winter diapause. In this chapter, I examined the ecological significance of summer diapause in this ground cricket.

The ability of embryos to enter summer diapause in A. fasciatus depends on the time of oviposition (Chapter III). Eggs laid in summer have this ability but most of those laid in autumn do not. The latter hatch without any interruption if incubated at high temperatures at which summer diapause is manifested in summer eggs. As an approach to the problem given above, eggs with and without summer diapause were compared for the survival rate, embryonic development, and water absorption at different levels of environmental moisture and temperature. The results will be described and discussed in relation to the temperature and moisture conditions to which the eggs of this species are exposed in the field.

Materials and Methods

Air temperature and precipitation data were derived from those accumulated by Oregon State University in Corvallis (44.3°N).

Soil temperature was measured from habitats of A. fasciatus in Corvallis. I chose a place (1.5 x 0.3 m) located in the middle of the

habitat (10 x 15 m) which contained sites where the ground was covered with green, wilted, or no plants. The measurements were made with a thermometer at the surface and 1 cm depth in the soil at these sites at 15:00 on August 9, 1981. I chose this day because of the high average air temperature (29.2°C, second highest of the year).

To determine the moisture content of the soil in habitats of A. fasciatus, 20 - 50 grams of soil (clay loam) were collected at different times of the year. Each sample was weighed before and after it was dried at 110°C for 24 hours and the moisture content was calculated from the difference divided by the initial weight.

The effects of soil moisture on the embryonic development and survival rate of eggs were examined at 30°C. The soil used was collected in summer from habitats of A. fasciatus. For each treatment, eggs were deposited at 1 cm in the soil put in a plastic container (4 x 4 x 3 cm) with an air tight-lid. After different periods of incubation in the soil, they were removed and placed on moist paper. The survival rate was determined 30 days later: the eggs which darkened or whitened were considered as dead. The methods for dissection of eggs and determination of their stages have already been described in Chapter III.

The effects of desiccation on the weight and survival of eggs at different ages were observed at 20°C. Eggs laid by females collected in August, 1981, were exposed to 50 % relative humidity for 1 or 5 days and otherwise kept on moist paper. The humidity was controlled using potassium hydroxide in desiccators (Solomon 1951). During the period of desiccation, eggs were placed on dry absorbent tissue. The survival rates after exposure to 50 % relative humidity were determined in the same way as described above except that eggs were kept at 20°C.

Results

Habitat: Figure 10 illustrates the seasonal fluctuations in the average air temperature and precipitation in Corvallis. The precipitation is the highest in January and decreases towards the middle of summer when the temperature is the highest. As described in Chapter II, A. fasciatus starts emerging as an adult at this driest time of

the year. One may, therefore, expect that both adults and the eggs of this species would be exposed to dry and warm conditions in summer. However, this should not be concluded until the environmental conditions of their micro-habitat are examined.

To obtain such information, the temperature and moisture content of the soil from about 1 cm in depth were measured from habitats represented by this species. The measurements were made at 4 different sites characterized as follows. At site 1, there were green forbs on the ground. Sites 2 and 3 were covered with wilted grasses, the density of which was higher in the former than in the latter. Site 4 was bare. All sites were exposed to the sun. The results given in Fig. 11 show that there was a great variation in temperature among the 4 microenvironments. The surface temperature was about 60°C at site 4 and 34 °C at site 1. The temperature at 1 cm depth in the soil at which eggs are probably deposited (p. 22) was 30.5°C at site 1. The surface of the ground was firmly caked and had many cracks. The moisture content of the soil from site 4 was only 5.5 % while it was 17 % in the sample from site 1. These and similar measurements made on different dates in August (Tanaka, unpublished data) suggest that the soil covered with green forbs is kept much cooler and contains more moisture than that covered with wilted grass or directly exposed to the sun.

To examine the seasonal changes in moisture content of the soil, samples were periodically collected from two sites of the same habitat used above. The results are summarized in Fig. 12. The moisture content in the exposed site was about 5 % in August while it was between 10 and 20 % in the site covered with green forbs. In 1981, no rain was recorded in August nor in the first half of September.

Survival Rate in the Soil: Eggs obtained from females collected in early August, 1982, were deposited in the soil with different moisture contents and incubated at 30°C to examine the survival rate under these conditions. Eight or 30 days after deposition, they were moistened on sheets of paper in Petri dishes and the survival rate was determined one month later. The results given in Table 9 show that the eggs did not survive when incubated in soil with a moisture content of

3.9 % for 8 days while those deposited in soil of 5.9 % or more moisture did. However, when kept in the soil for 30 days, mortality was observed in the latter except at a moisture content of 10.9 %. Some eggs were slightly collapsed but no eggs were swollen due to water absorption. Although the results are based on a rather small sample size, it seems that eggs laid in the soil with less than 10.% moisture would be faced with a high risk of death at high temperatures. This and the results in Figs. 11 and 12 indicate that only those eggs laid near or under green forbs would survive a summer without suffering a high mortality. The cooler and more moist conditions associated with green forbs or grasses seem to be also important for the survival of adults because they can rarely be found in those fields where all plants are wilted in summer.

Effect of moisture on embryonic development: To examine the embryonic development under wet-warm or dry-warm conditions, eggs were incubated at 30°C either in the soil collected from their habitat in summer or on moist paper, and dissected at different intervals to determine their embryonic stage. Eggs laid in summer and in autumn were tested by the same procedure. The results are set out in Table 10.

When autumn eggs were placed on moist paper, most of them absorbed water within a week and the embryos reached stage VIII by day 8. Apparently, their development was not suppressed because eggs hatched without interruption once stage VIII was reached (Chapter III). The rest embryos, on the other hand, remained at stage II or III. This was probably due to the induction of summer diapause by the high temperature. Embryos which appeared to enter summer diapause were also found among eggs deposited in soil (Table 10). Some autumn embryos remained at around stage IV or V by day 10 and further development did not take place. Instead, their body and appendages became thinner and smaller and it was not possible to distinguish precisely some of those which probably developed beyond stage III from those which had remained at stage II or III. All eggs became slightly smaller than the size at the time of being laid but few eggs were found to be completely collapsed

even after 30 days of incubation in the soil. The summer eggs deposited in the soil with 10.1 % moisture content all appeared to enter summer diapause in response to the high temperature (Table 10). As observed in autumn embryos, they were shrunken and some rounded their body when kept in soil for 30 days.

To observe embryonic development under moist conditions after exposure to dry conditions, eggs maintained at 30°C in the soil with 10.1 % moisture content for different periods were moistened and hatches were recorded at the same temperature until the 50th day after being laid. The eggs tested were derived from females collected in mid-September and about two-thirds of them hatched without diapause when continuously kept under wet conditions (or on moist paper) at 30°C. In eggs exposed to dry conditions in the soil, hatching began in two weeks after transfer to wet conditions and continued for several days. As shown in Figs. 13a and b, the eggs which hatched during this period were distinguished from the others which started hatching at least two days after the last hatch (indicated by the discontinuities of the lines drawn in Figs. 13a and b). The proportion of eggs in the first hatching group in each treatment, the time spent by them under wet conditions, and the total percent mortality are given in Table 11. When maintained in the soil for 2 days, the proportion of eggs in the first hatching group was slightly decreased and it became significantly lower when kept in the soil for 4 or 6 days than when incubated under wet conditions throughout the experimental period (Table 11 and Fig. 13a). This may indicate that the induction of summer diapause is more common under dry conditions than under wet. Those eggs which were developing without summer diapause appeared to enter a state of quiescence when water deficiency was encountered (Table 10). Upon transfer to the wet conditions, they absorbed water and resumed development to hatch under the influence of high temperature. Therefore, their hatching started later as the time of transfer to wet conditions was delayed (Fig. 13a) but the time spent under wet conditions before hatching was almost constant in those exposed to dry conditions for 4, 6, 8 or 10 days (Table 11).

When the period of exposure to dry conditions was prolonged for

15 days or more, the proportion of eggs in the first hatching group gradually increased (Fig. 13b). In those transferred to wet conditions 20 days after being laid, it was 84.4 % which was significantly higher than the percent hatching attained in the control during the same period of incubation at 30°C ($t = 6.63$; $P<0.05$). This suggests that the termination of summer diapause was accelerated under dry conditions so that an additional 20 percent of eggs hatched upon transfer to wet conditions. When exposed to dry conditions for 30 days, all surviving eggs but one appeared in the first hatching group. The time spent by the first hatching group after transfer to wet conditions increased as the time of transfer was delayed. This was probably because eggs exposed longer to dry conditions took longer to gain sufficient water to resume embryogenesis and because those which had been in summer diapause at an early stage joined the first hatching group.

The above results suggest that even without summer diapause embryos survived in the dry soil for one month in a state of quiescence and resumed development to hatch upon transfer to wet conditions. Eggs which entered summer diapause also hatched rapidly after transfer to wet conditions. However, this was true only when they had been kept under dry conditions for more than 10 days.

To examine if the same is true when eggs exposed to dry conditions are moistened at a lower temperature, eggs laid by females collected in late September were deposited in the soil with 10.1 % moisture content at 30°C and then removed to wet conditions at 20°C at different intervals after being laid. For comparison, groups of eggs were similarly transferred from 30 to 20°C under wet conditions. Instead of observing their hatching behavior, the number of embryos which had developed beyond the winter diapause stage was determined 40 days after transfer to 20°C in this experiment. This was done by examining the color of the compound eyes seen through the shell. Embryos develop pigment in the compound eyes when they rotate inside the shell (Rakshpal 1962). The results are given in Table 12.

When constantly kept under wet conditions, no embryo underwent revolution if transferred from 30 to 20°C within 6 days after being

laid, while more than a half did if the transfer was delayed for one day (Table 12). Those embryos which did not start revolution would have probably entered winter diapause at stage VII in response to the moderately low temperature (Table 8 in Chapter III). The above results indicate that there is a critical stage after which the embryo fails to enter winter diapause at 20°C and it is probably stage VII because eggs without summer diapause reached this stage by the 6th day after being laid (Table 10).

Similar results were obtained when eggs kept in the soil at 30°C were moistened at 20°C. No embryo underwent revolution during the experimental period if transferred to moist conditions at 20°C during the first 10 days (Table 12). However, when maintained in the soil for longer than 10 days, some eggs were found with pigment in the embryo's eyes during the observation period. In the eggs kept in the soil for 20 days, about 12 % underwent revolution and hatched but the rest did not. Preliminary observations suggest that no embryo underwent revolution within 40 days after transfer to 20°C if eggs laid in summer were treated in a similar way (n = 90). Therefore, it is likely that the eggs which underwent revolution after an exposure to dry conditions for more than 10 days in Table 12 were those which had not entered summer diapause at 30°C. In other words, some of the eggs which have survived a long period of dry and warm conditions in a state of quiescence (without entering summer diapause) would hatch without entering winter diapause even if moistened at 20°C (c.f. Fig. 13 b).

Tolerance to Dry Conditions at Different Stages: To examine the tolerance to dry conditions at different egg stages, eggs incubated on moist paper for different periods were exposed to a relative humidity of 50 % for 1 or 5 days at 20°C. For each treatment, three groups each consisting of 30 eggs were tested. After exposure to dry conditions, they were moistened and held at the same temperature for 30 days to determine the survival rate.

As shown in Fig. 14a, eggs absorbed water from outside and their weight doubled. This occurred during the period between days 14 and 20 when the embryos were at either stage III, IV, V, or VI (Chapter III).

The weight loss after one-day exposure to desiccation was the greatest in the 16-day old eggs, while it was only 10 % or less in those before or after water absorption (Fig. 14b). The degree to which weight was lost after desiccation was closely related to the mortality (Fig. 14 c). The eggs which were absorbing water were the most vulnerable to desiccation. Similar results were also obtained from eggs subjected to the dry conditions for 5 days, although the mortality at the beginning of the egg stage became as large as that in the eggs during water absorption in this case.

Water Absorption at Different Temperatures: If eggs become particularly vulnerable to desiccation once they start absorbing water, the summer diapause of this species might function to delay the time of water absorption until the dry season is over. This hypothesis is based on the assumption made by Browning (1965,1967) that water absorption occurs only at a particular stage of embryogenesis for each species. In order to test this hypothesis, groups of eggs laid in summer or autumn were incubated on moist paper at 20, 24, 27, and 30°C and the number of eggs which completed or almost completed water absorption (eggs larger than 0.67 mm in width) was counted daily for 30 days after being laid. If the above assumption is true, it would be expected that the higher the temperature the later the time of water absorption because embryonic development is suppressed more strongly at earlier stages as the temperature increases (Chapter III). The results in Fig. 15 clearly show that this was not the case. More than 50 % of summer eggs kept at 20°C absorbed water by day 17 and a similar level was reached later at 27°C but earlier at 23 and 30°C. For comparison, groups of autumn eggs were also incubated at 30°C. About 71 % of these eggs absorbed water in a week and the rest started doing so about 2 weeks later. Most of the former (59 out of 64 eggs) hatched in 16 days of incubation without any developmental suppression.

To confirm the above results, eggs were dissected to determine their stage within 24 hours after they absorbed water and reached 0.69 mm in width at different temperatures. Water absorption took place at any of those stages ranging from stage II to stage VI in summer eggs

(Table 13). However, it seems to have occurred at rather definite stages at each temperature. The time of water absorption tended to be earlier at higher temperature within the range between 20 and 30°C. As mentioned, some autumn eggs incubated at 30°C absorbed water in a week and hatched without any developmental interruption. When autumn eggs were dissected at the end of water absorption, some were at stage II or III and the others at stage V, VI, or VII (Table 13). The latter were probably those developing without diapause. Two of such embryos had already reached stage VII when dissected but they probably attained this stage after water absorption was completed. It is interesting that those developing without diapause did not absorb water at stage II or III where eggs in summer diapause did under the same conditions.

These results indicate that the time of water absorption varies not only among individuals but also with the incubation temperature and the time of oviposition. However, some summer eggs incubated at 27 or 30°C did not absorb water for at least a month. This suggests that summer diapause makes it possible for the eggs of this species to pass the summer without requireing water absorption, although some eggs may absorb water if it is available.

Discussion

The embryo of A. fasciatus enters summer diapause in early stages at high temperatures if laid in summer or during the early part of the laying season (Chapter III). Of those laid in autumn, some do not enter summer diapause but hatch without any interruption when incubated at a high temperature. The proportion of such eggs increases towards the end of laying season. It seems, therefore, reasonable to conclude that the summer diapause in this species would constitute an adaptation by which the eggs laid in summer can remain unhatched until autumn when they resume development and enter winter diapause. However, in all experiments in the previous chapters, the eggs were placed on moist paper so that they had always the free access of water. The climatological data (Fig. 10) indicate that the air temperature is the highest and precipitation the lowest in midsummer when adults of A. fasciatus begin

to appear and lay eggs in the field. This may suggest that eggs laid in summer would be exposed to not only warm but also dry conditions in summer. The surface of the ground in their habitat is firmly caked and has many cracks throughout the summer because of desiccation. Therefore, the possibility may exist that the summer diapause provides a means for the eggs of this cricket to withstand drought as well as high temperatures.

However, when summer eggs were deposited in the soil collected from the field and incubated at 30°C for 30 days, high rates of mortality were recorded in the soil with 5.9 % moisture or less (Table 9). Apparently, they are not resistant to desiccation and require a certain level of soil moisture to survive the summer. The soil with such a level of moisture can be found near or under green forbs in summer. This, together with the fact that adults are closely associated with the existence of green forbs or grasses, may suggest that eggs of this species are laid in the soil which is covered with green forbs or grasses so as to avoid the extremes of desiccation and heat which are otherwise inevitable in summer (Figs. 11 and 12). When autumn eggs are incubated in such soil at 30°C, most survived for at least one month in a state of quiescence (Tables 10 and 11). Therefore, it is not likely that summer diapause has been selected for in this species because it increases the resistance to desiccation.

One of the most important roles of summer diapause in A. fasciatus may be to avoid untimely hatching or non-diapause development in autumn which would ultimately lead to extinction. In summer, eggs laid near or under green forbs may survive but the moisture content of the soil is normally not high enough for them to absorb water. The eggs of this species absorb water during the embryogenesis (Fig. 14) as in all other crickets studied (Browning 1953; McFarlane et al. 1959; Rakshpal 1962; Masaki 1960). When water absorption was prevented, embryogenesis was interrupted and even those embryos which otherwise had developed rapidly until hatching survived in a state of quiescence for a month at a high temperature (Tables 10 and 11). Therefore, without summer diapause, the embryos become quiescent and their hatching is prevented in summer. Table 14 summarizes the responses to various environmental

conditions of eggs with or without the capability of entering summer diapause. Eggs without this capability become quiescent in response to dry conditions at 30°C (c). They resume non-diapause development and hatch whenever water is given at 30°C (d and f). However, when moistened at 20°C after a short exposure to dry conditions at 30°C, eggs enter winter diapause irrespective of the capability of entering summer diapause (e). When the exposure to dry and warm conditions is prolonged, winter diapause is not induced in any eggs if moistened at 30°C (f). If moistened at 20°C (g), however, eggs in summer diapause would resume development and then enter winter diapause but some of those which have failed to enter summer diapause also do not enter winter diapause. Without summer diapause, eggs may survive a dry summer but would start non-diapause development in autumn when enough water is available for further embryogenesis and would fail to overwinter.

Diapausing insects are generally known to be relatively resistant to environmental desiccation (Andrewartha 1952; Lees 1955; Danilevskii 1965). This possibility was tested in A. fasciatus by exposing eggs at different ages to a low relative humidity (Fig. 14). One or 5-day exposure to a relative humidity of 50 % caused less than 15 % mortality in those which had already reached the winter diapause stage. Similar low rates of mortality were also obtained at the middle of pre-diapause period at which summer diapause would occur if incubated at high temperatures. These results may suggest that the occurrence of diapause at these stages is a result rather than the cause of the relatively high resistance to desiccation. As reported in other species (Thompson and Bodine 1936; Birch and Andrewartha 1942), eggs of A. fasciatus were relatively susceptible to desiccation during the first several days after being laid and during the period of water absorption. However, the eggs shortly after being laid were relatively unsusceptible to a short period (i.e. 1 day) of desiccation (Fig. 14). High rates of mortality occurred only when the period of desiccation was extended for 5 days. Their susceptibility to desiccation decreased as the time of desiccation was delayed. This is probably related to the gradual deposition of serosal cuticle underneath the chorion (Tanaka, unpub-

lished data), as suggested by Laughlin (1957). In the eggs absorbing water, on the other hand, one-day exposure to desiccation resulted in about 40 % mortality which was not markedly increased by an additional exposure of 4 days. This indicates that eggs become susceptible to desiccation only for a short period around the time of water absorption and those before and after this period are relatively resistant to it.

Browning (1965,1967) suggested that water absorption occurs only at a particular stage of embryogenesis, although the stage at which water is absorbed differs from one species to another. Moriarty (1969) observed the pattern of water absorption by eggs of Chorthippus brunnens which varies greatly among individuals,and concluded that the time of water absorption does not have a close relationship with the stage of embryonic development. The embryonic stage at which water absorption occurred in the eggs of A. fasciatus varied not only among individuals but also with the temperature of incubation and the time of oviposition (Table 13), the results being contrary to the statement made by Browning (1965,1967). Nevertheless, water absorption did not happen at random. In eggs laid in summer, it occurred in earlier stages as the incubation temperature increased (within the range between 30 and 20°C)(Table 13). When eggs laid in autumn were examined at 30°C, they showed two groups differring from each other in the stage at which water was absorbed. One group absorbed water at stage II or III as those laid in summer did and the other group at stage V, VI, or VII. The latter eggs were apparently developing without diapause (Tables 10 and 13). It is obvious that the variation in the stage at which water is absorbed has to do with the induction of summer diapause. However, no correlation seems to exist between the time of water absorption and that of the initiation or termination of summer diapause. Eggs at 30 or 27°C absorbed water during the period of summer diapause in a sporadic fashion (Fig. 15). Some remained without water absorption for more than one month.

From the above discussions, it is concluded that summer diapause is important in stabilizing the univoltine life cycle in A. fasciatus which starts emerging as an adult and laying eggs in a dry summer. The

role of summer diapause is probably to prevent the initiation of non-diapause development before winter rather than to increase the resistance of eggs to dry and warm conditions. The eggs in summer diapause can remain under warm conditions without absorbing water which may not be available until summer is over.

Figure 10. Seasonal fluctuations in the average air temperature and precipitation in Corvallis, Oregon.

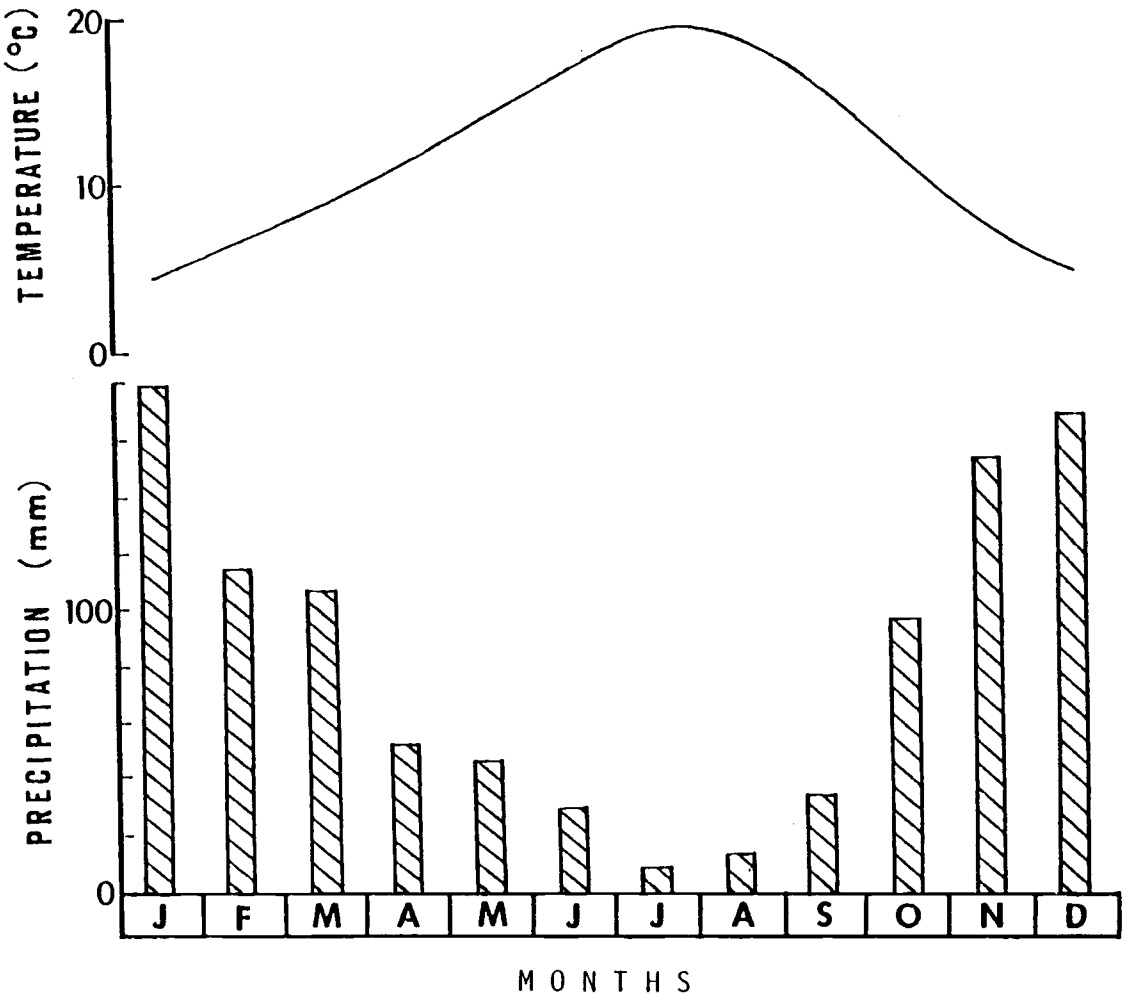
TEMPERATURE (°C)
20
10
0
PRECIPITATION (mm)
100
0
J
F
M
A
M
J
J
A
S
O
N
D
MONTHS

Figure 10

Figure 11. Variation in temperature at the surface (open columns) and at 1 cm depth in the soil (closed columns) and in the moisture content of soil at 1 cm in depth. Vertical lines indicate the standard deviation of the mean of 5 measurements.

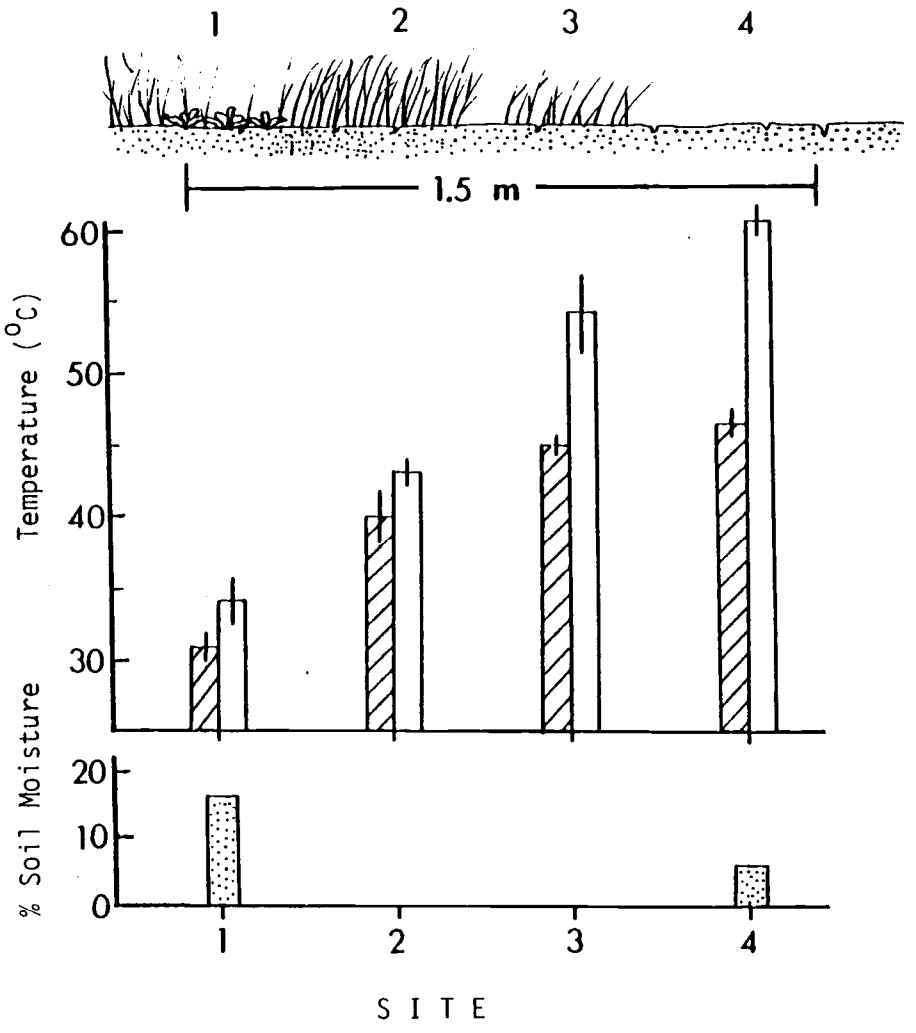
1
2
3
4
1.5 m
60
50
40
30
Temperature (°C)
20
10
0
% Soil Moisture
1
2
3
4
SITE

Figure 11

Figure 12. Seasonal fluctuations in the moisture content of soil from 1 cm depth of soil covered with green forbs (triangles) or exposed (circles) in a habitat of A. fasciatus. The data indicated by open circles were obtained in 1980 and those indicated by closed circles and triangles in 1981.

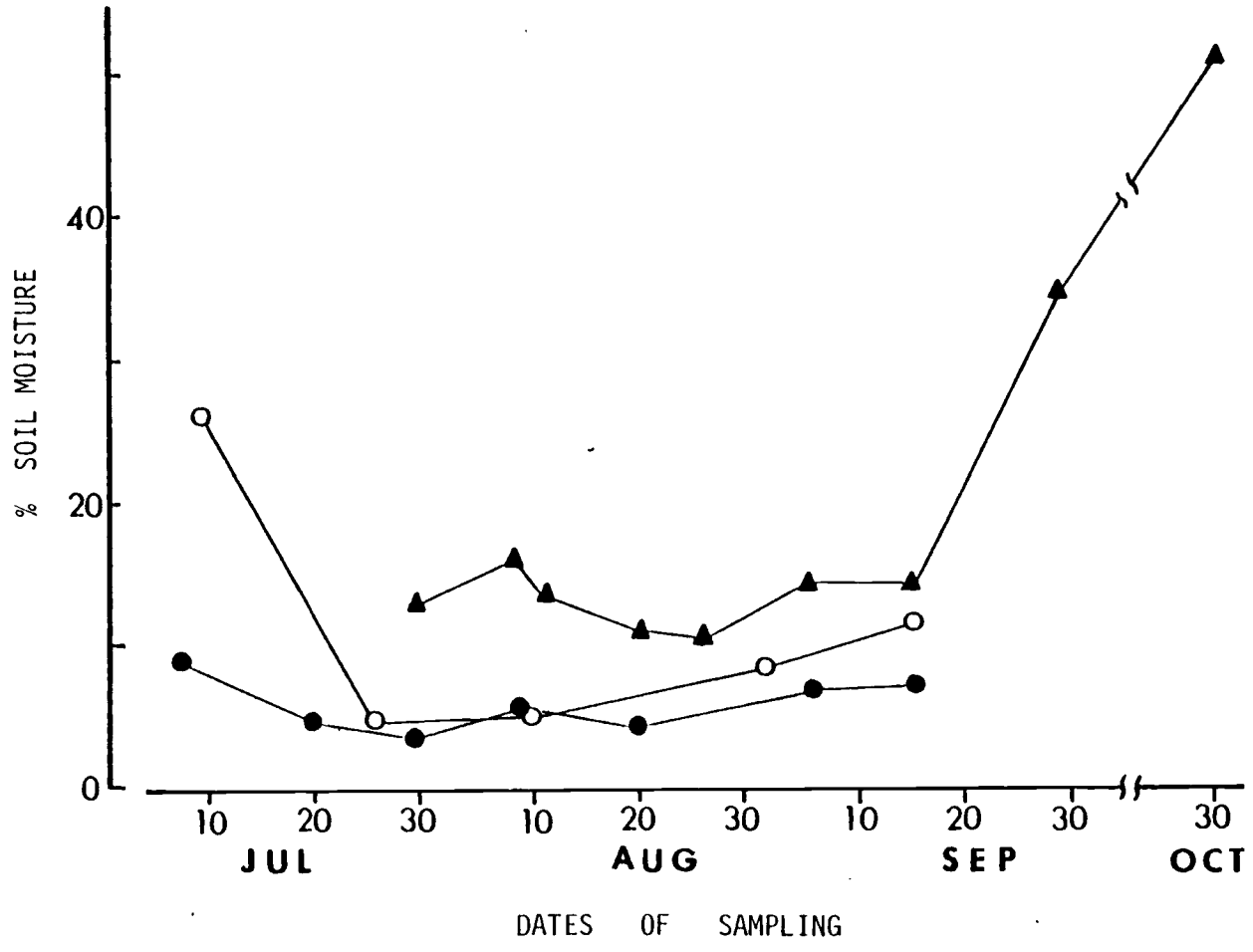
% SOIL MOISTURE
40
20
0
10
20
30
10
20
30
10
20
30
30
JUL
AUG
SEP
OCT
DATES OF SAMPLING

Figure 12

Figure 13a and b. Cumulative percentages of hatch in A. fasciatus eggs which were transferred from dry conditions to wet conditions at 30°C. Numbers in the figure indicate the times of the transfer. For explanation of two groups of eggs indicated by the discontinuities of the lines, see text.

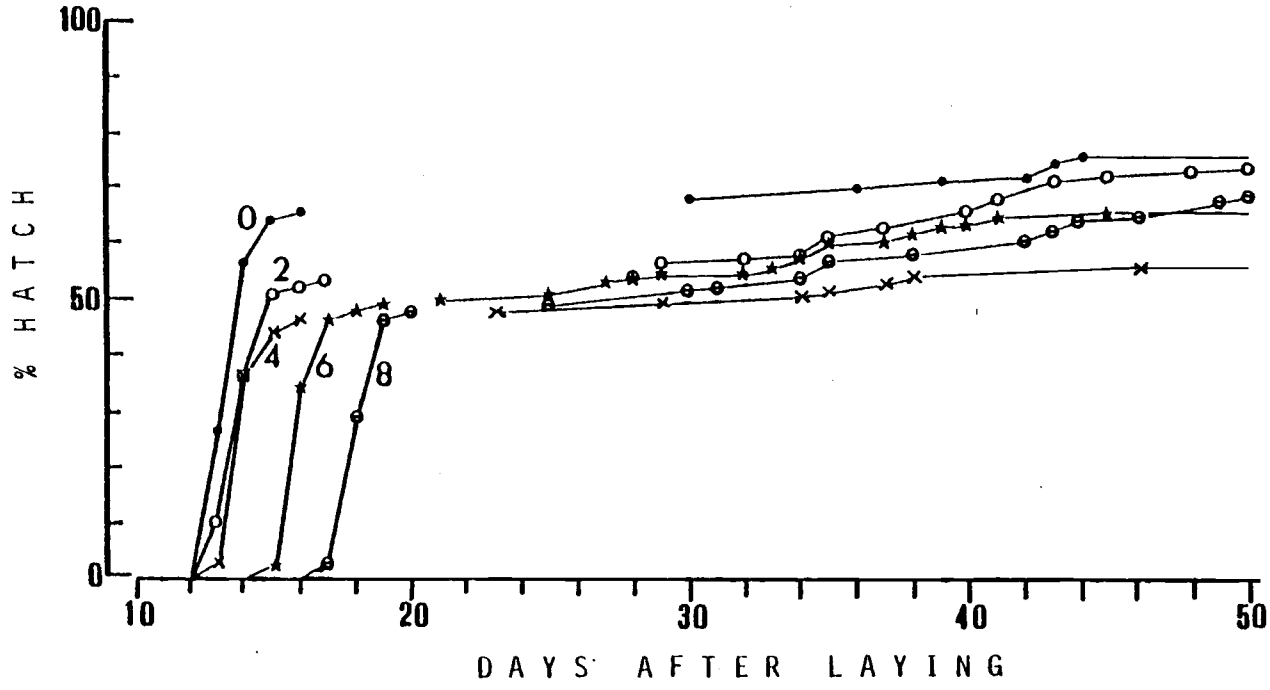

Figure 13a

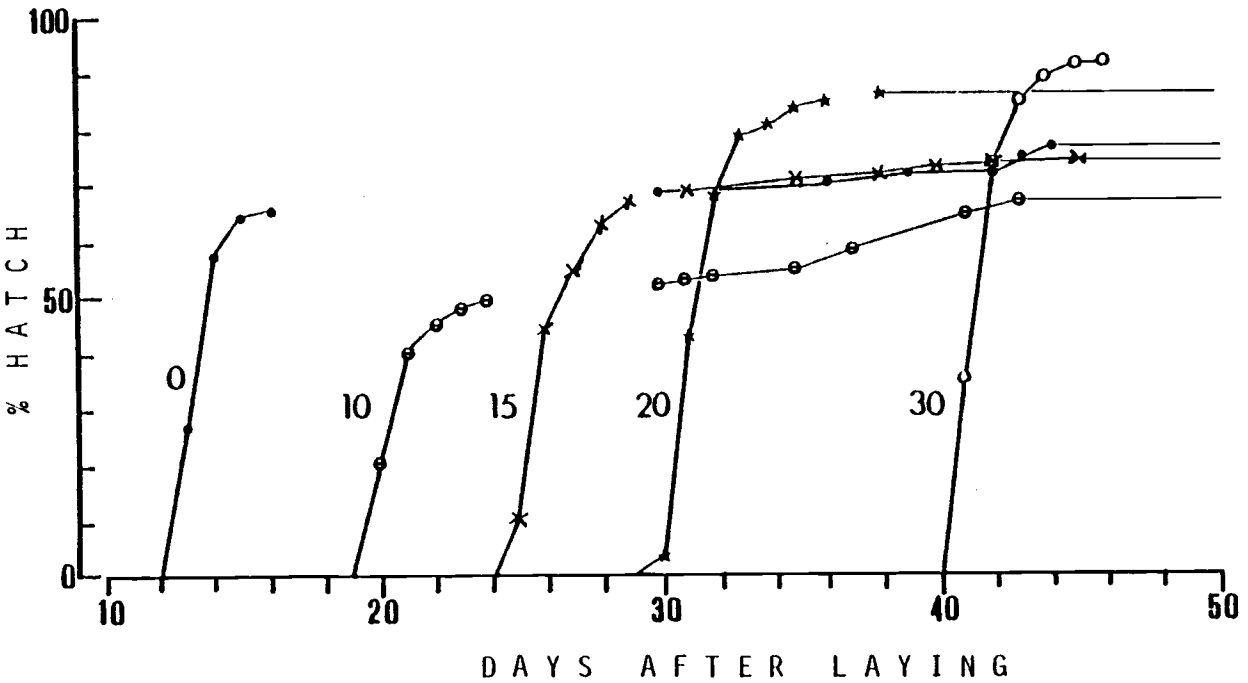

Figure 13b

Figure 14. The effects of exposure to a relative humidity of 50 % at different times during the egg stage on the percent weight loss (b) and mortality (c) in A. fasciatus (20°C). The embryonic stages and changes in egg weight are also shown (a). The data were based on 3 replicates each comprising 30 eggs.

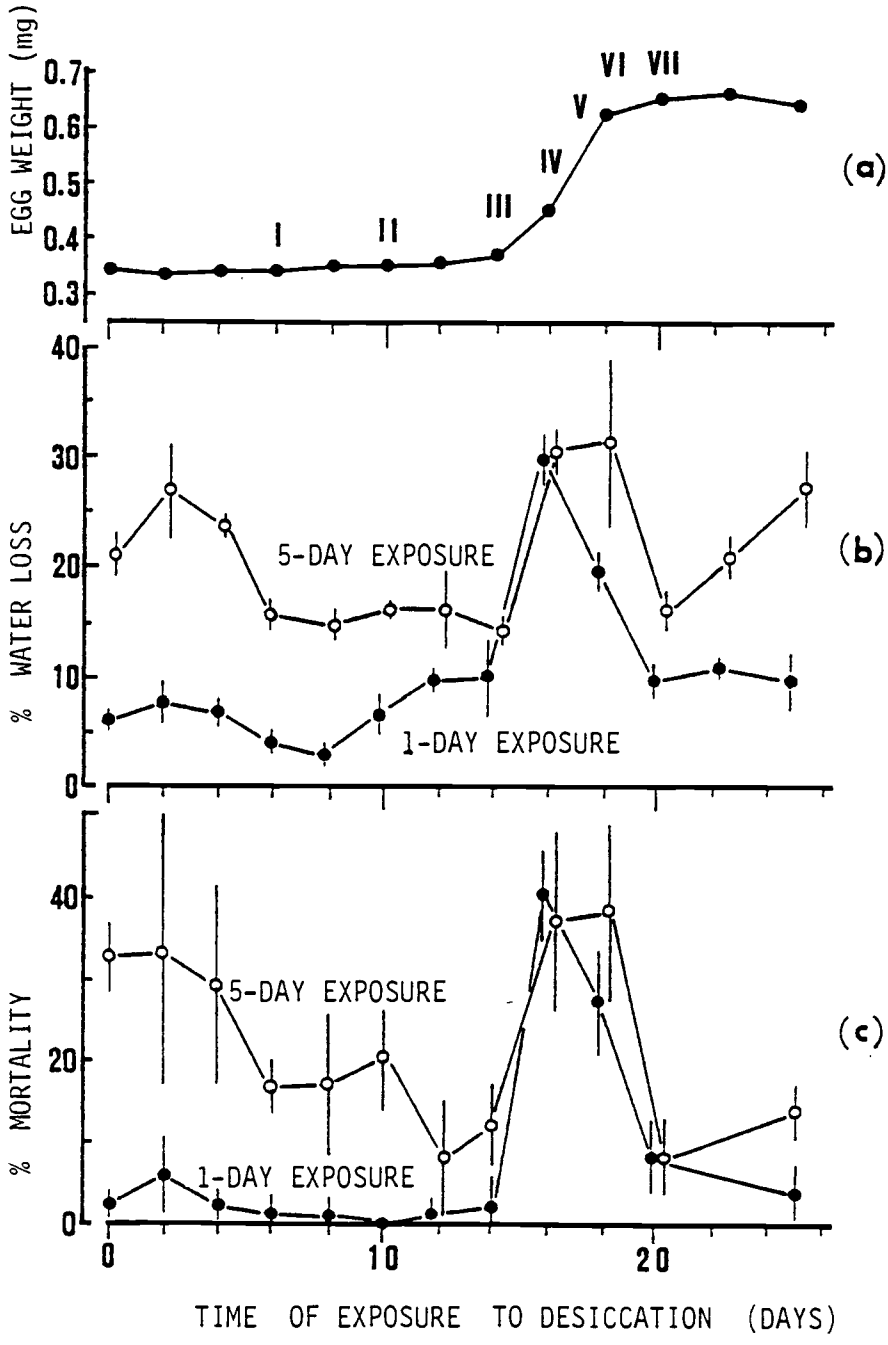
EGG WEIGHT (mg)
0.7
0.6
0.5
0.4
0.3
I
II
III
IV
V
VI VII
(a)
% WATER LOSS
40
30
20
10
0
5-DAY EXPOSURE
1-DAY EXPOSURE
(b)
% MORTALITY
40
20
0
5-DAY EXPOSURE
1-DAY EXPOSURE
(c)
0
10
20
TIME OF EXPOSURE TO DESICCATION (DAYS)

Figure 14

Figure 15. Cumulative percentages of A. fasciatus eggs which absorbed water at different temperatures. The data plotted were connected by solid lines for summer eggs and by interrupted lines for autumn eggs.

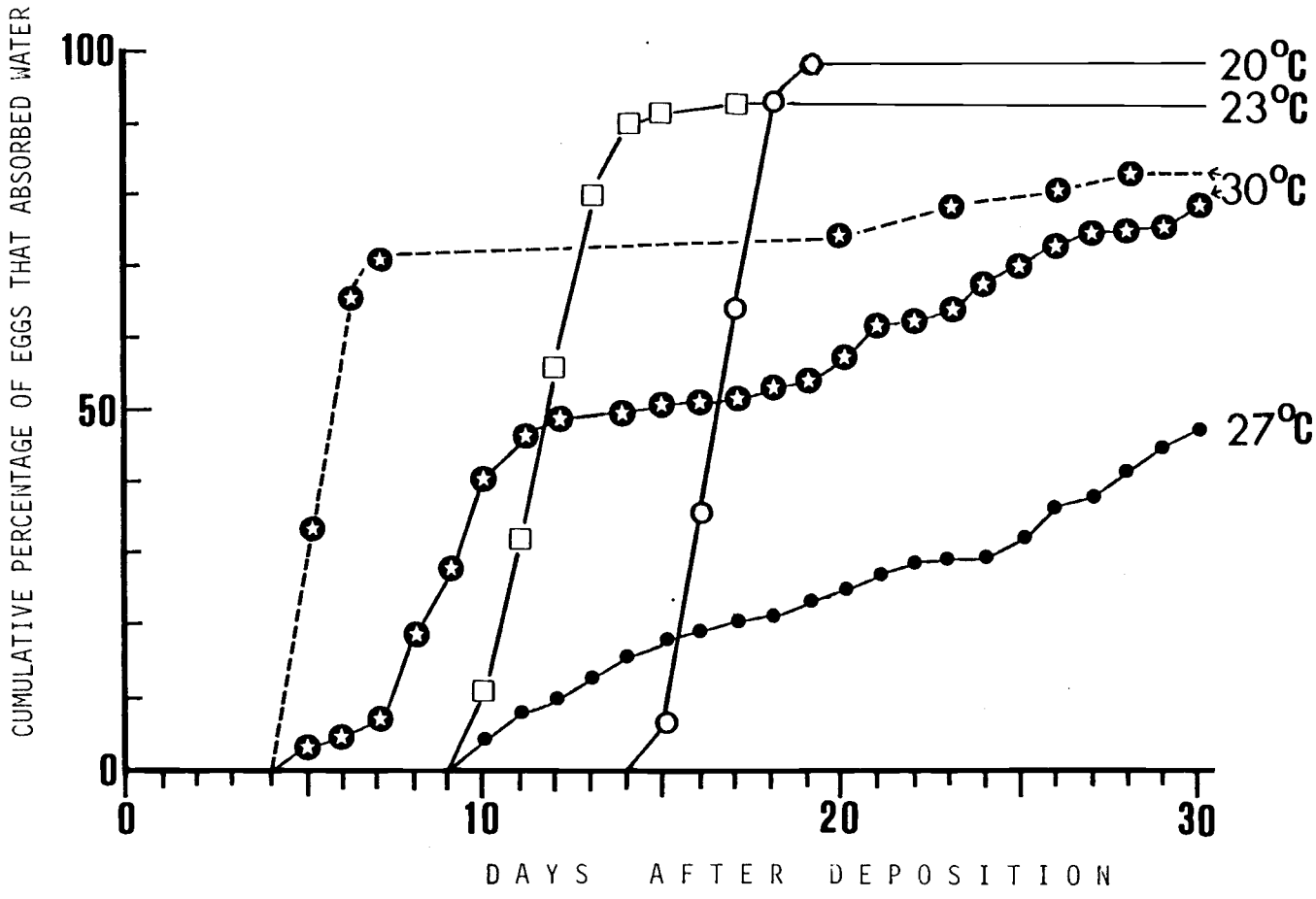
CUMULATIVE PERCENTAGE OF EGGS THAT ABSORBED WATER
100
50
0
0
10
20
30
DAYS AFTER DEPOSITION
20°C
23°C
30°C
27°C

Figure 15

Table 9. The effects of different levels of soil moisture at 30°C on the survival of eggs in A. fasicatus. The survival rates were determined 30 days after the removal of eggs from the soil and incubation on moist paper.

% soil water water	Time of incubattion in the soil	
	8 days	30 days
10.9	100[a](0/25)[b]	100 (2/25)
7.9	100 (0/25)	87 (11/23)
5.9	100 (11/23)	17 (23/23)
3.9	0 (24/24)	0 (25/25)

[a] % of survial.

[b](Number of collapsed eggs/ number of eggs tested).

Table 10. The embryonic development of A. fasicatus after different periods of incubation on moist paper and in soil with about 10 % moisture content at 30°C.

Days after being laid	Embryonic stage I	II	III	IV	V	VI	VII	VIII	No. eggs dissected
Autumn eggs on moist paper									
3		15							15
4		1	3	7					11
4.5		2		7	3	1	2		15
5		2		2	3	2	8		17
6		2	1				7		10
7		3					2	5	10
8		2						9	11
Autumn eggs in soil (10.1 %)									
3		10							10
4		4	4	2					10
5		4	1	4	1				10
6		2	7	3	2				14
8		1	2	2	5				10
10		1	1	3	4	1			10
15		2	4	3	1				10
20		3	1	4	2				10
30	1	2	3	4					10
Summer eggs in soil (10.9 %)									
8	1	9							10
15	2	8							10
30	8	1	1						10

Table 11. The effects of incubation in the soil with 10.1 % moisture content on the embryonic development of A. fasciatus at 30°C. The eggs were kept on moist paper after the removal from the soil.

Time of incubation in soil (days)	% eggs in the first group[a]	t	Days spent under wet conditions	% total mortality
0	65.6 ± 5.1[b]		13.7 ± 0.7[c]	2.2
2	53.3 ± 3.3	3.5	12.2 ± 0.8	0.0
4	46.7 ± 3.3	5.4*[d]	10.2 ± 0.6	0.0
6	48.9 ± 3.3	4.6*	10.3 ± 0.7	2.2
8	50.9 ± 12.4	1.8	10.4 ± 0.6	1.1
10	48.9 ± 10.2	2.5	10.9 ± 1.0	2.2
15	66.7 ± 8.8	0.2	11.4 ± 1.1	0.0
20	84.4 ± 3.8	5.1*	11.8 ± 1.2	2.2
30	91.1 ± 3.8	6.9*	12.0 ± 1.1	7.8

[a]For explanation of the first group, see Fig. 4.

[b]The mean ± S.D. of 3 replicas each consisting of 30 eggs.

[c]The mean ± S.D. for the eggs in the first group.

[d]Asterisks indicate that the % of eggs in the first group is significantly different from that in the control at 5 % probability level.

Table 12. The embryonic development of A. fasciatus at 20°C after incubation at 30°C either in the soil with 10.1 % moisture content (A) or on moist paper (B). All eggs were placed on moist paper at 20°C.

Time of transfer to 20°C (days)	% of embryos which underwent revolution during 40 days at 20°C	
	A	B
0	0.0 ± 0.0[a] (0.0)[b]	0.0 ± 0.0[a] (0.0)[b]
2	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
4	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
5	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
6	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)
7	——— ——	56.0 ± 10.4 (49.7)
8	0.0 ± 0.0 (0.0)	76.7 ± 8.8 (73.3)
10	0.0 ± 0.0 (0.0)	——— ——
15	5.6 ± 1.9 (5.6)	——— ——
20	12.2 ± 10.2 (12.2)	——— ——
30	5.6 ± 1.9 (5.6)	——— ——

[a]The mean ± S.D. of 3 replicates, each consisting of 30 eggs,

[b]The total % hatch in 40 days at 20°C.

Table 13. The embryonic stages of A. fasciatus at the end of water absorption at different temperatures.

Temperature (°C)		Embryonic stage I	II	III	IV	V	VI	VII	VIII	No. of eggs dissected	Age of eggs dissected (days)
30	A[a]		6	1		4	2	2		15	5 - 6
30	S[b]		13							13	7 - 20
27	S		3	11	5					19	14 - 40
24	S		4	16	22					42	11 - 14
22	S				14	4	2			20	12 - 17
20	S				3	6	11			20	17 - 18
15	S				10	3				13	50 - 60

[a]Eggs laid in autumn.

[b]Eggs laid in summer.

Table 14. Response to various moisture and temperature conditions of embryos with (A) or without the capability of entering summer diapause (B) in A. fasciatus. S, summer diapause; W, winter diapause; Q, quiescence; H, hatch.

	Treatment				Response	
	Initial Moisture	°C	Second Moisture	°C	A	B
(a)	wet	30	———	—	S	H
(b)	wet	20	———	—	W	W
(c)	dry	30	———	—	S	Q
(d)	short dry	30	wet	30	S	H
(e)	short dry	30	wet	20	W	W
(f)	long dry	30	wet	30	H	H
(g)	long dry	30	wet	20	W	W or H

V. MECHANISM FOR THE CONTROL OF THE INDUCTION AND INTENSITY OF DIAPAUSE

Introduction

The embryo of Allonemobius fasciatus shows seasonal variation in diapause characteristics (Chapter III). Eggs laid in summer enter diapause with a higher intensity than do those laid in autumn when incubated at 20°C. At a high temperature, some eggs hatch without entering diapause, although this species is strictly univoltine in the northern parts of North America (Fulton 1951; Chapter II). The proportion of such individuals is low in summer eggs and increases toward the end of laying season. Most eggs laid in late autumn hatch without showing any sign of developmental suppression at 30°C. These phenomena apparently suggest the involvement of some maternal factor(s) in determining the response of eggs to temperature. A model was proposed to explain these phenomena by assuming a substance called factor A produced and transmitted by adult females to their eggs (Chapter III). However, how this factor might influence the processes determining the onset and intensity of diapause is not known.

In studies of diapause in the univoltine field cricket, Teleogryllus commodus, Browning (1952) found that eggs hatched promptly without the intervention of any diapause at a high temperature when they had been exposed to a low temperature for a certain period in the pre-diapause stage. A similar phenomenon has been reported in several other species such as Melanoplus mexicanus (Parker 1930), M. bivittatus (Church and Salt 1952), Ephippinger cruciger (Dean and Hartley 1977), Leptophyes punctatissima (Deura and Warne 1982) and two species of Conocephalus (Hartley and Warne 1972). Browning (1952) in explaining this phenomenon in T. commodus proposed a hypothesis that diapause development would be completed during the low-temperature treatment even in the pre-diapause stage so that the eggs can complete embryogenesis without interruption upon transfer to a high temperature. This suggests that the failure to enter diapause after treatment of the pre-diapause eggs results from the same processes that cause the

termination of diapause at a low temperature. This view was also taken by Dean and Hartley (1977) working with E. cruciger. On the other hand, Hogan (1960) suggested the possibility that the physiological mechanisms of prevention and termination of diapause differ from each other even though low temperature is effective in each case. He found that no eggs of T. commodus averted diapause when constantly incubated at a low temperature of 13°C, indicating that a low temperature itself does not eliminate diapause. Furthermore, when the effect of low temperature on pre-diapause and diapause eggs were compared, those in diapause required considerably longer treatment for the prompt hatching upon transfer to a high temperature. However, there has been no clear evidence to suggest whether the porcesses that affect the onset of diapause and those that terminate diapause are the same or not (Hogan 1960).

The purpose of the present chapter is to describe the results of experiments to examine the effects of temperature on the onset and intensity of diapause and discuss the possible mechanisms underlying these phenomena in relation to the hypothetical factor assumed in Chapter III.

Materials and Methods

To examine the effect of temperature on egg hatching, freshly laid eggs were exposed to various temperature regimes in darkness. The temperatures used were kept constant within the range of ± 1°C. Female crickets were reared in the laboratory at 27°C and LD 16:8 hr or collected in the field as previously described. Egg batches laid by females collected in autumn contained some eggs which developed without interruption when held at 27°C while those laid by females collected in summer or by those reared in the laboratory all entered diapause and did not hatch for long periods of time. The former will be called autumn eggs and the latter summer eggs for convenience. Eggs were collected at 27°C and transferred to other temperatures within 24 hours of deposition. Further details of experimental methods will be described in appropriate sections.

Results

Onset of diapause: To test the effects of temperature on the initiation of diapause, freshly laid eggs were incubated at 16, 20, 23, and 30°C for various lengths of time and then transferred to 27°C. Although the rates of development varied depending upon how long the eggs were held at the first temperature, a bimodal pattern of hatching occurred which could be recognized by a probit analysis of the hatching records. Depending upon the initial temperature and the length of incubation, a proportion of the eggs hatched within 3 weeks (identified as "rapid hatching") after transfer to 27° and the reminder hatched sporadically over a period of months.

Figure 16 illustrates the proportion of eggs which developed rapidly among those transferred from 20 to 27°C at different times after being laid. Among eggs laid in autumn, the proportion increased the longer the eggs were held at 20°C and reached a maximum of 62 % when the transfer was made on day 12. The proportion then declined and reached a minimum when the eggs were kept for 25 days at the lower temperature before transfer. Thereafter, a shift in temperature again increased the proportion of rapidly hatched individuals and all eggs hatched shortly after transfer on day 50. Figure 17 shows that the time for development among rapidly developing eggs at 27°C varied depending upon how long they were held at 20°C before the transfer. When kept constantly at 27°C, eggs hatched about 18 days after oviposition. This developmental period decreased the longer the eggs were held at 20°C before transfer and reached a minimum after 20 days. No further delay was found through day 50, although a small change may occur after prolonged exposure to 20°C. These results suggest that embryonic development at 20°C continues for 20 days after oviposition and is suppressed thereafter. This conforms to the morphological observations that embryos took about 20 days at this temperature to reach the end of appendage-formation stage at which diapause was induced (Chapter III).

A different result was obtained when eggs laid by summer females were treated in the same way (Fig. 16). They did not respond to a

shift in temperature by rapid hatching until it occurred on day 40. That is, all individuals entered diapause. The proportions of eggs which rapidly hatched upon transfer to 27°C on day 40 and later were generally lower than those obtained from autumn eggs. This is probably because the former entered diapause with a higher intensity than did the latter.

The results of an experiment in which autumn eggs were transferred from other temperatures to 27°C are given in Figs. 18 and 19. When transferred from 16 to 27°C, the proportion of rapidly hatching eggs increased rapidly as the time of transfer was delayed, and reached a maximum after 30 days. This increase was continuous and did not decline after prolonged incubation at 16°C as it did after incubation for 20 days at 20°C (Fig. 16). As shown in Fig. 19, The time spent at 27°C by rapidly hatching eggs declined as the time of incubation was increased but did not reach a minimum of 12 days until eggs had been kept at 16°C for 50 days. This suggests that at this temperature it took 50 days for the eggs to reach the stage at which they could enter diapause, although they did not do so because they were transferred to the higher temperature (Fig. 18). When transferred from 23 to 27°C, on the other hand, the proportion of eggs that rapidly hatched did not increase during the first 30 days. These results, together with those in Fig. 16, suggest that more eggs hatched rapidly at 27°C as the initial temperature decreased when the transfer was made during the early stages of embryonic life.

As shown in Chapter III, high temperatures reduce the incidence of diapause in autumn eggs. Therefore, if a transfer is made from a high temperature to a low temperature, the proportion of rapidly hatching eggs would be expected to increase the longer the eggs are kept at the higher temperature. This was the case in eggs transferred from 30 to 27°C (Fig. 18). The proportion of rapidly hatching eggs was enhanced when the period of incubation at 30°C increased from 0 to 8 days, although the first 4-day exposure did not significantly increase the proportion. The value obtained from the eggs transferred on day 8 was not significantly different from that obtained when eggs

were constantly kept at 30°C (49 % on the average, not presented in the figure)($p>0.05$), suggesting that their fate had already been determined before the transfer. This is consistent with the morphological observations that embryos completed blastokinesis in 8 days at 30°C if no summer diapause had been induced (Chapter IV).

Intensity of Diapause: To examine if diapause is induced with different levels of intensity at different temperatures, summer eggs were exposed to 27°C for 5 days at different times of the egg stage and otherwise kept at 20°C. The mean time required for 50 % hatch in each treatment is given in Fig. 20. The results show that diapause intensity, measured by the duration of egg stage, was increased by a 5-day exposure to the high temperature. The sensitivity of embryos to this effect increased linearly as they developed and reached the maximum on day 20 when they attained the end of the appendage-formation stage where diapause was induced (Chapter III). This effect was less pronounced by still detected after day 25, suggesting that the high temperature intensified diapause after the diapause stage was reached. When eggs were kept at 20°C for 30 days or more, the mean time for 50 % hatch was shorter than that in the eggs maintained at 20 °C throughout the egg stage. In this case, the exposure to 27°C would have stimulated eggs to terminate diapause.

When constantly maintained at a high temperature, embryos enter a summer diapause before reaching the end of the appendage-formation stage (Chapter III). In order to examine the effect of high temperature during a period of summer diapause on the intensity of winter diapause, summer eggs were first subjected to 27°C for different period periods and then transferred to 20°C. The mean time required for 50 % hatch in 3 egg batches in each treatment is plotted against the time of transfer to 20°C in Fig. 21.

When eggs were exposed to 27°C for the first 4 or 8 days, the mean time for 50 % hatch was slightly longer than that in the eggs constantly held at 20°C, although the difference was not statistically significant (p 0.05). After day 8 when embryos had entered summer diapause (Chapter III), the duration of egg stage tended to increase

as the transfer to 20°C occurred later (slope= 0.81; r^2= 0.66; P<0.05). However, the time spend at 20°C was almost constant and there was no significant correlation between the time spent at 20°C and the time of transfer (r^2= 0.06; P>0.05). This suggests that embryos resumed development upon transfer to 20°C and entered winter diapause but a prolonged exposure to 27°C during summer diapause did not affect the intensity of winter diapause.

Figure 22 shows the results of transfer made in the other direction, i.e. from 20 to 27°C, with summer eggs. The data were derived from the experiment described in Fig. 16. When transferred to 27°C within 12 days after being laid, eggs took a much longer time to hatch than did those transferred later. When the transfer was made on day 16, the time required for 50 % hatch was reduced by about 50 days. It was reduced still further until the 50th day after which it increased. These results suggest that, although the eggs transferred to the high temperature within 12 days after oviposition would have entered summer diapause, those kept at 20°C and transferred on day 16 or later did not but entered winter diapause. This may also be supported by the fact that embryos reach the winter diapause stage after 20 days of incubation at 20°C (Chapter III).

Discussion

The data given in the present chapter suggest that some physiological processes responsible for the determination of diapause characteristics proceed in the egg of *A. fasciatus* and they are sensitive to temperature. When eggs laid in autumn were first exposed to low temperature and then transferred to 27°C at certain times during the pre-diapause period, the proportion of rapidly hatching individuals was higher than that when constantly kept at 27°C (Figs. 16 and 18). Browning (1952) attributed a similar phenomenon in the eggs of *T. commodus* to the completion of diapause development during the low temperature in the pre-diapause stage. However, this hypothesis was criticized by Hogan (1960) who repeated and extended the study on dia-

pause in the same species and found that an exposure to low temperature during the pre-diapause period itself did not have a diapause-eliminating effect. For the same reason, the hypothesis proposed by Browning (1952) can not be applied for the case in A. fasciatus: the eggs of this cricket enter diapause at low temperatures (e.g. 20°C) (Chapters III and IV).

In Chapter III, a model was proposed to explain the seasonal variation in both incidence and intensity of diapause in A. fasciatus by assuming a hypothetical factor A transmitted from adult females to their eggs. This model was based on the close relation between the greater tendency towards the initiation of diapause and the higher intensity of diapause. It assumed that eggs laid in summer receive more of this factor from their mother than do those laid in autumn, and that higher amounts of this factor increase both the tendency to diapause and the intensity of diapause. However, the temperature at which eggs were incubated was also found to influence the intensity of diapause (Fig. 20). For example, when eggs incubated for the first 12 days at 20°C were subjected to 27°C for 5 days and then returned to 20°C, the time required for 50 % hatch was about 145 days, or about 20 days longer than the time required for the eggs constantly held at 20°C. This can hardly be explained by the occurrence of diapause development in the pre-diapause stage at the low temperature (Browning 1952; Lees 1955), but seems to indicate the intensification of diapause by the high temperature as suggested in T. emma (Masaki 1962). The sensitivity of eggs to this diapause-intensifying action of high temperature increased as they developed and reached the maximum on day 20 when they attained the diapause stage (Fig. 20). This was less pronounced after day 25 and disappeared by day 30. The results are quite similar to what has been found in T. emma. That is, the dia pause-intensifying action of high temperature extends to the beginning pf diapause stage, although the two species of crickets enter diapause at morphologically different embryonic stages (Masaki 1962; Chapter III). These results indicate that the assumption of factor A alone does not account fully for the diapause phenomenon in A. fasciatus.

In order to explain the complicated responses to temperature of eggs in this species, a model was constructed as shown in Fig. 23. In this model, factor A is assumed to be converted in eggs to a substance, B, which is required for the production of another substance, C. The induction of summer diapause depends upon factor A and that of winter diapause upon substance C. Fig. 23a illustrates the relationship betwen the amount of factor A and the probability of entering summer diapause. The model assumes that summer diapause can be induced if eggs are exposed to a high temperature within a certain period after being laid (H - S). This is based on the results of the experiment described by Fig. 22 where eggs which were transferred from 20 to 27°C before day 16 required almost the same period of time for 50 % hatch as that required by those kept constantly at 27°C. They retained the responsiveness to a high temperature during the first 12 days or so at 20°C and entered summer diapause upon transfer to 27°C. Those transferred on day 16 or shortly before reaching the winter diapause stage required a much less time to hatch at 27°C. They would have failed to enter summer diapause but entered winter diapause. After eggs are laid, factor A gradually decreases in amount at 20°C (Fig. 23a). Summer eggs do not reach the critical level below which summer diapause is not induced upon transfer to 27°C within the period beteen H and S. On the other hand, eggs laid in autumn receive smaller amounts of factor A from their mother. Therefore, some individuals may not enter summer diapause even if placed at 27°C immediately after being laid. As they develop at 20°C, the amount of factor A declines, which decreases the probability that they enter summer diapause upon transfer to 27°C. This could explain the absence of rapidly hatching eggs laid in summer and the increasing proportion of rapidly hatching eggs laid in autumn when transferred from 20 to 27°C during the first 12 days after being laid (Fig. 16).

The rate of conversion of factor A to substance B is assumed to be higher at lower temperatures (Fig. 23b). This would explain that eggs held at a lower initial temperature showed a greater proportion of eggs which hatched rapidly without entering diapause upon transfer to

27°C during the early stages (Figs. 16 and 18): When transfer was made on day 8, the proportion of rapidly hatching eggs was about 70 % if the initial temperature was 16°C but it was about 25 % and less than 10 % if the initial temperature was 20 and 23°C, respectively. In other words, embryos lost their capability of entering summer diapause at earlier stages as the initial temperature was lower.

Rapid conversion of factor A to substance B at a low temperature could result in a high production of substance C and induce winter diapause with a high intensity. However, the intensity of winter diapause was increased when a brief exposure to a high temperature was given to eggs otherwise kept at 20°C (Fig. 20). This can be explained if the production of substance C is also temperature dependent but greater at higher temperatures as shown in Fig. 23 b. When eggs entered summer diapause, however, there was little additional effect of prolonged exposure to high temperature on the intensity of winter diapause (Fig. 21). This indicates that little production of substance C takes place during the period of summer diapause even at a high temperature.

The induction of winter diapause involves an all-or-none response. Because winter diapause which would otherwise have been induced at 20°C was prevented if the eggs were transferred to a higher temperature during the pre-diapause stage (Fig. 16), the critical level of substance C for the induction of winter diapause would be higher at a higher temperature. The stage in which the induction of winter diapause is determined is not known. However, the rapidly hatching eggs transferred from 20 to 27°C markedly decreased the time to hatch at 27°C when the transfer was made within 12 days after oviposition but they did so only at a lower rate when transferred on day 16 (Fig. 17). This suggests that developmental rate is gradually reduced as embryos approach the diapause stage which is normally reached by day 20 at 20 °C (Chapter III). Therefore, the responsiveness to temperature for the induction of winter diapause appears at least several days before the diapause stage. Then, it seems to be retained at least until the beginning of the diapause stage. This is based on the fact that

autumn eggs which would otherwise have developed without diapause at 30°C entered winter diapause when transferred to 20°C at the beginning of diapause stage or before this stage (Chapter IV). Therefore, it is assumed that the induction of winter diapause depends on the amount of substance C assumulated and the temperature experienced during this critical period which starts shortly before the diapause stage and ends at the beginning of diapause stage.

Figures 23c, d, and e illustrate a model based on the above assumptions to explain the possible mechanism for the determination of the induction and intensity of winter diapause. The amount of substance B increases linearly with time at 20°C until factor A is depleted (i in Fig. 23c). When eggs were transferred to 27°C, the rate of conversion from factor A to substance B is reduced because it is inversely related to temperature (Fig. 23b). The production of substance C depends upon temperature (Fig. 23b) and the amount of substance B. At a given temperature, e.g. 20°C, it increases in amount as more of substance B is produced (i in Fig. 22d). When transferred to 27°C, the amount of substance B does not increase significantly over a short period. Therefore, the amount of substance C increases almost linearly with time after transfer to 27°C and the rate of increase depends upon the amount of substance B which is more available as the time of transfer is delayed. The higher temperature also accelerates embryonic development so that the amount of substance C attained by the beginning of diapause stage is greater as the time of transfer is delayed. This might explain the positive correlation between the intensity of winter diapause and the time of exposure to 27°C (Fig. 20). That is, the amount of substance C produced during the 5-day exposure to 27°C is greater as the exposure occurs later. It seems also likely that the availability of the precursor(s) of substance C may be one of the limiting factors responsible for the different effects of the high temperature at different embryonic stages on the intensity of winter diapause.

Figure 23e shows the increases in the amount of substance C plotted against the embryonic stages instead of the absolute time.

As assumed above, the induction of winter diapause is determined by the amount of substance C and the temperature experienced during the critical period (R - D in Fig 23e). The former is greater as the transfer to the high temperature occurs earlier during this period (ii and iii in Fig. 23e). However, an earlier transfer to the high temperature would expose eggs to a higher average temperature. Therefore, as the transfer is delayed, the amount of substance C required for the induction of winter diapause would become smaller (p. 78), increasing the probability of eggs to enter winter diapause. This might explain the decreasing proportion of rapidly hatching eggs obtained when transferred from 20 to 27°C after day 12 (Fig. 16).

The transmission of an inhibitory substance from female adults to eggs was first suggested by Watanabe (1924) to explain the diapause in Bombys mori. Muroga (1951) calculated the consumption coefficient of such an inhibitory substance at different temperatures and found that a temperature of 7.5°C was the most effective in breaking down this substance. His theory was based on the assumption that the inhibitory substance transmitted from adult females decreased in amount in eggs under low temperatures. However, his data also indicate that the effect of chilling varied with the age of embryos, i.e the proportion of eggs that hatched upon transfer from a chilling temperature to a high temperature decreased as the pre-chilling period at 25°C was prolonged (Muroga 1951). This Lees (1955) referred to the intensification of diapause by the pre-chilling temperature of 25°C. If the termination of diapause results from the disappearance of some growth inhibitor from the egg, the intensification of diapause could mean the production of this substance in the egg. If this is the case, it seems difficult to imagine that the inhibitory substance transmitted from adult females is the same substance as the one determining the intensity of diapause as in the case of A. fasciatus. As Takami (1958) described, it is not unreasonable to suppose that the inhibitory substance postulated by Watanabe (1924) has some connection with the diapause hormone secreted by the suboesophageal ganglion of the adult females (Hasegawa 1951,1957; Fukuda 1951; Isobe et al. 1973; Kubota

et al. 1976; Yamashita et al. 1981). Although the diapause hormone can be found both in the ovaries of adult females and in laid eggs (Kai and Kawai 1981), how this hormone might be involved in the control of the actual onset and intensity of diapause is not known.

Ando (1978) has explained the diapause phenomenon in the egg of Atrachya menetriesi by assuming a diapause factor determining both induction and intensity of diapause. According to his model, the diapause factor begins to be produced from another substance, the precursor, transmitted from adult females, by a certain metabolic system which is established a few days after being laid (at 25°C). A low temperature treatment (7.5°C) during the pre-diapause stage destroys not only the diapause factor produced but also the metabolic system producing it so that diapause is not induced upon transfer to a higher temperature (25°C). This model does not account for the prevention of diapause in A. fasciatus as a result of transfer from 20 to 27°C because, as mentioned, diapause was not prevented by a constant exposure to the low temperature. However, the present results favor his idea that the induction and intensity of (winter) diapause are determined by a common factor which is different from the substance(s) which might be transmitted from adult females.

Figure 16. The effect of temperature shift from 20 to 27°C on the proportion of rapidly hatching eggs of A. fasciatus laid in summer (small dots) or in autumn (large dots). Each dot is based on 25 eggs in summer eggs or 50 in autumn eggs.

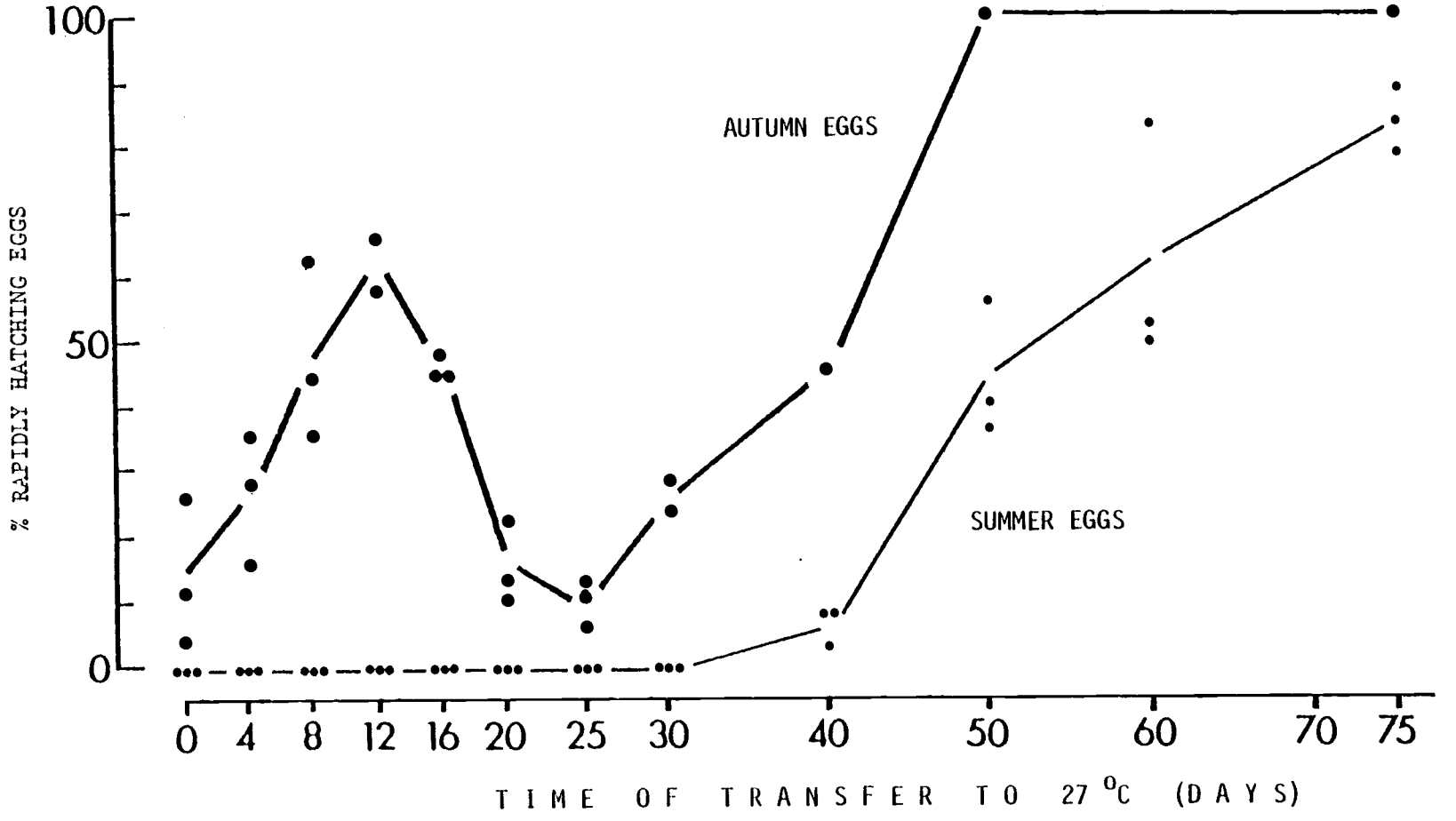
100
50
0
% RAPIDLY HATCHING EGGS
AUTUMN EGGS
SUMMER EGGS
0 4 8 12 16 20 25 30 40 50 60 70 75
TIME OF TRANSFER TO 27 °C (DAYS)

Figure 16

Figure 17. The time apent at 27°C to hatch by the eggs of A. fasciatus which hatched rapidly upon transfer from 20 to 27°C. Open circles indicate the means for summer eggs and closed ones for autumn eggs. Vertical lines indicate the standard deviation.

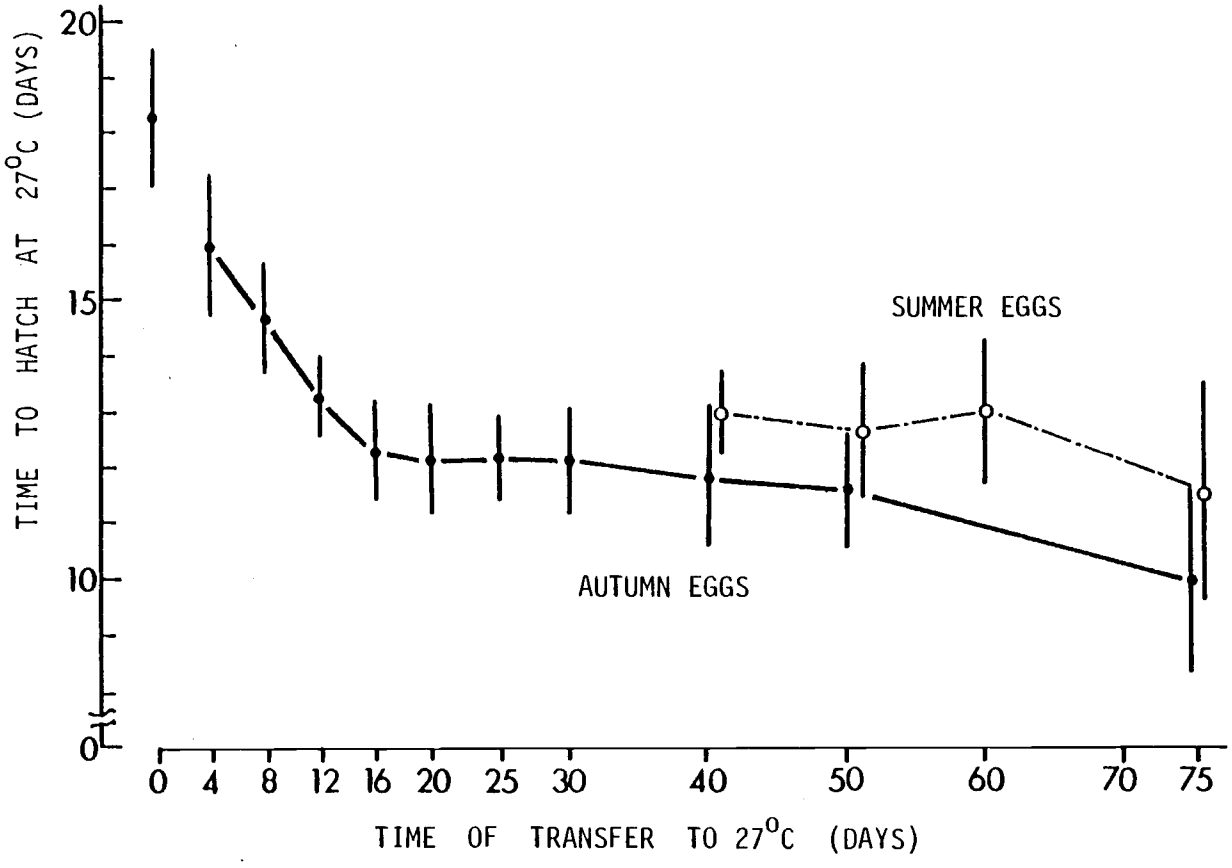
TIME TO HATCH AT 27°C (DAYS)
20
15
10
0
SUMMER EGGS
AUTUMN EGGS
0 4 8 12 16 20 25 30 40 50 60 70 75
TIME OF TRANSFER TO 27°C (DAYS)

Figure 17

Figure 18. The effect of temperature shift from 16, 23, or 30 to 27°C on the proportion of rapidly hatching eggs of A. fasciatus laid in autumn. Each dot or circle is based on 50 eggs.

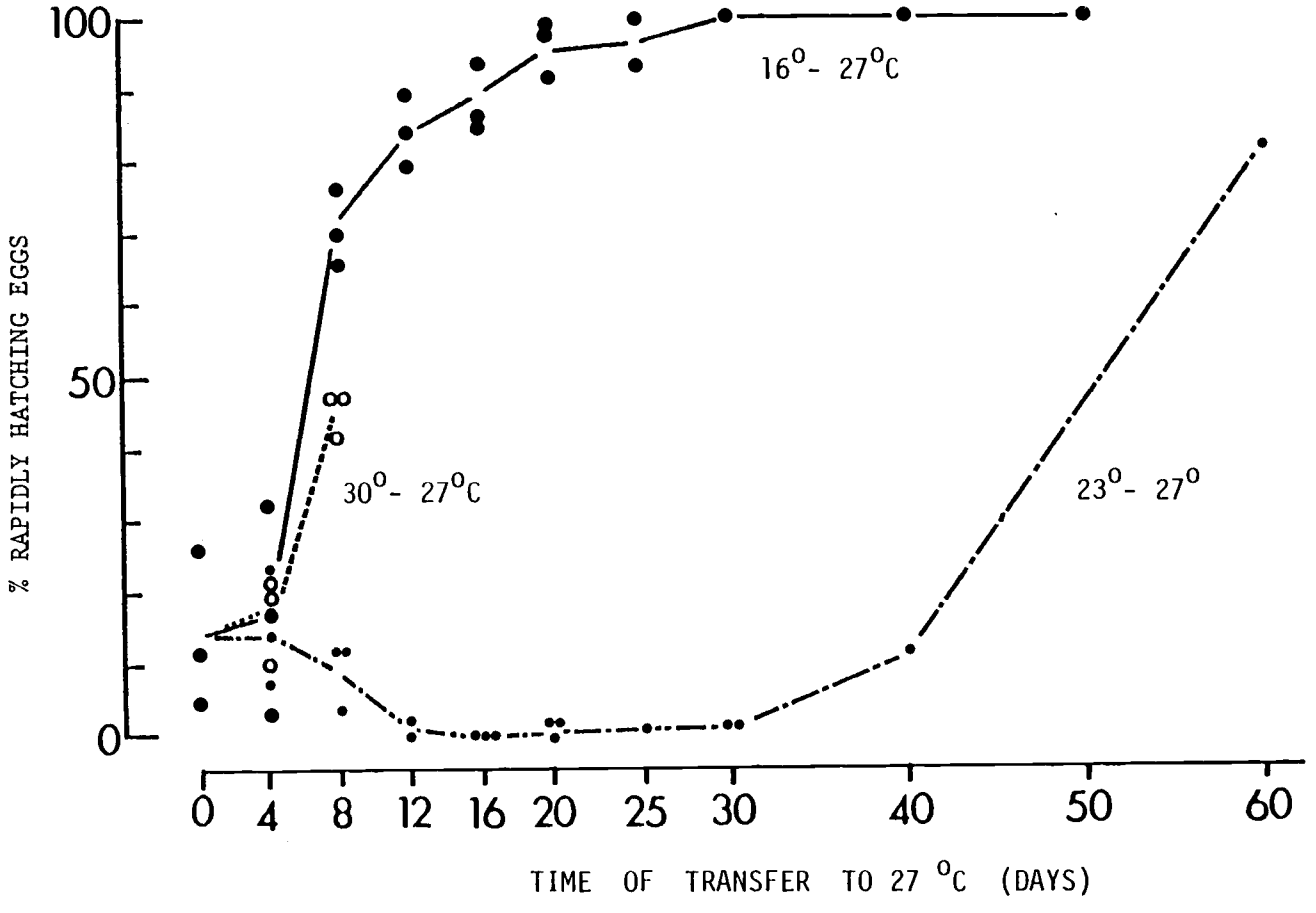
100
50
0
% RAPIDLY HATCHING EGGS
16°- 27°C
30°- 27°C
23°- 27°
0 4 8 12 16 20 25 30 40 50 60
TIME OF TRANSFER TO 27 °C (DAYS)

Figure 18

Figure 19. The time spend at 27°C to hatch by the eggs of A. fasciatus which hatched rapidly upon transfer from 16, 23, or 30 to 27°C. Vertical lines indicate the standard deviation. The dots without vertical lines are based on less than 3 eggs.

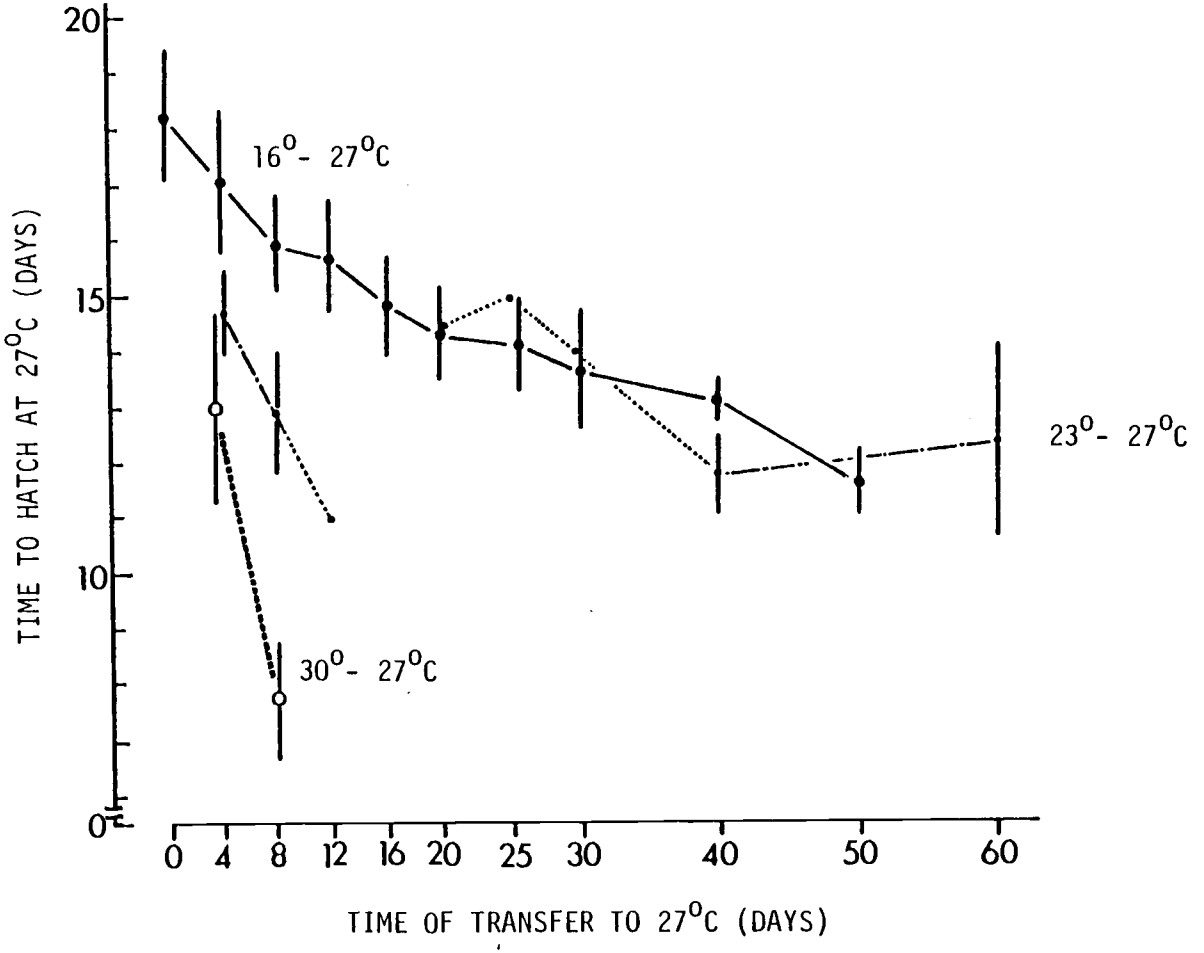
20
15
10
0
TIME TO HATCH AT 27°C (DAYS)
16°- 27°C
23°- 27°C
30°- 27°C
0 4 8 12 16 20 25 30 40 50 60
TIME OF TRANSFER TO 27°C (DAYS)

Figure 19

Figure 20. The effect of a 5-day exposure to 27°C on the time required for 50 % hatch in summer eggs of A. fasciatus otherwise kept at 20 °C. Each dot is based on 25 eggs.

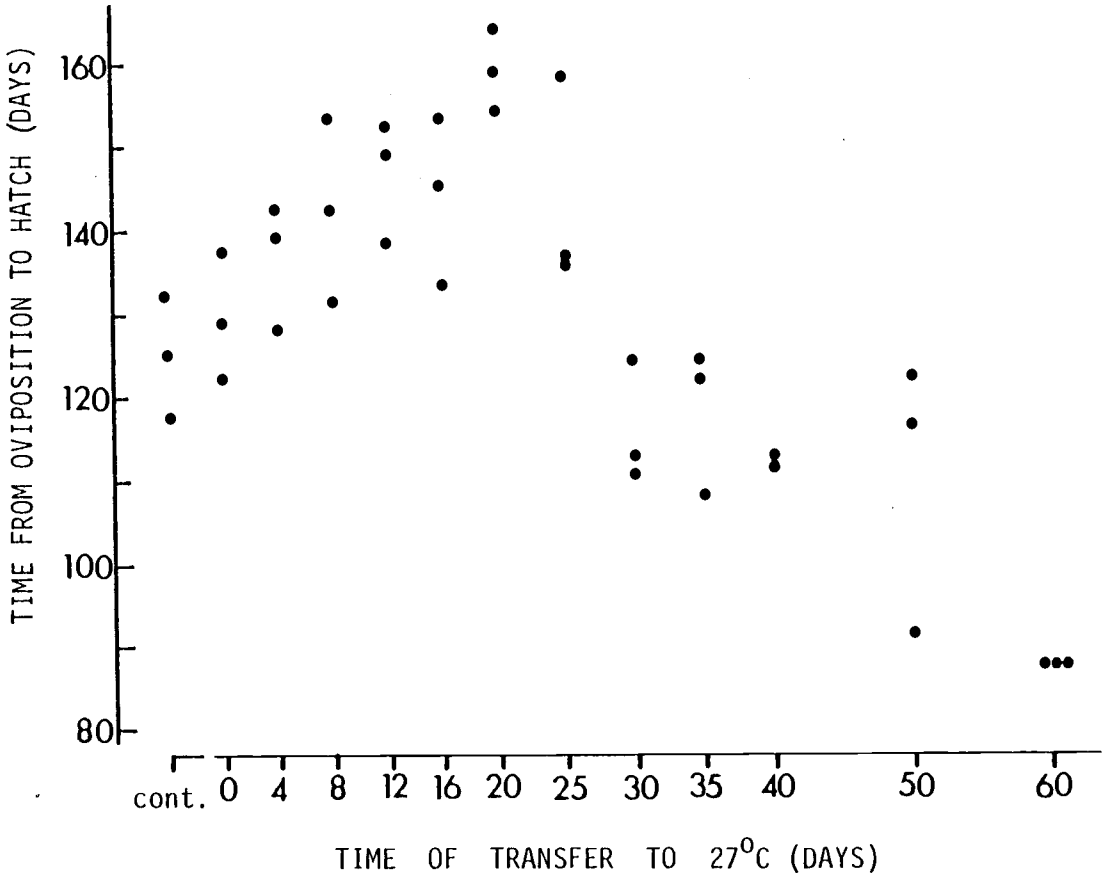
TIME FROM OVIPOSITION TO HATCH (DAYS)
160
140
120
100
80
cont. 0 4 8 12 16 20 25 30 35 40 50 60
TIME OF TRANSFER TO 27°C (DAYS)

Figure 20

Figure 21. The effect of temperature shift from 27 to 20°C on the time required for 50 % hatch in summer eggs of A. fasciatus. Each dot indicates the mean of 3 replicates, each consisting of 25 eggs. Vertical lines indicate the standard deviation.

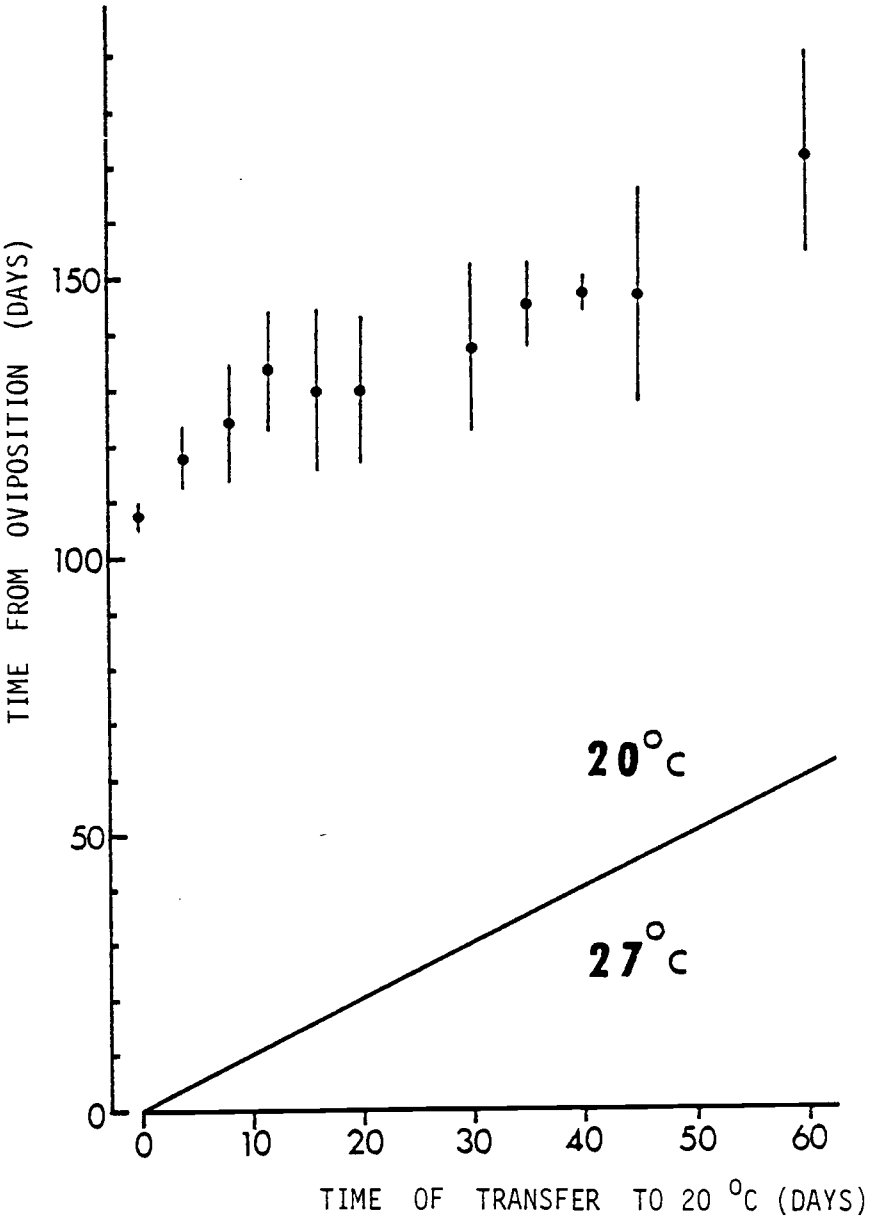
TIME FROM OVIPOSITION (DAYS)
150
100
50
0
20°C
27°C
0
10
20
30
40
50
60
TIME OF TRANSFER TO 20 °C (DAYS)

Figure 21

Figure 22. The effect of temperature shift from 20 to 27°C on the time required for 50 % hatch in summer eggs of A. fasciatus. Each dot indicates the mean of 3 replicates, each consisting of 25 eggs. Vertical lines indicates indicate the standard deviation.

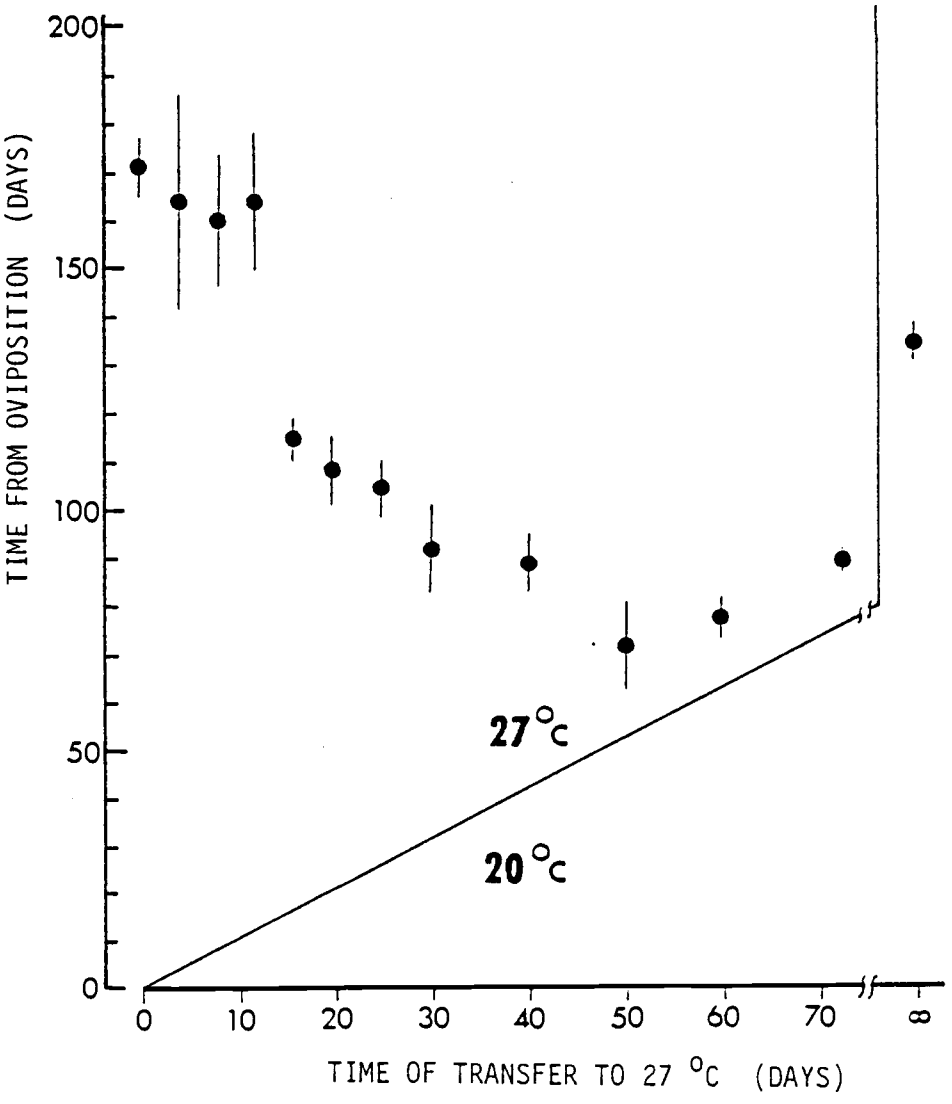
200
150
100
50
0
TIME FROM OVIPOSITION (DAYS)
27 °C
20 °C
0
10
20
30
40
50
60
70
∞
TIME OF TRANSFER TO 27 °C (DAYS)

Figure 22

Figure 23. A model of the mechanism determining the induction and intensity of diapause in A. fasciatus. (a): the possible mechanism for the control of induction of summer diapause. Summer diapause is determined during the period from H to S. Changes in amount of factor A in summer eggs and autumn eggs are expressed by broken lines and solid lines, respectively. (b): Relative rates of conversion of factor A to substance B (B) and production of substance C (C) at different temperatures. (c - e): The possible mechanism for the control of induction and intensity of winter diapause; Changes in amount of substances B and C are given in (c) and (d) when the egg has been kept at 20°C (i) or when transferred from 20 to 27°C (ii and iii). Arrows indicate the time of transfer. The relationship between the amount of substance C and the critical stage (R - D) is expressed in (e) by plotting the changes in amount of substance C against the embryonic stage. For further explanation, see text.

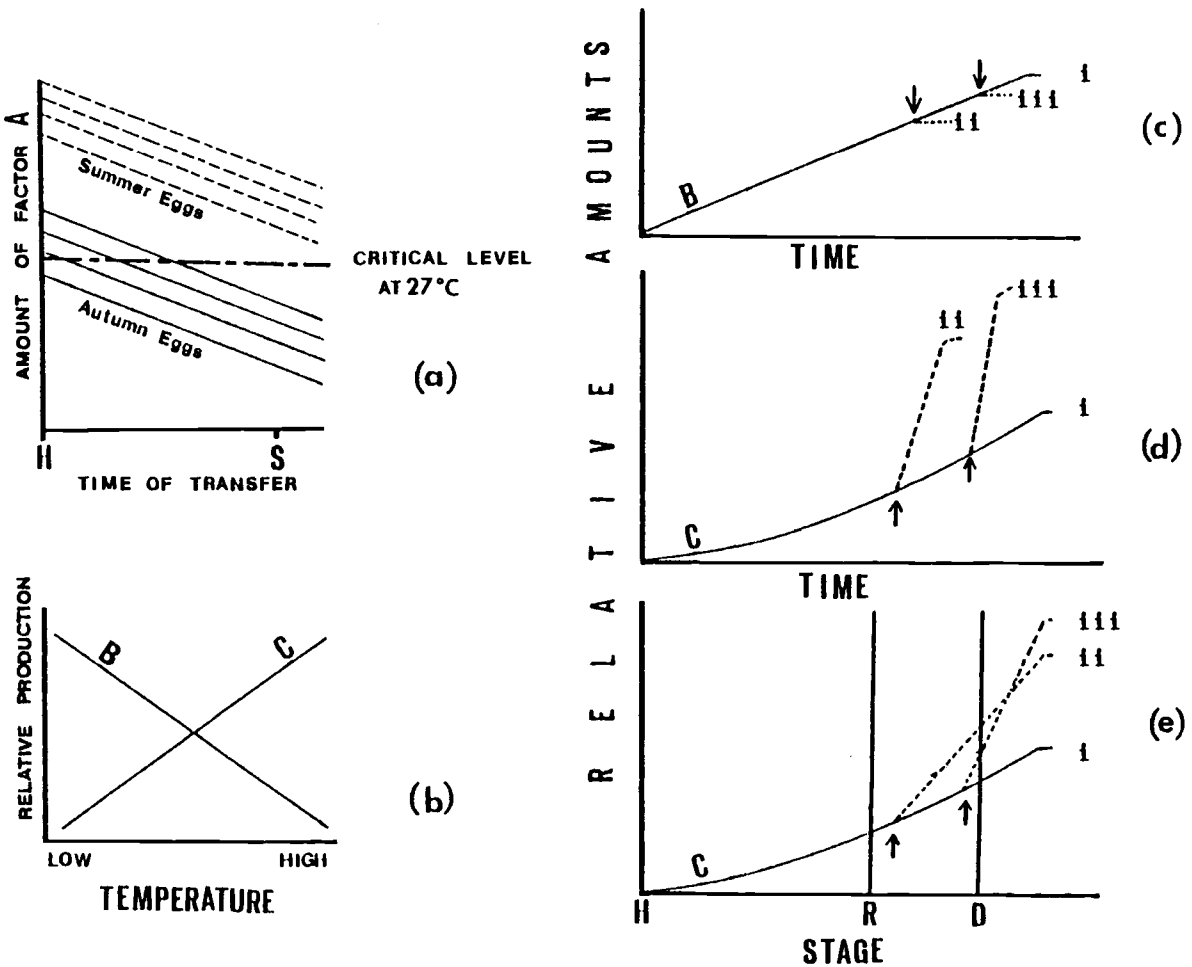
AMOUNT OF FACTOR A
Summer Eggs
Autumn Eggs
CRITICAL LEVEL AT 27°C
(a)
H
S
TIME OF TRANSFER
RELATIVE PRODUCTION
B
C
LOW
HIGH
TEMPERATURE
(b)
RELATIVE AMOUNTS
B
TIME
(c)
C
TIME
(d)
H
R
D
STAGE
(e)
i
ii
iii

Figure 23

VI. SUMMARY AND CONCLUSIONS

Allonemobius fasciatus maintains a univoltine life cycle with an embryonic diapause over an altitudinal gradient of as large as 1,000 m. A more than 1.5 month difference exists in hatching time between the lowest and highest altitudes studied. Little or no difference was detected among populations from different altitudes when developmental traits such as diapause intensity, postdiapause development, and nymphal development were compared in the laboratory. The phenotypic plasticity as found in the photoperiodic control of nymphal development seems to play an important role in compensating for the shorter growing season at higher elevations, making it possible for this species to occur over the relatively large gradient of altitude. Photoperiod changed the duration of the nymphal stage by influencing the duration of each instar and the number of instars.

The embryo of A. fasciatus showed seasonal variation in diapause characteristics when eggs laid at different times of the season were placed on moist paper and incubated at constant temperatures. The intensity of diapause at 20°C was greater in eggs laid early in the season than in those laid later. At 20°C, all embryos entered diapause at the end of the appendage-formation stage. At higher temperatures, on the other hand, embryonic development was suppressed before that stage. This early developmental suppression was called "summer diapause" to distinguish it from diapause mentioned above. The latter was called "winter diapause". The induction of summer diapause depended not only upon the incubation temperature but also upon the time of ociposition. The capability of entering summer diapause at a high temperature was decreased as the time of oviposition was delayed. Embryos which failed to enter summer diapause hatched in a few weeks at a high temperature while those which had been in summer diapause at a high temperature resumed embryogensis and enterd winter diapause upon transfer to 20°C.

The life cycle strategy of an insect can not be described based on the data obtained in the laboratory. Information about environ-

mental conditions in its micro-hatitat is essential. A large variation was observed in soil moisture and temperature among different micro-environments in a habitat of A. fasciatus. Although little rain falls in summer, the soil which is covered with green forbs was found to be kept relatively moist and cool. It was moist enough for eggs to survive for at least a month at 30°C but too dry for them to absorb water required to complete embryogenesis. Eggs laid in summer would enter summer diapause in response to high temperatures and survive until autumn without absorbing water. When eggs laid in autumn were deposited in such dry soil and kept at 30°C, they failed to enter summer diapause but survived at least for a month in a state of quiescence. After a certain period of desiccation in the soil, some of them hatched without entering winter diapause when moistened at 20°C. Therefore, the ecological significance of summer diapause in this species is probably not to ensure the survival of eggs during the dry, warm season but to avoid untimely hatching or non-diapause development before winter. Eggs laid in autumn may encounter warm weather. However, their hatching before winter would be prevented by the decreased but still high intensity of winter diapause. In other words, the two types of diapause provide important mechanisms for this species to stabilize the univoltine life cycle. The present study provided the first example of summer diapause in the family, Gryllidae.

A shift in temperature was effective in preventing eggs from entering diapause. This effect depends upon the time of oviposition, the level of incubation temperature, and the time of shift in temperature. Diapause intensity was influenced by a brief exposure to high temperature and the time of this exposure during the egg stage. These results suggest that some physiological processes responsible for the determination of diapause characteristics proceed in the laid egg and they are sensitive to temperature.

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