Oecologia (Berl.) 17, 1—10 (1974)

Diapause in a Migrant Insect, the Milkweed Bug *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae)

Hugh Dingle

Department of Zoology, University of Iowa, Iowa City, Iowa 52242

Received May 6, 1974

Summary. Oncopeltus fasciatus exhibits a facultative reproductive diapause which is triggered by short photoperiods. The duration of the triggering photoperiod (the "critical photoperiod") is a function of the environmental conditions under which populations are reared. Some individuals are apparently sensitive to critical photoperiods in the early instars, but maximum sensitivity, indicated by 100% diapause, occurs in the late 5th instar during development of the pharate adult. Sixty per cent retain sensitivity into the first five days of adulthood. Temperatures of 27°C can prevent diapause. Once initiated, diapause can be broken either by raising the temperature or increasing the day length; it is broken abruptly, for once oviposition starts, it proceeds at maximum rate. Diapause contributes significantly to a migratory strategy in the temperate parts of the range of *O. fasciatus* and probably does so in the tropics as well.

Many insects respond to changes in daylength by entering a period of suspended development or delayed reproduction known as diapause. In some species there is little or no diapause in the population on long days while on short days most or all individuals enter diapause; in other species the reverse is true. The transition photoperiod is frequently abrupt with a specific day length, the "critical day length" or "critical photoperiod", causing more than half of the population to diapause. The exact duration of the critical photoperiod and the proportion of the population entering diapause frequently vary with temperature and over the geographic range of the species (Danilevsky, 1965; Danilevsky *et al.*, 1970; Beck, 1968; Tauber and Tauber, 1972). Some insects are sensitive to changes of as little as 15 minutes in the duration of the light period (Lees, 1955).

The large milkweed bug, *Oncopeltus fasciatus*, responds to photoperiods by altering age at first reproduction (Dingle, 1968a, 1972). This insect is a wide ranging New World species occurring from Canada to Argentina (Slater, 1964). At least in the northern parts of its range it is a long distance migrant overwintering in the southern United States, the West Indies, and Mexico and invading temperate zones in spring and summer. In the fall the northern populations leave before severe frost. Short days and low temperatures delay reproduction (*i.e.* induce diapause) thus providing both sufficient time to migrate elsewhere (Dingle, 1966) and added stimulus to migration (R. L. Caldwell, unpublished); oviposition reduces the tendency of this species to make long distance flights (Dingle, 1972, 1974; Caldwell, 1974; Rankin, 1974).

Diapause in *O. fasciatus* is therefore of interest because it is a significant attribute of an overall migration and life history strategy. It is also highly labile both environmentally, as will be discussed here, and apparently genetically. Selection experiments indicate that diapause in *O. fasciatus* is highly responsive to selection, *i.e.* as a trait it has a high proportion of additive genetic variance, with, therefore, important consequences for the evolution of life history strategies (Dingle, 1974). This paper considers environmental influences in diapause in *O. fasciatus* and their relation to the migration and life history of the species. The genetic characteristics will be discussed elsewhere in a later paper (Dingle and Hegmann, in prep.; a preliminary discussion appears in Dingle, 1974).

Methods

The insects were kept in controlled environment chambers with regulated photoperiod, temperature, and humidity. Photoperiod and temperature were varied according to the requirements for the experiment in progress while humidity was maintained between 30 and 60 per cent R. H. Within this range the bugs are unaffected by humidity fluctuations.

The measure of diapause used was the presence or absence of oviposition by the females since this can be readily monitored. Diapause in males can be determined by their readiness to mate, but this is considerably less easy to monitor and is more subject to experimental error. Hence only data concerning oviposition were used in this study. All females used were kept with males as bisexual pairs in covered plastic petri dishes with holes punched to allow air circulation. They were supplied with a constant amount of milkweed seed as food, a cotton wick for drinking, and a small amount of dry cotton for oviposition. Life history statistics such as age at first oviposition (the indicator of diapause), dates oviposition occurred during the life span, number of eggs per clutch, age at death, and so forth were recorded for each female. Stocks from which pairs were drawn were maintained at appropriate temperature and photoperiod and were cleaned and supplied with fresh cotton, water, and seed at regular intervals.

Details of method for each experiment are given in the appropriate sections below.

Results

1. The Diapause Response Curve

Oncopeltus fasciatus is typical of many temperate zone insects in that it enters diapause as days shorten with impending autumn. This is illustrated in Fig. 1 which shows the diapause response curves of populations reared under four different photoperiod and temperature conditions. To determine the curve for a population reared under a given regimen, females from stocks in that regimen were transferred as eggs to a different photoperiod cycle and the age at first oviposition of the resultant adults (reared as pairs) under the experimental conditions was determined. For example, the data in Fig. 1 show that females from stocks kept on a daily schedule of 16 hrs of light and 8 hrs of dark (LD 16:8) at a constant 23° C have a mean age at first oviposition of 14.4 days when maintained under the same LD 16:8 conditions; but in two replicates transferred as eggs to LD 12:12 at 23° C, the mean ages at first oviposition were 64.3 and 69.1 days, indicating diapause. In bugs from stocks maintained at LD 16:8 but transferred as eggs to LD 13:11 the mean age at first oviposition was 32.0 days, and was 27.9 days when the transfer was to LD 12.5:11.5. There is thus some manifestation of diapause under these regimens, but the major shift in age at first oviposition, to over 60 days, comes between 12 and 12.5 hrs in the daily light period. The "critical photoperiod" thus occurs between 12 and 12.5 hrs of light.

The diapause response curves for females from stocks reared at LD 24:0 (*i.e.* constant light) and 12:12 are also given in Fig. 1. Note here that the critical

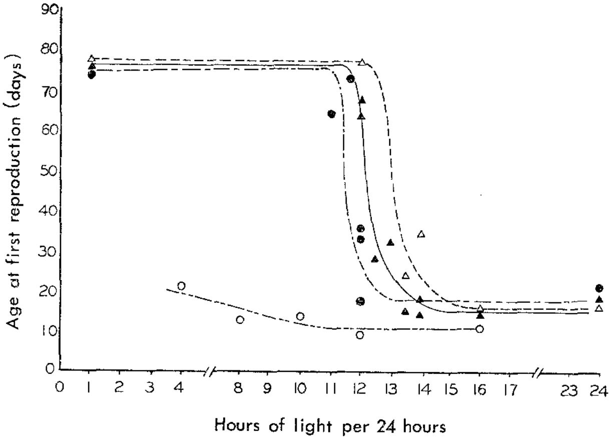

Fig. 1. Diapause response curves for different laboratory populations of *Oncopeltus fasciatus*. Right hand curve (open triangles) for bugs maintained at constant light and 23°C, middle curve (closed triangles) LD16:8 and 23°C, and left hand curve (closed circles) LD12:12 and 23°C. Lower curve (open circles) is for results at 27°C. In all cases bugs were transferred as eggs from their rearing conditions to experimental photoperiods

photoperiod for bugs reared in constant light has shifted to the right toward a longer daylength while that of the bugs reared at LD12:12 has shifted to the left. These shifts are similar to those found in geographical races of many species of insect; more northern populations show a critical photoperiod at a longer daylength and more southern populations at a shorter (Danilevskii, 1965; Danilevsky *et al.*, 1970; Tauber and Tauber, 1972). Selection experiments suggest that in *O. fasciatus* these shifts are the result of genetic changes (Dingle, 1974). These experiments will be discussed in detail in a subsequent paper (Dingle and Hegmann, in prep.).

Finally, the experiments were repeated at 27°C using eggs transferred from LD16:8 to 4 additional regimens. Under these conditions the diapause response while present was much reduced. At LD4:20 the mean age at first oviposition was 20.4 days as opposed to 13.8 days at LD8:16 ($t = 1.68$, $df = 45$, $P < 0.10$). A temperature of 27°C thus largely, but apparently not completely, overrides the diapause response. *O. fasciatus* reproduces successfully up to 31°C (Dingle and Caldwell, 1971) at which temperature diapause is essentially absent. Overriding of diapause by temperature is a common phenomenon in insects (Lees, 1955; Beck, 1968).

Once diapause had begun, it could be broken by transferring bugs to an appropriate photoperiod or temperature. With respect to the former, adults derived from eggs transferred at 23°C from LD16:8 to LD12:12 (*i.e.* which normally would be expected to diapause) were transferred back to LD16:8 when they were 15 days post-eclosion. The females showed a mean age at first oviposition of 31.4 days or 16.4 days after transfer. This mean was significantly different from that of diapausing bugs maintained at LD12:12 ($t = 2.56$, $df = 61$, $P < 0.02$) indicating that diapause had been broken. Similarly females transferred as eggs

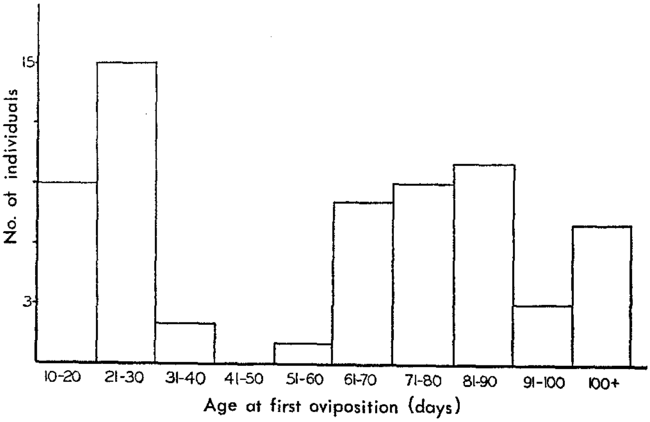

Fig. 2. Distribution of ages at first oviposition in females transferred as newly molted adults from LD16:8 23°C to LD12:12 23°C. Age 0 is taken as time of adult molt

from LD16:8 to LD12:12 in 23°C, were transferred again at 15 days post-eclosion to a temperature of 27°C still at LD 12:12. These females began ovipositing on the average at 32.9 days again significantly different from diapause bugs ($t = 2.27$, $df = 60$, $P < 0.05$). Again diapause was broken by the appropriate environmental conditions, in this case temperature.

2. The Sensitive Period

Most insects are sensitive to photoperiodic change during a specific stage (or stages) of the life history (Lees, 1955; Beck, 1968). Five experiments were run to test for a "sensitive period" in *O. fasciatus*. In the first three, bugs were transferred from LD16:8 23°C to LD12:12 23°C at the beginning of the 5th instar, 5 days after eclosion to the 5th instar, and as newly eclosed adults (within one hour of eclosion). In both cases where transfer occurred in the 5th instar, the adult females diapaused with mean ages of first oviposition of 69.4 days and 70.9 days respectively. In the case of bugs transferred as newly eclosed adults, mean age at first reproduction was 59.9 days suggesting diapause. A closer inspection of the data, however, revealed an interesting result; approximately one-third of the females showed little or no diapause (mean age at first oviposition 21.7 days, range 16–31 days) while the remaining two thirds showed complete diapause (mean age at first oviposition 84.8 days, range 58–146 days). The distributions within and between the two groups are shown in Fig. 2. In the 4th experiment bugs were transferred from LD16:8 to LD12:12 after they had been adults for 5 days; in this case there was no diapause (mean age at first oviposition 16.3 days).

In the 5th experiment of this series, bugs were transferred as eggs from LD16:8 to LD12:12. At the beginning of the 5th instar (within one hour of ecdysis), they were transferred back to LD16:8 and kept there until they were 5 day old adults at which point they were again transferred to LD12:12. The results are indicated in Fig. 3. The majority of bugs in this experiment (21 of

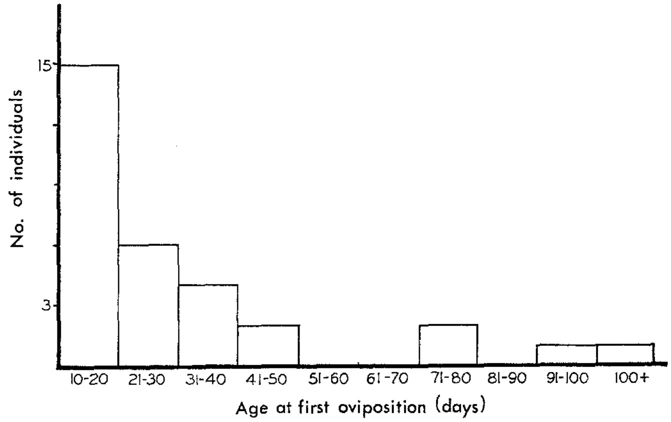

Fig. 3. Distribution of ages at first oviposition in a sample of females transferred as eggs from LD16:8 23°C to LD12:12 23°C, as newly molted 5th instars from LD12:12 to LD16:8, and as 5 day old adults from LD16:8 to LD12:12. Age 0 is time of adult molt

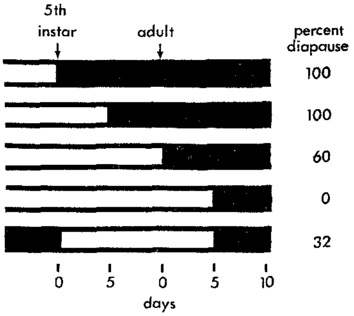

Fig. 4. Diagram of sensitive period experiments and their results. Open bars indicate time spent at LD16:8 23°C, solid bars time spent at LD12:12 23°C. Arrows indicate eclosion to 5th instar and adult, respectively

a total of 31) showed no diapause and began ovipositing before 30 days post-eclosion, while the remainder showed varying degrees of delay in oviposition; the last bug to lay eggs did so at 102 days. Since bugs transferred to LD12:12 as 5 day old adults failed to diapause (see above), the fact that some diapausing occurred here implies a residual effect of the LD12:12 photoperiod experienced from the egg through the 4th instar. The data in Fig. 3 also suggest that under the conditions of this experiment, there is a continuous gradation from non-diapause to diapause in contrast to the discontinuous distribution in Fig. 2.

The results of these 5 experiments can be summarized as follows and are diagrammed in Fig. 4. If bugs experience a short photoperiod during the last days of the 5th instar, all will diapause. It is at this time that the pharate adult is developing, and this stage is thus a sensitive period. In addition, 60% of the bugs diapaused when they experienced a short photoperiod immediately after adult eclosion (Fig. 2) indicating that in some bugs photoperiodic sensitivity continues into early adulthood. Finally, approximately one-third of the bugs which experienced a short photoperiod from egg through 4th instar also diapaused (Fig. 3) suggesting some photoperiodic sensitivity in these early stages. There thus appears to be no single sensitive period in *O. fasciatus*, but rather increasing sensitivity rapidly rising to a peak in the late 5th instar at the time the pharate adult is developing and rapidly falling in the first days of adulthood. Five day old adults will no longer enter diapause if exposed to short days, but will break diapause if exposed to long days.

3. The Pattern of Diapause Emergence

In most reported studies of diapause the response is recorded as some proportion of the experimental (or occasionally natural) population entering into or emerging from the diapause state. Rarely is variability in rate or pattern of entry or emergence reported although there are some exceptions (*e.g.* Morris and Fulton, 1970; Waldbauer and Sternburg, 1973). The major problem has been the lack of a convenient or ready measure of such rates or patterns.

In *O. fasciatus* a similar problem exists with respect to entry into diapause, but there is a ready measure for emergence from it. This is the time course of egg-laying by the females. Two basic possibilities exist. First, individual females could emerge from diapause in all or none fashion so that once oviposition occurs, it continues thereafter at more or less maximal rate. Secondly, females could emerge gradually either by laying initially smaller clutches of eggs gradually increasing to maximal clutch size or by having initially longer intervals between clutches and gradually decreasing inter-clutch interval.

The oviposition pattern of *O. fasciatus* was analyzed by plotting mean clutch size against days for two groups of females. The first group consisted of bugs transferred as eggs from LD16:8 to LD12:12 (*i.e.* which diapaused), and the second consisted of bugs from a culture maintained for many generations without transfer at LD12:12 (*i.e.* which did not diapause); temperature was 23° C. Day 0 was taken as the first day a female laid a clutch of eggs; thus chronological age was ignored. Means were computed in two ways to test for the two basic time courses of egg laying indicated above. First, mean clutch sizes were computed only for those females which actually laid eggs on a given day. Thus if initial clutches are small, mean clutch size should increase to a maximum. This was not the case as indicated by the upper pair of regression lines in Fig. 5. Once egg-laying began, clutch sizes remained essentially the same throughout life in both diapause and non-diapause groups with only a very slight decline with age. The lower pair of regression lines was obtained by plotting the mean clutch size of all females alive on a given day following the onset of oviposition (*i.e.* failure to lay was counted as a clutch size of 0). If inter-clutch interval decreases over an interval following the onset of egg laying, then the means should again initially be small (because on a given day only a small portion of the females would be laying), rising as inter-clutch interval decreased and more females per day oviposited. There is no evidence for this as indicated in the lower pair of lines in Fig. 5. Inter-clutch interval increased slightly with age, but otherwise showed no change throughout egg-laying life in either the diapause or non-diapause groups.

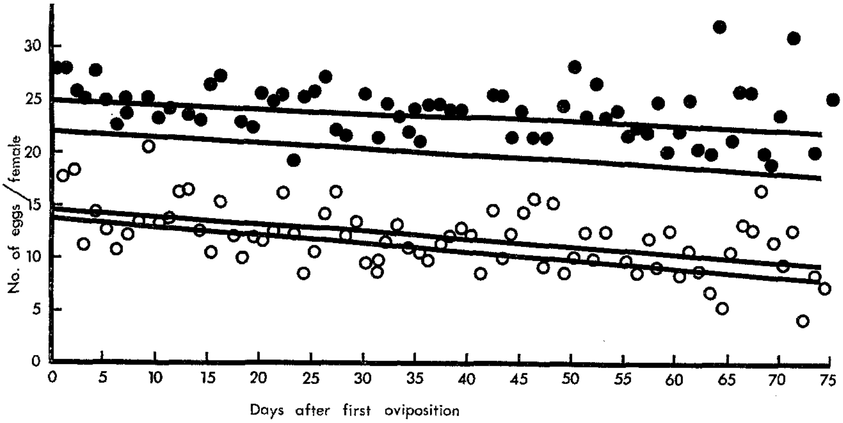

Fig. 5. Clutch size as a function of days after first oviposition (day 0 taken as day first eggs laid) in diapausing (upper of each pair of regression lines) and non-diapausing (lower line of each pair) *O. fasciatus*. Upper pair of lines are regressions through mean clutch sizes when only females laying eggs contributed to the mean; lower pair regressions through means with all females contributing. The points are for the diapausing population; those for the non-diapausing population are omitted for clarity

Discussion

Oncopeltus fasciatus exhibits a facultative reproductive diapause. This diapause is triggered by short photoperiods below a critical level (Fig. 1) which is a function of the environmental conditions under which the bugs develop. The photoperiodic response is largely overridden if the temperature at which cultures are maintained is raised from 23° C to 27° C (Fig. 1). The bugs are most responsive to short photoperiod during the late 5th instar (when the pharate adult is developing) but some individuals are sensitive as young adults or between egg and 4th instar (Fig. 4). Once diapause has been initiated, it can be broken by transferring either to a longer photoperiod (LD16:8) or a higher temperature (27° C). It is broken abruptly, for once oviposition starts, egg-laying proceeds at maximum rate with only a slight decline with age (Fig. 5). Physiologically diapause probably results from juvenile hormone levels too low to stimulate egg development since supplying the bugs with hormone results in the rapid onset of oviposition (Rankin, 1974).

Previously (Dingle, 1968a), I had referred to *O. fasciatus* as a non-diapausing insect but with an "incipient diapause" resulting from the delay in oviposition with short photoperiod. In view of the results reported here, however, this no longer seems valid. The responses to photoperiod and temperature are so similar to those reported for a wide variety of diapausing species, that they are best considered an adult reproductive diapause in the sense that the term is used in the insect physiology literature (Lees, 1955; Beck, 1968).

What is particularly interesting about the diapause of *O. fasciatus* is the fact that populations maintained under different environmental regimens show different critical photoperiods (Fig. 1). The demonstration of this phenomenon is not new. For example, Danilevskii (1965) and Danilevsky *et al.* (1970) list several species, particularly Lepidoptera, in which the critical photoperiod shows considerable geographic variation from north to south with northern populations entering diapause in longer photoperiods than southern. A similar observation is reported by Tauber and Tauber (1972) for the neuropteran *Chrysopa carnea.* In each case the photoperiod chosen is the best predictor for the time of onset of unfavorable temperatures. In addition, laboratory selection experiments with Lepidoptera (Harvey, 1957; Beck and Apple, 1961; Barry and Adkisson, 1966) and Hemiptera (Honek, 1972) have altered the critical photoperiod. Selection has also decreased the critical photoperiod for *O. fasciatus* (Dingle, 1974) suggesting that the changes observed in populations in different laboratory regimens (Fig. 1) were the result of varying selective pressures.

In this connection it is of interest to note that *O. fasciatus* is a pan-tropical species extending into both the North and South Temperate Zones (Slater, 1964). It is therefore subject over much of its range to photoperiods of less than LD 16:8. On the basis of the above results, one would predict that equatorial populations would exhibit critical photoperiods of less than 12 hrs. To the north of the equator, however, photoperiods are shorter in the dry season than in the wet season so that a photoperiodic response could be a good strategy for *O. fasciatus* populations occurring there. Under unfavorable conditions migration to a new habitat and subsequent colonization would be promoted by reproductive delay. In the tropics *O. fasciatus* is probably a continuous colonizer especially since *Asclepias* populations develop asynchronously over much of Central America (R. B. Root and S. J. Chaplin, personal communication). Life history characteristics in these regions, however, remain to be studied.

O. fasciatus is certainly a long distance migrant in that portion of its range where it is subject to wide fluctuations in photoperiod (Dingle, 1972). In spring and summer when photoperiods are long, reproduction is rapid allowing the insect to take maximum advantage of the previously empty habitats it colonizes (Dingle, 1968a). In the autumn transition from long to short days would result in the experiencing of light durations less than the critical photoperiod, and diapause would ensue causing reproductive delays comparable to those reported here. This delay both promotes flight in more individuals (R. L. Caldwell, unpublished) and permits more time to migrate elsewhere (Dingle, 1968b) with immediate maximum reproduction and hence colonization once a favorable habitat is found and diapause broken (Fig. 5). Iowa bugs brought in from the field in October do in fact show reproductive delays while those captured in mid-summer do not. In view of the correspondence of short photoperiod and dry season in much of Central America, the ability to predict season by photoperiod may have been a pre-adaptation in this pan-tropical insect allowing it to invade temperate regions. Additional support for this view comes from the fact that a species confined to the tropics, *O. unifasciatellus*, also shows a photoperiodic diapause response (Dingle, unpublished observations).

Finally, there also would appear to be selective advantage in timing maximum sensitivity to short photoperiod to occur in the late 5th instar during development of the pharate adult. In a species which combines adult reproductive diapause with migration this is the optimal time to respond to the appropriate environmental stimuli. If sensitivity were higher earlier in larval life, the individual might later enter diapause when in fact conditions were favorable for reproduction (*e.g.* when temperatures were suitable even though photoperiod was short). Even if the resultant diapause were broken by current environmental conditions, the resultant delay in reproduction would be a disadvantage in a colonizing species (*i.e.* an "*r* strategist"). Delaying the decision to diapause until later in the adult stage could result in some reproduction as conditions were becoming unfavorable and a delay in migration to more favorable areas. On balance, then, selection should favor maximum sensitivity in the pharate adult which is in fact when it occurs. The timing of sensitivity to short photoperiod is thus seen as part of the overall diapause-migration strategy of *O. fasciatus* (Dingle, 1968a).

Acknowledgements. I thank R. L. Caldwell and J. P. Hegmann for discussion and comments, and Toni Yeager, Nigel Blakley and Ann Warren for giving the bugs excellent care. Supported by grants GB-8705 and GB-40822 from the U.S. National Science Foundation.

References

Barry, B. D., Atkinson, P. L.: Certain aspects of the genetic factors involved in the control of the larval diapause of the pink bollworm. Ann. ent. Soc. Amer. **59**, 122–125 (1966)

Beck, S. D.: Insect photoperiodism. New York: Academic Press 1968

Beck, S. D., Apple, J. W.: Effects of temperature and photoperiod on voltinism of geographical populations of the European corn borer, *Pyrausta nubilalis*. J. econ. Ent. **54**, 550–558 (1961)

Caldwell, R. L.: A comparison of the migratory strategies of two milkweed bugs, p. 304–316. In: L. Barton Browne, ed., Experimental analysis of insect behaviour. Berlin-Heidelberg-New York: Springer 1974

Danilevskii, A. S.: Photoperiodism and seasonal development of insects. Edinburgh: Oliver and Boyd 1965

Danilevsky, A. S., Goryshin, N. I., Tyschenko, V. P.: Biological rhythms in terrestrial arthropods. Ann. Rev. Ent. **15**, 201–244 (1970)

Dingle, H.: Some factors affecting flight activity in individual milkweed bugs (*Oncopeltus*). J. exp. Biol. **44**, 335–343 (1966)

Dingle, H.: Life history and population consequences of density, photoperiod, and temperature in a migrant insect, the milkweed bug *Oncopeltus*. Amer. Natur. **102**, 149–163 (1968a)

Dingle, H.: The influence of environment and heredity on flight activity in the milkweed bug *Oncopeltus*. J. exp. Biol. **48**, 175–184 (1968b)

Dingle, H.: Migration strategies of insects. Science **175**, 1327–1335 (1972)

Dingle, H.: The experimental analysis of migration and life history strategies in insects, p. 329–342. In: L. Barton Browne, ed., Experimental analysis of insect behaviour. Berlin-Heidelberg-New York: Springer 1974

Dingle, H., Caldwell, R. L.: Temperature and reproductive success in *Oncopeltus fasciatus*, *O. unifasciatellus*, *Lygaeus kalmii*, and *L. turcicus*. Ann. ent. Soc. Amer. **64**, 1171–1172 (1972)

Dingle, H., Hegmann, J. P.: The nature of genetic variance influencing diapause in a migrant insect, *Oncopeltus fasciatus*. In preparation.

Harvey, G. T.: The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Canad. J. Zool. **35**, 549–572 (1957)

Honěk, A.: Selection for non-diapause in *Aelia acuminata* and *A. rostrata* (Heteroptera, Pentatomidae) under various selective pressures. Acta ent. Bohemoslov. **69**, 73–77 (1972)

Lees, A. D.: The physiology of diapause in arthropods. Cambridge: Cambridge University Press 1955

Morris, R. F., Fulton, W. C.: Heritability of diapause intensity in *Hyphantria cunea* and correlated fitness responses. Can. Ent. **102**, 927–938 (1970)

Rankin, M. A.: The hormonal control of flight in the milkweed bug, *Oncopeltus fasciatus*, p. 317–328. In: L. Barton Browne, ed., Experimental analysis of insect behaviour. Berlin-Heidelberg-New York: Springer 1974

Slater, J. A.: A catalogue of the Lygaeidae of the world. Storrs: University of Connecticut Press 1964

Tauber, J. J., Tauber, C. A.: Geographic variation in critical photoperiod and in diapause intensity of *Chrysopa carnea* (Neuroptera). J. insect Physiol. **18**, 25–29 (1972)

Waldbauer, G. P., Sternburg, J. G.: Polymorphic termination of diapause by Cecropia: genetic and geographical aspects. Biol. Bull. **145**, 627–641 (1973)

Dr. Hugh Dingle
Department of Zoology
University of Iowa
Iowa City, Iowa 52242, USA