THE INFLUENCE OF ENVIRONMENT AND HEREDITY ON FLIGHT ACTIVITY IN THE MILKWEED BUG *ONCOPELTUS*

By HUGH DINGLE

Department of Zoology, University of Iowa, Iowa City, Iowa, U.S.A.

INTRODUCTION

Previous work has shown that on a 16 hr.-light, 8 hr.-dark photoperiod at 23° C. flight activity in the large milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae), as determined by mean flight duration, is strongly age-dependent (Dingle, 1965, 1966). At 8–10 days following adult eclosion a distinct peak of flight activity is present; there are some later long flights, mostly by males, but these occur infrequently and in relatively few individuals (Dingle, 1966). The 8–10-day flight peak follows the cessation of growth-ring deposition in the cuticle and therefore is a post-teneral phenomenon (Johnson, 1963, *et seq.*); it also occurs prior to the onset of reproduction. Because the period of maximum flight duration is post-teneral, pre-reproductive, and several times the mean duration of flights earlier or later in the life-history, it was concluded that it in fact represents migration in *Oncopeltus* (Dingle, 1965). Calculations based on the flight capacity of individual bugs indicate that a migrant *Oncopeltus* is capable of traversing a considerable distance (Dingle, 1966).

Not all bugs in a population are migrants, however (Dingle, 1966). When flights of 30 min. or longer were used as criterion for 'migration', it was found that only about 30% of the females and 20% of the males of a 'wild-type' population tested at 2–3-day intervals for the first 45 days of adult life are migrants; the remainder fly only for brief periods no longer than a few minutes. There is thus present a behavioural polymorphism with recognizable 'flyers' and 'non-flyers'; the former are presumably colonizers serving the function of adaptive dispersal while the latter would maintain a population in a currently suitable habitat. The purpose of this paper is to examine the basis for the behavioural polymorphism.

The experiments undertaken were designed to explore the extent to which this polymorphism is genetically or environmentally induced. The environmental parameters analysed for their effect on flight were photoperiod, temperature and food level, all of which have major consequences for life-history and population (Dingle, 1967) and are therefore relevant to migration as well. Time of day influences reproductive and feeding activity (Caldwell & Dingle, 1967), and it has therefore also been examined in regard to flight. To uncover a possible genetic basis, selective breeding experiments were performed to try to increase the proportion of 'migrants' in the population. The work reported here is part of a continuing study of migration and other aspects of behaviour and the life-history of *Oncopeltus*.

MATERIALS AND METHODS

The 'wild-type' bugs used in this study were descendants of insects collected in the field at several locations in Michigan, Iowa and Illinois; in subsequent generations there was free hybridization between populations so that no genetically distinct lines were established. In addition to the previously used 16 hr.-light, 8 hr.-dark photo-period at 23° C. (16 L–8 D, 23° C.), three other conditions were used: 16 L–8 D, 27° C.; 12 L–12 D, 27° C.; and 12 L–12 D, 23° C. Dry milkweed seeds were supplied thrice weekly, and water was continually available from cotton wicks. Adults were colour-coded with spots of quick-drying paint on the day following eclosion; their ages were thus known, and they could be individually identified.
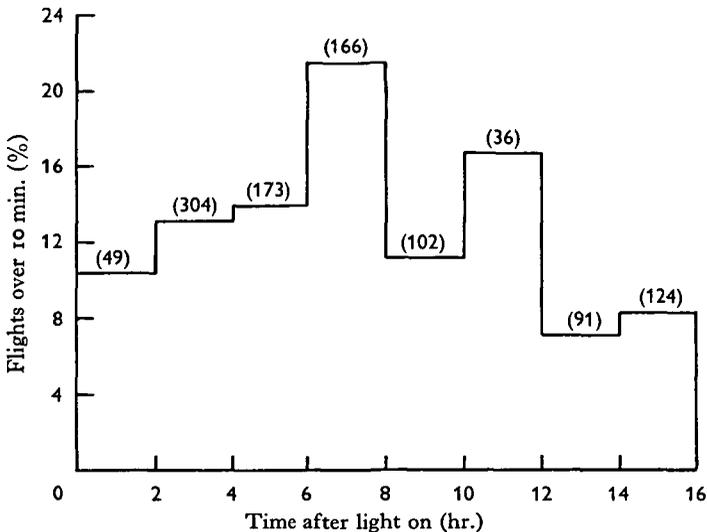

Fig. 1. Percentage of flights lasting 10 min. or longer as a function of time of day. Numbers in parentheses are sample sizes for each interval.

The method of flight testing, by attaching a stick to the pronotum with wax, was the same as previously described (Dingle, 1965). Again, the sum of the durations of the first five flights was used as the measure of flight activity. The specific methodological details and rationale of each of the experiments are given in the appropriate section of the Results.

RESULTS

Time of day

To determine if time of day influenced the duration of flight, all data for bugs reared at 16 L–8 D, 23° C. were analysed without regard to age or sex. Most of these insects were in fact not migrants, since long flight is largely restricted to certain ages, so the criterion for 'flight' was taken as 10 min. or longer rather than 30 min. as used for the determination of presumptive migrants. The data were divided into 2 hr. blocks, and the percentage of those tested which flew for at least 10 min. was calculated for each 2 hr. These values were then plotted against time in hours following the onset of the light portion of the light–dark cycle (Fig. 1).

The resulting histogram indicates that bugs are more likely to fly for 10 min. or longer during the middle of the day than at the beginning or end; they are least likely to fly in the evening. A χ^2 test on the data showed that the observed numbers flying were significantly different from the expectation that flyers would be evenly distributed throughout the day ($P < 0.05$). Duration of flight is therefore, in part at least, a function of time of day. Since these data include all bugs raised at 23° C. regardless of age, it is not possible, strictly speaking, to make such a statement for migrants. There is no *a priori* reason, however, why it should not apply to them, and most migration flights probably also occur during the midday hours.

Temperature

Bugs were flight-tested after rearing at three constant temperatures: 19°, 23° and 27° C. Previous results had shown that at 23° C. approximately 30 % of females and 20 % of males were migrants on a criterion of a 30 min. or longer flight time (Dingle, 1966). These have now been repeatedly confirmed. Four different experimenters, each unaware of the results obtained by the others, have flight-tested bugs at 23° C.; all four showed roughly 30 % of females and 20 % of males migrating (differences between experimenters were not significant with $P > 0.50$ using χ^2). These values were obtained regardless of whether bugs were reared on 12 hr. or 16 hr. photoperiods (see below).

Table 1. *Flights of bugs reared at 27° C.*

Photoperiod	Sex	Age*	Sample size	No. flying 30+ minutes	Percentage flying	P†
12 L–12 D	Males	6–10	27	0	0·0	< 0·01
12 L–12 D	Females	6–10	25	2	8·0	< 0·04
16 L–8 D	Males	6–10	55	7	12·7	> 0·30
16 L–8 D	Females	6–10	43	3	7·0	< 0·005

* In this and succeeding tables age is expressed in days following adult eclosion.
† P values when compared with bugs reared at 23° C.

In bugs reared at 27° C. flight was depressed. Table 1 gives the results of flight-tests on bugs reared on either 12 hr. or 16 hr. photoperiods. Testing was done 6, 8 and 10 days following adult eclosion because at this temperature deposition of cuticular growth rings ceases at 6 days (unpublished observations). In all cases but one, χ^2 tests comparing data from 23° and 27° C. rearing were statistically significant. The one exception is the sample of males reared on a 16 hr. day. This may mean that flight in these bugs is not depressed by raising the temperature, but in view of the fact that in all three of the other cases higher temperatures did seem to inhibit flight, the trend toward less flight in these males is probably real. On the basis of the sum of the evidence it seems safe to say that migration occurs less often in bugs maintained at 27° C. than at 23° C.

Finally, females only from cultures reared at 19° C. on a 12 hr. photoperiod were flight-tested at 10 and 14 days following eclosion; they were allowed 2 hr. to warm up to 23° C. before the trials. From a sample of 44 just 5 or 11·4 % flew. This was significantly different from the value for females reared at 23° C. (using χ^2, $P < 0.03$) indicating that low temperatures as well as high seem to depress flight.

Photoperiod

Bugs reared on a daily regimen of 12 L–12 D, 23° C. were flight-tested at 8–10 days following adult eclosion; at this time insects reared at the same temperature on a 16 hr. light period show a distinct flight peak (Dingle, 1965, 1966). With either photoperiod cuticular deposition ceases at 8 days at 23° C. (unpublished observations). The results are given in Table 2, and for both sexes are essentially no different from those obtained with 16 hr. of light. Again approximately 20 % of males and 30 % of females flew for long enough to be considered migrants.

Table 2. *Flights of bugs reared on* 12 L–12 D, 23° C.

Sex	Age	Sample size	No. flying 30+ minutes	Percentage flying	P*
Males	8–10	56	11	19·6	> 0·50
Females	8–10	58	16	27·6	> 0·50
Females	25	50	17	34·0	< 0·05

* When compared to bugs of the same age and sex reared at 16 L–8 D, 23° C.

On 12 hr. of light, however, oviposition by the females is considerably delayed when compared with 16 hr. animals (Dingle, 1967). Since migration is usually a pre-reproductive phenomenon (Kennedy, 1961; Johnson, 1965), females were in addition flight-tested at 25 days post eclosion. Bugs reared on 16 hr. of light are ovipositing at this time and have essentially stopped flying (Dingle, 1965, 1966); 12 hr. bugs had not yet begun to oviposit. These data are also presented in Table 2 and indicate that the proportion of migrants is the same as that at 8–10 days on either photoperiod, but is considerably higher than that of 16 hr. females of the same age. Extension of the pre-reproductive period thus also extends the time during which migration takes place.

To put pre-reproductive flight in ecological perspective, I have previously used the concept of 'reproductive value'. This term is a measure of the maximum contribution of an individual of specified age to future population increase and as such is also a measure of sensitivity to natural selection (see Dingle, 1965 for a more complete discussion). It is calculated from the expression

$$\frac{V_x}{V_0} = \frac{e^{rx}}{l_x} \sum_{x+1}^{\infty} e^{-rx} l_x m_x,$$

where V_x is the reproductive value at age x, V_0 is this value at age 0 (= 1), e is the base of natural logarithms, x is age, l_x is the proportion of those alive at age 0 still alive at age x, m_x is the birth rate expressed as the number of female eggs laid per female of age x (i.e. during the interval $x-\frac{1}{2}$ to $x+\frac{1}{2}$), and r is an expression of the growth potential of a population usually referred to as 'the intrinsic rate of (natural) increase'. All these values for *Oncopeltus* on a 12 L–12 D, 23° C. regimen have been reported elsewhere (Dingle, 1967). Reproductive values for adult females on this regimen are plotted in Fig. 2.

These values do not reach maximum until some time after the onset of migratory activity. In nature, therefore, a bug would presumably make its major contribution to future population growth after it had for the most part completed its flight or, in

other words, it would be an effective colonizer. These bugs were not tested beyond 25 days post eclosion, but it is likely that flight activity is considerably depressed once reproduction begins. This was the case with the 16L–8D, 23° C. regimen when reproduction was initiated much earlier (Dingle, 1965, 1967). Since they are flying when reproductive values are low, bugs on the shorter photoperiod are also less sensitive to selection during the migration period than are the insects reared on the longer days.
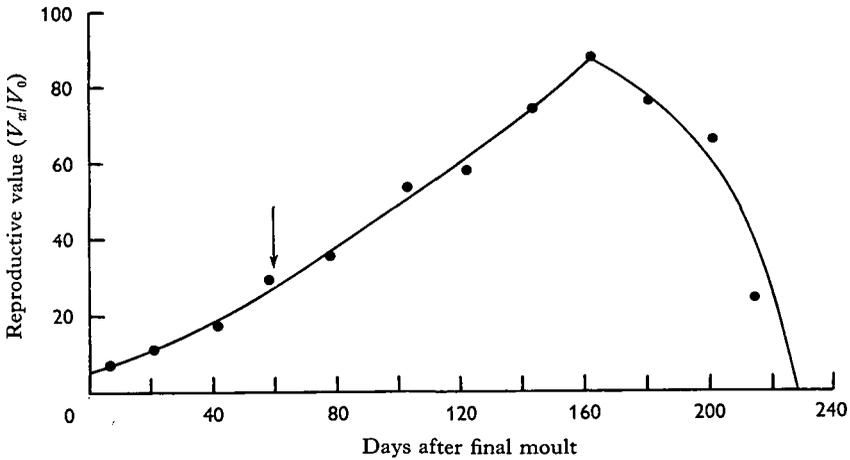

Fig. 2. Reproductive values for *Oncopeltus* females reared at 12L–12D, 23° C. Arrow indicates onset of oviposition.

Table 3. *Influence of food deprivation on flight*

Sex	Age starved	Age flight tested	Sample size	No. flying 30+ minutes	Percentage	No. flying 60+ minutes	Percentage
Males	5–8	8	74	33	44·6	20	27·0
Females	5–8	8	74	37	50·0	24	32·4
Females	20–25	25	58	24	41·4	19	32·8
Females	20–28	28	50	16	32·0	13	26·0

Food deprivation

Unfavourable environmental conditions would be expected to enhance migratory activity in insects (Southwood, 1962; Johnson, 1965, 1966). Accordingly, bugs of different ages were deprived of food for a few days to see if this treatment either increased the proportion of the population showing long flights or increased flight activity in insects which would not be expected to fly because of age or reproductive condition. One group was deprived of food for 4 days, days 5–8, shortly after adult eclosion and was then tested for flight at the end of this period. A second group was deprived from days 20 to 28, and the females only were flight-tested, first on day 25 and again on day 28. In both instances water was constantly available as usual; the regimen was 16L–8D, 23° C. The results of these experiments are given in Table 3.

If flights of 30 min. or more are taken as indicating migration, as has been done previously with *Oncopeltus*, then a short period of starvation early in adult life does

increase the proportion of migrants. In males the proportion increases from approximately 20 to 44·6 % and in females from approximately 30 to 50 %. These results are significantly different at $P < 0.01$ in both instances. During testing, however, it was noted that, although there was more flight, this was chiefly due to short bursts of activity. There did not, in fact, seem to be much if any increase in the number of long 'cruising' flights lasting an hour or more. Accordingly food-deprived bugs which flew for an hour or longer were compared with their counterparts having constant access to food. In this case the differences were not statistically significant ($P > 0.15$ for males, $P > 0.20$ for females). The effect of food deprivation on 5- to 8-day-old bugs seems to make them in general more likely to fly, but not necessarily to make them undertake long 'cruising' flights (as defined above) if they would not otherwise have done so. In other words, food deprivation did not activate a 'switch mechanism' converting non-flyers to flyers, but rather shifted a flight duration continuum toward somewhat longer flights. Note that the definition of 'migration' in this and preceding papers is an operational one; there is in fact a continuum between non-migratory and migratory flights in many insects (Kennedy, 1961) including *Oncopeltus*.

With regard to older bugs, females which are deprived of food beginning on day 20 soon cease to oviposit. When flight-tested at day 15 or day 28, they showed enhanced flight activity (Table 3) comparable to that shown by 25-day, and hence pre-reproductive, females on a 12 L–12 D regimen (Table 2). Taking flights of 30 or more minutes, the proportions flying at either day 25 or day 28 were significantly different from the results for 16 L–8 D, 23° C. females which were not food-deprived. Again this seemed to be in part a result of general but not necessarily migratory flight activity. The proportion flying for at least an hour at 25 days, however, was still significantly different from that of fed bugs. It thus seems to be the case that the tendency to migrate, depressed when reproduction starts, can reappear if reproduction shuts down as a result of food deprivation. The proportion capable of migrating is apparently not substantially changed.

Selection

By far the greatest effect on proportion migrating was produced by the appropriate selection and breeding for migratory capability. The P_1 generation consisted of four individuals, two males and two females, reared at 16 L–8 D, 23° C. and isolated from the opposite sex for the first 45 days following adult eclosion. During this time they were flight-tested at 3-day intervals and accumulated a total flight time of at least 40 hr. At day 46 they were placed together in a container and allowed to breed. The resulting offspring were then tested at 8 and 10 days post imaginal eclosion. Those flying on both trials were tested twice more and the two males and two females showing the most flight were again isolated, and their offspring in turn tested at 8 and 10 days following eclosion. The first four strong flyers of the F_3 generation were then the parents of F_4 from which ten bugs of each sex were tested to make sure the proportion of flyers remained high. Extensive sampling of the two later generations, F_3 and F_4, was not carried out because there was no obvious improvement of performance, and the procedures are somewhat laborious and time consuming.

The results of this selection for flight capacity are given in Table 4. The proportion of bugs flying for 30 min. or more increased from 20 and 30 %, respectively, for males

and females to at least 60 % in all cases. The differences between this strain and the 'wild-type' are highly significant in both the F_1 and F_2 generations and are evidently maintained through the F_4. Flight capacity is thus a heritable trait, and the behavioural polymorphism of migrants and non-migrants is to a great extent genetic.

Table 4. *Effect of selection on flight*

Sex	Generation	Sample size	No. flying 30+ minutes	Percentage flying	P*
Males	F_1	22	15	68·2	≪ 0·001
Females	F_1	25	15	60·0	< 0·01
Males	F_2	36	23	63·9	≪ 0·001
Females	F_2	41	28	68·3	≪ 0·001
Males	F_4	10	7	70·0	—
Females	F_4	10	6	60·0	—

* When compared with 'wild type' population of same age and sex.

DISCUSSION

Daily flight periodicities occur in a wide variety of insects including many migrants (Lewis & Taylor, 1965). Although *Oncopeltus* seems to be the first case demonstrated with tethered flight in the laboratory, the presence of maximum activity in the middle of the day is consistent with the observed field periodicities of other Heteroptera (Lewis & Taylor, 1965). In other insects the occurrence of flight during specific periods, in cases where it has been studied in any detail, seems to result from an interaction between emergence times and environmental factors; there is no evidence for an underlying timing mechanism although emergence itself may be endogenously timed (Johnson, 1965). In *Oncopeltus* mating, feeding and oviposition also exhibit daily peaks (Caldwell & Dingle, 1967). Of these the oviposition peak occurs at midday; conflict with migratory flight is unlikely, however, since migration is usually pre-reproductive. Mating and feeding, which in *Oncopeltus* are not mutually exclusive, reach simultaneous maxima late in the day and are thus out of phase with the flight peak. The adaptive advantage of the various cyclic activities in *Oncopeltus* may be that they allow dispersal of the population yet assure reproductive success by bringing the sexes together at feeding sites after flight (migration) has taken place (Caldwell & Dingle, 1967). The mechanisms underlying the cyclic behaviour are currently being investigated.

Whether or not a particular individual *Oncopeltus* will be capable of migrating is in large part determined by genetic factors (Table 4), and a relatively small proportion, 20 % of males and 30 % of females in a 'wild-type' population under the conditions of these experiments, seem to have this capability. Similar proportions of 'migrants' were found in the overwintering generation of a related species, *Lygaeus kalmii*, flight-tested immediately following its emergence in the spring (R. L. Caldwell, unpublished observations). I do not know why these values for *Oncopeltus* and *Lygaeus* are not higher, but they are consistent with the data of Lewis & Taylor (1965) who found that Heteroptera in general were among the least active flyers when compared with other common flying insects. Tethering, on the other hand, is a highly artificial situation, and in the present case no stimuli save absence of substrate contact were used to

maintain flight. If additional stimuli such as wind are used, some 'non-flyers' can be induced to fly for appreciable periods (unpublished observations), and the values given could thus be underestimates of what happens in nature. Suffice it to say, however, that there does seem to be a genetic behavioural polymorphism since the proportion of 'flyers' can be significantly increased by appropriate selection and breeding.

Of the environmental factors tested, temperature alone seems to alter the proportion of migrants in the population; both high and low temperatures depress long flights. In the case of high temperature, 27° C., lack of flight activity may represent an adaptive response in that the population would grow rapidly in a currently suitable habitat since reproduction begins early and reaches a peak soon after (Dingle, 1967). Possible reasons for reduced migration at low temperature, 19° C., are less clear. At this temperature, however, *Oncopeltus* is near the threshold where reproduction is possible (Lin, Hodson & Richards, 1954), and the absence of flight may indicate general physiological depression. No more satisfactory explanation is at present apparent.

The enhanced flight, but not necessarily migratory, activity caused in young adults by starvation (Table 3) would be adaptive in that it would allow short-range displacement of much of the population to habitats with greater resources. Food deprivation and photoperiod, however, affect migration mainly by lengthening the period during which it occurs; older bugs thus fly at a time when their counterparts, well-fed or on long days, do not. Ordinarily the period of migration, in females at least, is bounded at the beginning by the completion of cuticle development (Dingle, 1965) and at the end by the onset of oviposition, although in certain instances later inter-reproductive flights may occur. It is thus both post-teneral and pre-reproductive (or more strictly pre-ovipositional; see also Kennedy (1961), and Johnson (1963), *et seq.*). Short photoperiod and low food level extend the pre-oviposition period in *Oncopeltus* females, and as a result extend the flight period as well. The data, except in the case of low temperature noted above, are thus consistent with Johnson's (1963) 'hypothesis that migration is evoked, prolonged or suppressed in genotypical migrants by environmental factors affecting ovary development'. In some insects, e.g. aphids (Kennedy & Booth, 1963) and frit flies (Rygg, 1966), flight itself can shorten the pre-oviposition period.

Ecologically, the fact that most migration is pre-ovipositional means that females at its completion have a high 'reproductive value' (Fig. 2). They will, in other words, make a maximum contribution to the future growth of the population and are, therefore, colonizers and not refugees (Dingle, 1965). In cases, such as that of *Oncopeltus*, where extended migration may allow escape from the oncoming winter, habitats only seasonally available can be exploited (Dingle, 1967). This is a natural outgrowth of the presumed original function of migration, that of adaptive dispersal in insects living in any sort of temporary habitat (Southwood, 1962). Because of their high reproductive values, however, even the escapees from winter are colonizers when they reach their destinations.

In *Oncopeltus* starvation is known to prevent egg production by inhibiting production and release of corpus allatum (juvenile) hormone (Johansson, 1958). It is also likely that short photoperiod, which delays reproduction, acts in a similar manner. Johnson's (1963, *et seq.*) contention that migration is ultimately controlled by

endocrine factors is thus given additional support. The crucial question, however, is not answered. This is, simply, does the hormone (or hormones) involved act only indirectly on migration by affecting the reproductive system or does it act directly on the behaviour itself irrespective of reproductive effects? Experiments are currently in progress to explore this problem.

SUMMARY

1. Most long flights of *Oncopeltus*, tested using tethered flight in the laboratory, took place during the middle of the day. This is consistent with field data from other Heteroptera.

2. In bugs reared at 23° C., regardless of length of photoperiod, 20 % of males and 30 % of females were migrants. Temperatures of 19° and 27° C. reduced these proportions.

3. A short photoperiod of 12 hr. of light failed to increase the proportion of migrants over that present at 16 hr. The period in the life-history during which migration took place, however, was lengthened considerably. In spite of the lengthened flight period and a delay in oviposition, migrants arrive at their destinations with reproductive value high and are therefore colonizers.

4. Food deprivation may increase somewhat the proportion of migrants if it occurs shortly after eclosion, although most of the extra activity seems to be due to additional non-migratory flight. If it occurs after oviposition has begun, food deprivation can result in migration at a time when it would ordinarily have ceased.

5. The proportion of migrants could be significantly increased by appropriate selection and breeding. The basis for migratory behaviour is thus, in part at least, genetic.

6. The data in general support the hypothesis that migration is elicited, enhanced, or suppressed in genotypical migrants by those environmental factors which influence ovary (and corpus allatum) development.

REFERENCES

CALDWELL, R. L. & DINGLE, H. (1967). The regulation of cyclic reproductive and feeding activity in the milkweed bug *Oncopeltus* by temperature and photoperiod. *Biol. Bull. mar. biol. Lab., Woods Hole* (in the Press).

DINGLE, H. (1965). The relation between age and flight activity in the milkweed bug, *Oncopeltus*. *J. exp. Biol.* **42**, 269–83.

DINGLE, H. (1966). Some factors affecting flight activity in individual milkweed bugs (*Oncopeltus*). *J. exp. Biol.* **44**, 335–43.

DINGLE, H. (1967). Life history and population consequences of density, photoperiod, and temperature in a migrant insect, the milkweed bug *Oncopeltus*. *Am. Nat.* (in the Press).

JOHANSSON, A. S. (1958). Relation of nutrition to endocrine reproductive functions in the milkweed bug *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *Nytt Mag. Zool.* **7**, 1–132.

JOHNSON, C. G. (1963). Physiological factors in insect migration by flight. *Nature, Lond.* **198**, 423–7.

JOHNSON, C. G. (1965). Migration. In *The Physiology of Insects*, vol. II, ch. 3, pp. 187–226. Ed. M. Rockstein. New York: Academic Press.

JOHNSON, C. G. (1966). A functional system of adaptive dispersal by flight. *Ann. Rev. Ent.* **11**, 233–60.

KENNEDY, J. S. (1961). A turning point in the study of insect migration. *Nature, Lond.* **189**, 785–91.

KENNEDY, J. S. & BOOTH, C. O. (1963). Coordination of successive activities in an aphid. The effect of flight on the settling responses. *J. exp. Biol.* **40**, 351–69.

LEWIS, T. & TAYLOR, L. R. (1965). Diurnal periodicity of flight by insects. *Trans. R. Ent. Soc. Lond.* **116**, 393–469.

LIN, S., HODSON, A. C. & RICHARDS, A. G. (1954). An analysis of threshold temperatures for the development of *Oncopeltus* and *Tribolium* eggs. *Physiol. Zoöl.* **27**, 287–311.

RYGG, T. D. (1966). Flight of *Oscinella frit* L. (Diptera, Chloropidae) females in relation to age and ovary development. *Ent. exp. appl.* **9**, 74–84.

SOUTHWOOD, T. R. E. (1962). Migration of terrestrial arthropods in relation to habitat. *Biol. Rev.* **37**, 171–214.